0273-2289/95/5152—0649$06.40

A Combined Chemical-Enzymatic Method to Remove Selected Aromatics from Aqueous Streams

XIAODONG XU,[1] VIJAY T. JOHN,*[,1]
GARY L. MCPHERSON,[2] DEBORAH A. GRIMM,[3]
JOSEPH A. AKKARA,[4] AND DAVID L. KAPLAN[4]

[1]Department of Chemical Engineering; [2]Department of Chemistry; and [3]Coordinated Instrumentation Facility, Tulane University, New Orleans, LA 70118; and [4]US Army Natick Research, Development and Engineering Laboratories, Natick, MA

ABSTRACT

Aromatics are major pollutants found in aqueous environments and in sediments. Although there are many chemical and biochemical processes to remove and/or treat these contaminants, they have to be considered in light of the economcs and the time scales for treatment. We describe our initial work on a hybrid chemical-enzymatic technique to remove aromatics from aqueous streams. The aromatic is first converted to the corresponding phenol through classical Fenton-type chemistry involving catalysis by Fe(II). The phenol is subsequently polymerized through an enzymatic mechanism, using horseradish peroxidase as the oxidative enzyme. The polymer is insoluble in water and can be easily recovered. In addition, such phenolic polymers are useful products with varied applications in coatings and resins technologies. Thus, the pollutants can be eventually converted to useful products.

Index Entries: Aromatics; aqueous streams; phenolics; Fenton reaction; enzymatic polymerization.

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*Author to whom all correspondence and reprint requests should be addressed.

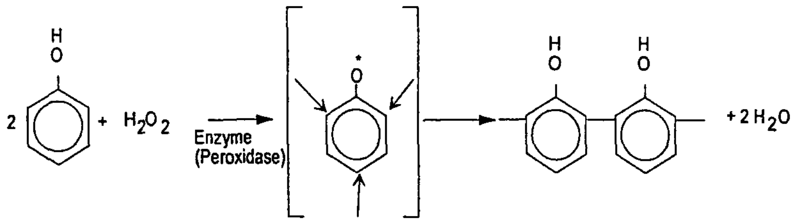

Fig. 1. A simplified mechanism for the enzymatic polymerization of phenols. We note the 1:1 stoichiometry of H_2O_2 reacted to chain linkage, and a 2:1 stoichiometry of phenol reacted to H_2O_2 reacted.

INTRODUCTION

Aromatics are major organic pollutants found in aqueous waste streams. Phenols, for example, are prevalent in waste streams from coal-conversion processes, and are generated during coal pretreatment steps prior to combustion. Physical methods to remove such contaminants include air stripping of volatile organics and adsorption, but the techniques do not result in destruction of the contaminant compounds unless followed by catalytic oxidation (*1*). The compounds can be degraded biologically, but the extensive times for complete destruction are a detrimental factor to be considered. Chemical methods include ozonation, peroxidation, photocatalysis, and hybrid versions of these techniques. (*2*).

In 1983, Klibanov and coworkers (*3*) pioneered a novel enzymatic approach to remove phenolics from waste streams. Here, an oxidative enzyme, horseradish peroxidase, was used to couple phenolics resulting in a polymer that is water-insoluble. The polymerization mechanism is shown in Fig. 1; polymer formation follows oxidative coupling where the addition of H_2O_2 leads to the initial formation of phenoxy-type radicals and final linkage at positions *ortho* or *para* to the hydroxyl, to form an insoluble polymer chain. The polymer is thus precipitated out of solution and is easily recovered. The polymer is relatively nontoxic compared to the monomer, and can be easily compacted for landfill or incineration. Phenolic polymers are widely used in resins and coatings, and a viable option is the use of these recovered polymers for such materials applications. The conjugation present in the enzymatically synthesized polymers also indicates potential applications to electrooptics (*4*).

The question that arises is whether other aromatics can be thus coupled together to form useful polymers. We attempt to do this by hydroxylating aromatics to make them amenable to peroxidase catalysis. Essentially, the method consists of chemically converting the aromatic to the corresponding phenol, and subsequently using the enzyme to couple the phenols and remove them from solution. Such chemical oxidation of aromatics can, in principle, be accomplished by a variety of methods where the

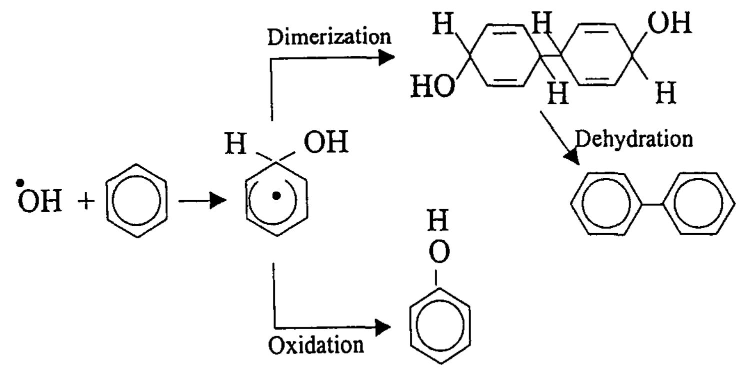

Fig. 2. The Fenton reaction for benzene oxidation (from Walling and Johnson, 1974).

common factor is the generation and reaction of hydroxyl radicals. Indeed, most of the techniques listed above for oxidative destruction of organics (e.g., ozonation, photolysis, peroxidation) involve the initial creation of a hydroxyl radical (OH$^\bullet$), which subsequently reacts with the organics (*2,5*). The objective in all such techniques described in the literature is the complete oxidation of the organic to carbon dioxide and water. To our knowledge, we are unaware of work where partial oxidation of aromatics to the corresponding phenol is carried out to make them susceptible to polymerization.

This article thus describes the use of a hybrid technique to remove aromatis from solution. The classical Fenton reaction (*6*) is employed to oxidize aromatics partially to the corresponding phenol, and enzymatic polymerization is then carried out to convert the phenols to the insoluble polymer. The Fenton reaction (*7,8*) involves the oxidation of Fe(II) to Fe(III) and the consequent formation of a very reactive hydroxyl radical (OH$^\bullet$) through the redox sequence below (*9*):

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + HO^- + OH^\bullet \tag{1}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^\bullet + H^+ \tag{2}$$

$$OH^\bullet + H_2O_2 \rightarrow H_2O + OH_2^\bullet \tag{3}$$

$$HO_2^\bullet + Fe^{3+} \rightarrow Fe^{2+} + H^+ + O_2 \tag{4}$$

$$HO_2^\bullet + Fe^{2+} \rightarrow HO_2^- + Fe^{3+} \tag{5}$$

$$OH^\bullet + Fe^{2+} \rightarrow Fe^{3+} + HO^- \tag{6}$$

Reactions of the hydroxyl with the aromatic are complex. For benzene, a pathway established by Walling and Johnson (*8*) involves the initial formation of the hydroxycyclohexadienyl radical followed by dimerization and dehydration to biphenyl, or further oxidation to phenol, as illustrated in Fig. 2. Biphenyl is insoluble and is therefore removed from solution.

However, the phenol remains in solution and our objective is then to couple the phenol molecules enzymatically to remove phenol from solution as a useful polymer. Since the reactions of benzene are reasonably well understood, we have used benzene as the model compound in our initial studies described here.

EXPERIMENTAL

Materials

All chemicals were purchased from Aldrich Chemicals (Milwaukee, WI) with a purity of at least 99.8% and were used as such. The enzyme, horseradish peroxidase (type II: mol wt 40,000, activity 200 U/mg), hydrogen peroxide (30%), and HEPES buffer were purchased from Sigma Chemical Company (St. Louis, MO). Deionized and doubly distilled water was used in all preparations.

Methods

The reaction mixture containing benzene and $FeSO_4 \cdot 7H_2O/FeCl_3$ was placed in 40-mL EPA standard vials with PFTE septa/phenolic screwed caps and magnetically stirred. The overhead dead space of the vials was minimized to avoid loss of the substrate through evaporation. For the Fenton reaction, the solution was buffered to pH 5.5 with HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]) at a concentration of 0.01*M*. The acidic medium is necessary to maintain Fe(II) solubility and prevent insoluble iron hydroxide formation. The Fenton reactions were initiated with the injection of H_2O_2 using a microsyringe. At least 3 h were allowed to complete the Fenton reaction before initiation of enzymatic polymerizations. At the end of the Fenton reaction, the pH is about 4.0. Fenton reactions were also run with 0.01*M* HCl (pH 2). For the enzymatic polymerization, the pH was adjusted to 8.2 using NaOH prior to reaction initiation.

Conversion of the substrate was monitored using a gas chromatograph (Varian 3410) equipped with a flame ionization detector. A 15-m fused silica capillary column with a phenylmethyl silicone stationary phase (1.5-μm film thickness) was used for analysis. Benzene and phenol concentrations were directly determined by injecting a 0.5 μL aqueous phase sample into the column held at isothermal conditions of 160°C, maintaining a flow rate of 26 mL/min (splitless). Both peaks eluted within 1 min and were well separated. Biphenyl, which is insoluble in water, was extracted subsequent to reaction using diethylether. Analysis of biphenyl involved injection at an initial column temperature of 115°C, which was immediately ramped to 140°C (5°C/min).

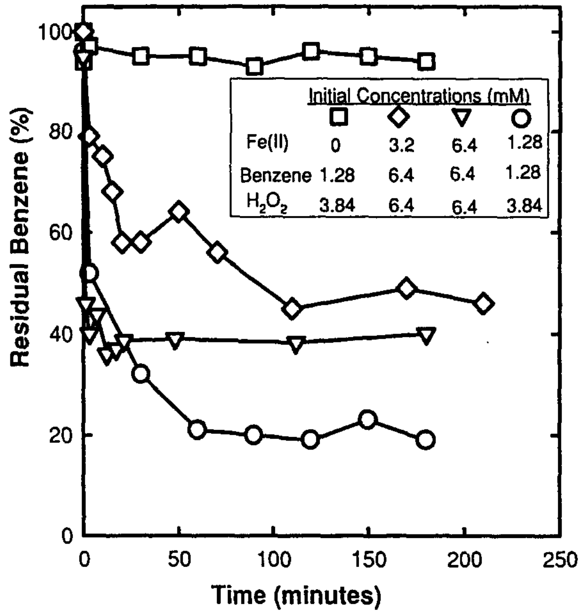

Fig. 3. Initial kinetics of the Fenton reaction. System pH adjusted to 5.4 with 0.01*M* HEPES before all experiments but one (O) where the pH is adjusted to 2 with 0.01*M* HCl. The same trend (rapid initial kinetics and appreciable conversion levels) is seen over the pH range 2–5.5.

Identification of products was accomplished by GC/MS. The GC/MS was performed on a Kratos Profile Mass Spectrometer outfitted with a Shimadzu 14A GC and operated with the Kratos Mach3 software. The GC column was a DB1 (J&W Scientific) 30 m × 0.25 mm with film thickness of 0.25 μm. Split injection (30:1) was performed, and carrier flow rate was about 30 mL/min. The following temperature program was used: initial temperature was 70°C for 1 min, first ramp was 10°C/min to 190°C, held for 1 min and second ramp was 20°C/min to 270°C, held for 2 min. The injector, MS re-entrant line, and interface were all at 270°C. The positive electron ionization (EI) spectra were acquired in the nominal mode with the magnet scanned over 50–500 amu at 0.3 sec/decade and with a mass resolution of 600.

RESULTS AND DISCUSSION

Figure 3 illustrates the typical rates of the Fenton reaction. In our experiments, the benzene level was kept below saturation to allow uniform sampling while the mixture was stirred. The control experiment (without addition of Fe(II)) indicates no reaction. On addition of Fe(II), the Fenton reaction proceeds very rapidly When a 1/1 Fe(II)/benzene initial ratio is used, over 50% of the final conversion level is reached within 3 min. To quantify such rapid kinetics roughly, a pseudo-first-order rate constant is calculated as 0.2/min. On a turnover basis (in terms of iron content), the reaction rate is approx 0.003 mmol benzene reacted/min·mg Fe(II). At all conditions listed in Fig. 3, the reaction is essentially complete within the first hour. The highest final conversion level of 80% (unfilled circles) is obtained when an excess of H_2O_2 (H_2O_2/benzene of 3/1) is used. The fast initial reaction rates and reasonable final conversion levels indicate the viabilty of the Fenton reaction in conversion of the aromatic.

Parameter variation experiments are summarized in Table 1 to characterize further the reaction. The effect of varying H_2O_2 is of particular interest, since the ratio of H_2O_2 to substrate (benzene) determines final conversion. We note that the amount of hydrogen peroxide needed to convert benzene to phenol does not follow a 1:1 stoichiometry as would be required by the simplified route to phenol ($H_2O_2 + C_6H_6 \rightarrow C_6H_5OH + H_2O$) and significantly high conversions (>90%) are obtained only at high excess concentrations of H_2O_2 (>6/1 H_2O_2/benzene). We also note that the material balance between total conversion and the amounts of phenol + biphenyl formed is approximately satisfied only at H_2O_2/benzene stoichiometric ratios of unity and lower. At these relatively lower ratios, all the precipitate is soluble in diethyl ether and is identified as biphenyl. At the higher ratio of 3/1 H_2O_2/benzene (row 7), we do not find closure of the material balance with just biphenyl and phenol as the sole products. In addition to biphenyl, a small amount of precipitate is observed under these conditions that is not extractable in diethylether. This precipitate on elemental analysis reveals carbon, hydrogen, and oxygen in the approximate weight ratio 12:1:4 indicating incorporation of oxygen into the hydrocarbon product. At H_2O_2/benzene ratios of 6/1 (row 8) and higher, practically no precipitate is formed, and solution analysis reveals only remnant benzene and the formed phenol. Based on these observations, we conclude that at high stoichiometric ratios, some of the H_2O_2 is used up in further oxidations to oxidize the aromatic completely. We are carrying out studies with ^{14}C-labeled benzene to attempt to quantitate CO_2 evolution at very high H_2O_2/benzene ratios. The oxygenated precipitate observed at the 3/1 ratio is perhaps a result of side reactions linking partially oxidized species to an insoluble oligomer.

A complete understanding of the reactions that take place at high H_2O_2/benzene ratios has not been attempted here. This is because of the fact that our objective is to maximize the efficiency of the reaction to produce phenol that can then be enzymatically polymerized. We thus examine just the results of the 1/1 (and lower) H_2O_2/benzene ratios where the material balances closes with an appreciable yield of phenol. The fact that a 1/1 stoichiometry leads to a maximum of 57% benzene reacted (row 4) implies the occurrence of the overall reaction ($2\,H_2O_2 \rightarrow 2\,H_2O_2 + O_2$) (9) that may deplete H_2O_2 available for reaction with the aromatic.

Table 1
Effect of Parameter Variations on Benzene Conversion and Product Yields Through the Fenton Reaction*

Run no.	Fe(II) conc., m*M*	H_2O_2 conc., m*M*	Benzene B, conc., m*M*	%Benzene conversion, mol B reacted/ mol B in feed, x100	%Conversion to phenol, P, mol P formed/ mol B in feed, x100	%Conversion to biphenyl, BP, mol BP formed/ mol B in feed, x100
1	6.4	2.13	6.4	21	14	5
2	2.13	6.4	6.4	48	39	5
3	3.2	6.4	6.4	51	38	10
4	6.4	6.4	6.4	57	38	14
5	19.2	6.4	6.4	40	26	7
6	38.4	6.4	6.4	28	14	8
7	6.4	19.2	6.4	82	54	10
8	6.4	38.4	6.4	95	10	<1

*Results are the average of three experiments. The maximum error is ±3 in conversion percentages. For example, the first value in percent benzene conversion (21%) can range from 18 to 24%.

At constant H_2O_2 and benzene initial concentrations of 6.4 m*M*, we note that the conversion goes through a maximum with Fe(II) addition (rows 2-6 in Table 1). The reactions of Fe(II)/Fe(III) are complicated, but the Fenton reaction involves competition for hydroxyl radicals by Fe(II) (reaction 6 of the redox sequence, $Fe^{2+} + OH^{\bullet} \rightarrow Fe^{3+} + OH^{-}$) leading to the depletion of hydroxyl radicals available for benzene oxidation.

With these considerations, rows 2, 3, and 4 in Table 1 illustrate the optimal conditions for the Fenton reactions with benzene. Row 2 perhaps represents the optimal conditions because the slightly lower conversion of benzene (48%) is offset by the significantly lower Fe(II) requirements (2.13 m*M*) and the high phenol/biphenyl selectivity (approx 8/1).

The enzymatic reaction is then carried out to reduce phenol levels. To determine if the Fenton reaction and enzymatic polymerization could be combined, two experimental approaches were considered. The first approach involved introducing the enzyme into the reaction medium prior to the Fenton reaction, and the second involved introduction of the enzyme after completion of the Fenton reaction. Both approaches, however, involved adjustment of the pH to 8.2 prior to initiation of the enzymatic reaction, since the enzyme is inactive at the acidic pH of the Fenton reaction. In the first approach, we observed no polymerization and reduction of phenol content, indicating that the enyzme was inactivated during the course of the Fenton reaction. The observation can be understood from the mechanism for peroxidase catalysis (*10*).

$$\text{Peroxidase} + H_2O_2 \rightarrow \text{Compound I} \tag{7}$$

$$\text{Compound I} + SH_2 \rightarrow SH^{\bullet} + \text{Compound II} \tag{8}$$

$$\text{Compound I} + SH_2 \rightarrow SH^{\bullet} + \text{peroxidase} \tag{9}$$

Compounds I and II are intermediate protein structures; compound I involves an oxo-ligated iron(IV) protoporphyrin π-cation radical, $(^{+\bullet}P)Fe^{IV}O$, and compound II is an iron(IV)-oxoprotoporphyrin IX species, $(P)Fe^{IV}O$. The substrate is the electron-donating species SH_2 (phenol). The free radical species $SH^{\bullet}$ combine to form the chain linkage. In the presence of excess H_2O_2, compound I is intercepted into an active form of the protein according to:

$$(^{+\bullet}P)Fe^{IV}O + H_2O_2 \rightarrow (P)Fe^{III}O + H_2O + O_2 \tag{10}$$

Thus, it is important that this shunt does not occur and that the substrate access to the enzyme is maintained. We believe that the enzyme is inactivated during the Fenton reaction during the addition of H_2O_2, when there is an insufficient amount of the phenolic substrate.

When the reaction is done following a two-step approach, where the Fenton reaction is completed, the pH adjusted, the enzyme introduced, and H_2O_2 added in small increments to initiate polymerization, we find that the reaction is efficient. Over 95% of the phenol formed during the Fenton reaction is removed from solution by enzymatic polymerization.

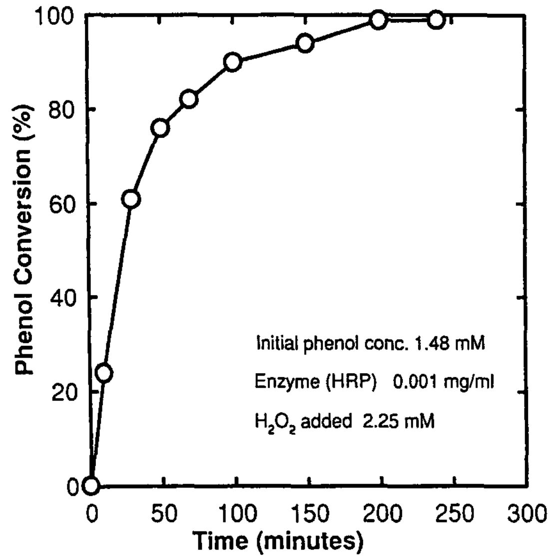

Fig. 4. Kinetics of a typical enzymatic polymerization. System pH adjusted to 8 with HEPES/NaOH. The H_2O_2 (2.25 m*M*) was added in one single step rather than in pulses. Phenol conversion was calculated on the basis of phenol disappearance.

Figure 4 illustrates model kinetics with a "clean" system containing just phenol (no iron species) prior to initiation of the enzymatic step. With an enzyme concentration of 0.001 mg/mL, the reaction is sufficiently rapid, with final conversion being reached in 3–4 h. The conversion here is calculated on the basis of phenol disappearance. At such low enzyme concentrations (0.001 mg/mL), we do not see much of a polymer precipitate. On the other hand, when the enzyme concentration is increased by an order of magnitude, the material balane for phenol disappearance is well satisfied by the mass of the insoluble polymer. Our recent experiments indicate that the enzyme is trapped in the polymer that is forming. At low enzyme concentrations, this entrapment leads to a loss of activity before chains can grow to the point of insolubility. In other words, at very low enzyme levels, conversion of phenol is primarily to low-mol-wt oligomers that remain marginally soluble.

A calculation from the results of Fig. 4 indicates that the pseudo-first-order rate constant for phenol conversion under these conditions is 0.03/min. The pseudo-first-order rate constant is an order of magnitude slower than rates for the Fenton reaction. On an enzyme turnover basis, however, the reaction rate is approx 0.02 mmol phenol reacted/min·mg enzyme. Thus, on the basis of catalyst mass, the enzymatic reaction is almost an order of magnitude faster than the Fenton step.

Thus, our results indicate the feasibility of the chemical-enzymatic method to remove benzene from the aqueous phase. The process can be visualized as a two-stage process (Fig. 5), where the Fenton reaction is

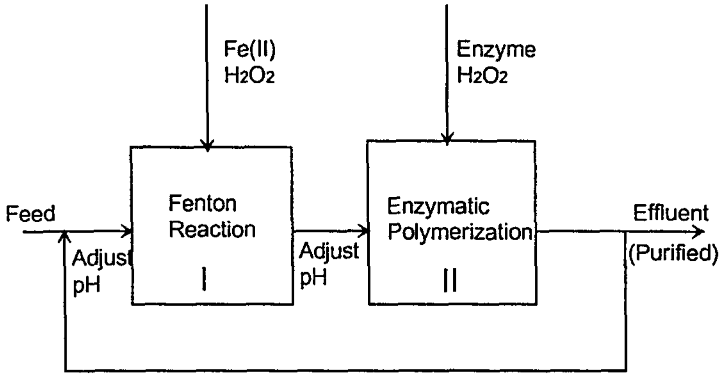

Fig. 5. Schematic of a two-stage process for phenol removal. We note the existence of a precipitate of biphenyl in Stage I and a precipitate of polyphenol in Stage II that have to be recovered intermittently.

carried out in the first stage and enzymatic polymerization in the second. To attain higher conversions of the aromatic, a recycle-type system or a train of two-stage reactors can be employed. Some additional considerations of the two-stage process of Fig. 5 are mentioned. In Stage I, it may be possible to introduce multiple pulses of H_2O_2 to increase benzene conversion beyond the 50% level of the first pulse (rows 2 and 3 in Table 2) and still maintain phenol yields. Smith and Norman (*11*) have shown that the selectivity of phenol to biphenyl is considerably increased by reducing the rate of H_2O_2 addition. Studies are in progress to add H_2O_2 for the Fenton reaction in pulses to increase conversion and phenol selectivity.

A second consideration is the pH adjustment required for Stage II. If the effluents from Stage I are brought into Stage II, the increased pH in Stage II leads to the formation of insoluble iron hydroxides. Thus, Fe(II) has to be continuously added to Stage I, and for continuous operation, this may not be a very viable prospect. It is therefore necessary to immobilize Fe(II) in Stage I. Preliminary work in this laboratory, on ion-exchanging Fe(II) to Nafion-type membranes, shows that the membranes become catalytically active for the Fenton reaction. A full study of this aspect of the heterogeneously catalyzed Fenton reaction is in progress.

CONCLUSIONS

This work has shown the possibility of combining two existing technologies, the Fenton reaction and enzymatic polymerization, to remove benzene efficiently from solution and to convert it to a useful polyphenol, but there are some important aspects to be examined before extrapolating the concept to full-scale technology. First, it is important to understand the nature of the combined process to the removal and polymerization of substituted benzenes and to mixtures of such aromatics. Initial work appears to show the feasibility of hydroxylating aromatic amines and alkyl substituted benzenes. Applicability to chloro- and nitrobenzenes would be most useful from an environmental viewpoint. A second question is the continued viability of the enzyme. Recent work by Nakamoto and Machida (*12*) has shown that additives, such as gelatin and polyethylene glycol, can minimize peroxidase inactivation by preventing polyphenol adsorption on the enzyme. Such aspects as applied to the combined process have to be examined. A third aspect is whether phenol selectivity in Stage I can be improved. In addition to adding H_2O_2 in small pulses as mentioned above, Walling and Johnson (*8*) have shown that small amounts of Cu^{2+} also appears to favor phenol selectivity. These factors have to be examined carefully, and refinements of the two-stage process described here need to address such concepts. Another aspect is the nature of polyphenol formed in Stage II, its molecular weight, and physical properties as relevant to applications in resins and coatings technologies or as advanced materials. Here again, we have found that small amounts of anionic surfactants added to the polymerization step help sustain chains in solution for longer extents owing to surfactant hydrogen bonding with the monomer and growing chain. Again, a full study of surfactant effects on chain growth may help design polyphenols with appropriate mol-wt distributions.

It is our opinion that this initial study has shown the potential feasibility of combining chemical partial oxidation of an aromatic to subsequent enzymatic polymerization, to produce a useful product, and to remove aromatics from aqueous streams. The process is environmentally benign, since Fe(II) in small quantities is nontoxic and H_2O_2 is a benign oxidant. Other hybrid processes of generating hydroxyl radicals (e.g., photocatalysis) can also be visualized. At this point, however, it remains to be seen if the process described above or its variants are economically viable.

ACKNOWLEDGMENTS

Support from the US Army, the National Science Foundation, and the Department of Energy/Tulane Environmental Management Program is gratefully acknowledged.

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