Peroxidase-Catalyzed Oxidative Coupling of Phenols in the Presence of Geosorbents

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Introduction

Phenolic contaminants are of major environmental concern because of their multiple toxicity effects, and because they are generally highly soluble and thus environmentally mobile (1,2). Phenolic contaminants can be introduced to the natural environment through a variety of agricultural and industrial activities, such as pesticide applications, industrial discharges, and as a result of the incomplete biodegradation of certain other widespread contaminants, e.g., polyaromatic hydrocarbons. A particularly intriguing aspect of the environmental behavior of this class of organic contaminants is that they are able to undergo extensive oxidative coupling reactions both among themselves and with other organic substances, reactions mediated by such naturally occurring catalysts as peroxidases, laccases, tyrosinases, and oxides of iron and manganese (1-7). Enhancement of the enzymatic coupling reactions of phenolic chemicals has in fact been examined as a potentially promising means for both subsurface immobilization of these compounds (1-4) and their transformation in wastewater treatment processes (5-7).

This study focuses on investigating peroxidase-mediated phenol coupling reactions in the presence of soil/sediment materials. The goal was to mechanistically understand the influences of geosorbent materials on enzymatic coupling reactions, and to develop methods for predicting such influences. Experiments of phenol coupling reactions were performed under strategically selected conditions in systems containing different model geosorbent materials, using horseradish peroxidase (HRP) as the surrogate catalyst, and phenol the target contaminant. Three organic polymeric materials, cellulose, lignin and polymethylstyrene (PMS), and one inorganic material, silica sand were investigated as model geosorbent materials. The three organic materials differ significantly in their elemental compositions and structural features, and represents respectively natural organic materials at distinctly different diagenesis stages. The experimentally observed effects of the sorbent materials on phenol coupling were interpreted based on a comprehensive mechanistic treatment of the elemental reactions involved in the process, and the geosorbent effects were correlated to their chemical characteristics. Results of the study provide information critical for engineering application of enzymatic coupling reactions in soil/subsurface remediation practice.

Materials and Methods

Formation of non-extractable products under varying reaction conditions was examined to assess sorbent effects on phenol coupling. Phenol-coupling reactions were performed at room temperature in 13×100-mm glass test tubes operated as CMBRs. Each reactor contained 3 mL of 10-mM phosphate buffer (pH=7.0) solution comprised by a 500-μM mix of ^{14}C-labeled and unlabeled phenol, 2-mM H_2O_2, and a predetermined dosage of HRP. The reactor also contained a model sorbent at one of a series of selected sorbent/water ratios. Triplicate experiments were conducted for each reaction condition. After a 2-hr reaction period during which enzyme activity was completely depleted, 3 mL of methanol was added to each reactor and mixed with the

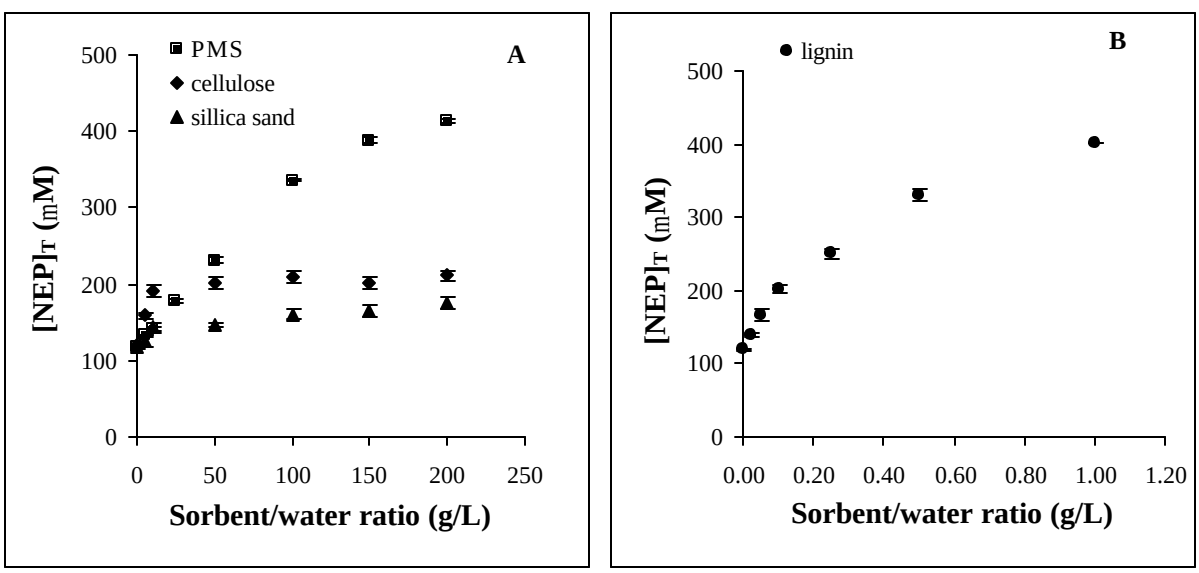
contents for 30 minutes. After extraction, a 1.5-mL sample of solution was withdrawn from the reactor and centrifuged at 20,800g for 20 min to separate the liquid and solid phases. A 0.5-mL aliquot of the liquid phase was sampled and mixed with 3 mL of ScintiSafe Plus 50% liquid scintillation cocktail, and its radioactivity then measured using a Beckman LS6500 liquid scintillation counter (Beckman Instruments, Inc.). The radioactivity remaining in the solid phase was calculated by mass balance and the NEP concentration calculated and expressed in terms of molar equivalents of phenol in the original solution. Blank control tests indicated that physical losses during the 2-hr reaction time and subsequent extraction and analysis were negligible.

Enzyme inactivation rates during phenol coupling reactions were examined separately with non-radiolabeled phenol at selected conditions. Samples of 0.05-mL volume were taken at predetermined times, and immediately measured for enzyme activity by the ABTS method described earlier (3). In this ABTS method, 0.05 mL of sample was added to a cuvette containing a 3-mL volume of phosphate buffer solution (pH = 6.0), followed by addition of 0.3 mL of 20-mM ABTS and 0.3 mL of 10-mM hydrogen peroxide to start the assay. The absorbance change at 405 nm was monitored by a 6405 UV/Vis spectrophotomer (Jenway Inc., Princeton NJ). One unit of peroxidase activity is defined as that amount catalyzing the oxidation of one µmol of ABTS per minute.

Results and Discussion

Figure 1 presents concentrations of total NEP formation ($[NEP]_T$) for systems having a constant HRP dosage and containing cellulose, silica sand, PMS (A) and lignin (B) at varied sorbent/water ratios. NEP formation was enhanced by increasing sorbent/water ratios in the case of each sorbent investigated. For cellulose and silica sand (Figure 1A), NEP formation initially increases sharply with increasing sorbent/water ratio up to a value of approximately 10 g/L, and then either levels off or increases more modestly at higher sorbent/water ratios. For PMS (Figure 1A), NEP continues to increase significantly with increasing sorbent/water ratio until, at the highest ratio tested (200g/L), a yield of 414-µM equivalents of NEP was obtained, representing nearly a four-fold increase over that formed in the absence of a sorbent. The influence of lignin is even more significant, as evidenced in Figure 1B by the comparable increase in NEP yield obtained with addition of only a two-order of magnitude lower sorbent mass.

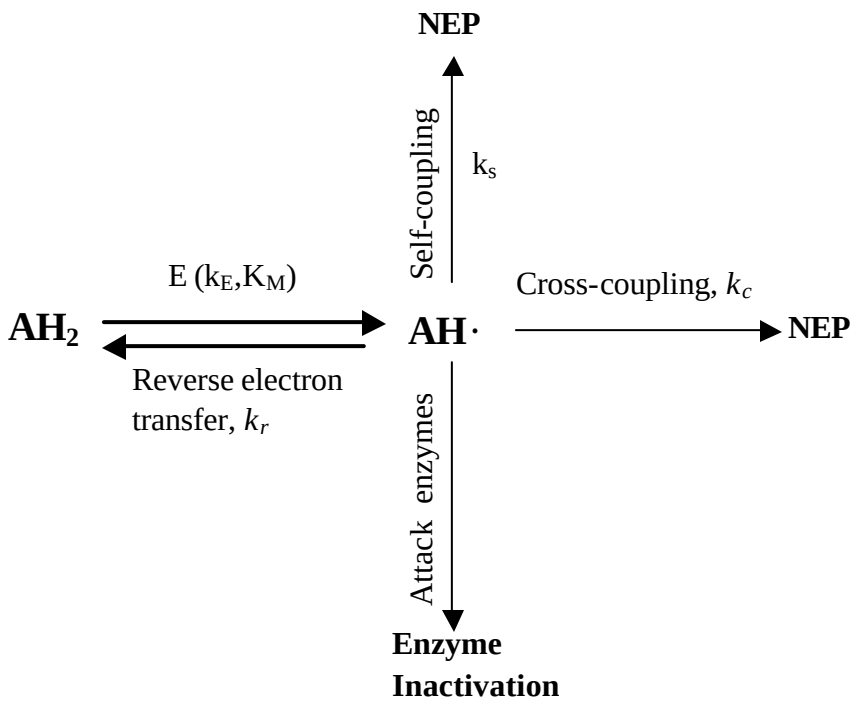
FIGURE 1



It was also experimentally observed in the study that three of four sorbent materials examined had mitigation effects on HRP inactivation in the enzymatic reaction system investigated, i.e., cellulose, silica sand, and lignin, but not polymethylstyrene (PMS).

A conceptual model depicted schematically in Figure 2 was developed to describe rate relationships for enzymatic phenol coupling in the presence of reactive sorbents. This model presumes that phenoxy radicals (AH•) are generated in a reversible process in the enzymatic reaction step, which then participate in several post-enzymatic reactions that include: i) attack and inactivation of the enzyme; ii) self-coupling of radicals; and/or, iii) cross-coupling of radicals with reactive sorbent materials. A system of equations designed to articulate this conceptual model was shown to successfully capture the rate behaviors observed experimentally for both enzyme inactivation and NEP formations.

FIGURE 2



The conceptual model was used to quantify and interpret the experimentally observed influences of different natural and model geosorbent materials on the enzymatic phenol-coupling reactions. The results lead to a conclusion that sorbent materials, depending on their chemical characteristics, influence peroxidase-mediated phenol coupling through one or both of two principal mechanisms; i.e., i) mitigation of enzyme inactivation, and/or ii) participation in cross-coupling reactions.

The influences of sorbent materials were further correlated to their chemical characteristics. It was found that sorbents may mitigate the inactivation of horseradish peroxidase by forming certain types of enzyme-ligand associations, and that such enzyme-ligand associations tend to be readily formed between HRP and materials comprising relatively hydrophilic polymeric matrix containing oxygen atoms. The study also suggests that aromatic or unsaturated C-C bonds may be necessary features for a material to participate in cross-coupling reactions. Substituent groups contained in the monomeric structure of a polymer material appear to have significant influences on its cross-coupling reactivities. In general, diagenetically “young” humic-type soil organic matters containing abundant oxygen-containing functionalities tend to be more reactive with respect to

participating in cross-coupling reactions than do diagenetically “old” kerogen-type materials having relatively reduced chemical structures.

Results obtained in this study lay a mechanistic framework for evaluating the reaction behaviors and efficiencies of peroxidase-mediated phenol coupling processes in systems containing varied soil/sediment materials. The results are thus critical for potential engineering implementation and enhancement of enzymatic coupling reactions in soil/subsurface remediation practice. Since enzymatic coupling reactions occur extensively as an important class of reactions in soil humification processes, the study also provides a strong scientific basis for understanding the natural transformation and fate of phenolic contaminants in environmental systems.

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