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Transformation of catechol in the presence of a laccase and birnessite

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Abstract

The transformation of naturally occurring phenols to humic polymers through oxidative coupling reactions may involve oxidoreductive enzymes and soil minerals as catalysts. There is limited information on the possible inhibitory or synergistic interactions between oxidoreductases and mineral catalysts as they participate in oxidative coupling of phenolic substrates. In this study, a ternary system was investigated, in which a fungal enzyme (*Trametes villosa* laccase), birnessite (δ-MnO_2), and a naturally occurring phenolic compound (catechol) were reacted together to model soil processes. Binary systems (catechol/laccase and catechol/birnessite) were included for comparison. In the absence of the mineral, *T. villosa* laccase (950 katal ml^{-1}) transformed 31% of catechol, whereas birnessite (1 mg ml^{-1}) in the absence of the enzyme showed a 24% catechol transformation. The percentages of catechol transformation in the binary systems did not accumulate in the ternary system; instead, birnessite and laccase tested together transformed only 36% of catechol. This suggested that birnessite had an inhibitory effect on substrate transformation by laccase catalysis. Enzyme assays indicated that inhibition was a result of enzyme deactivation by humic-like polymers produced by birnessite, and by Mn^{2+} ions released from the mineral. These observations underscore the importance of considering enzyme-soil mineral-organic matter interactions in studies of humus formation and contaminant removal.

Keywords: Birnessite; Catechol transformation; Laccase; Ternary systems

1. Introduction

Phenolic compounds occurring in soil environments are subject to oxidative coupling, a reaction that is important in humus formation (Bollag, 1992). The underlying mechanism of oxidative coupling is the oxidation of phenolic substrates to free radicals or quinones that subsequently undergo polymerization or binding to the existing humus (Dec and Bollag, 2000). Phenol oxidation may be mediated by oxidoreductive enzymes (e.g. laccase, peroxidase, tyrosinase) or abiotic catalysts (Bollag, 1992; Fillazzola et al., 1999; Naidja et al., 1999; Dec and Bollag, 2000). Birnessite is among the abiotic catalysts in soil that participate in oxidation, reduction, hydrolysis, polymerization, and complexation of many organic compounds, including catechol (McBride, 1987; Zhang and Huang, 2003). Previous studies demonstrated the formation of humus-like polymers in the presence of this mineral (Shindo and Huang, 1984). Substrates tested for birnessite-mediated oxidative coupling reactions include hydroquinone, resorcinol, catechol, or pyrogallol (Shindo and Huang, 1984; Kung and McBride, 1988; Naidja et al., 1999; Majcher et al., 2000; Wang and Huang, 2000a,b). Polyphenols involved in these reactions underwent partial ring cleavage with release of CO_2, as they formed coordinate complexes with mineral surfaces via hydroxyl groups at the adjacent carbons (Shindo and Huang, 1984; Majcher et al., 2000; Wang and Huang, 2000a; Dec et al., 2001). Another effect of birnessite-mediated oxidative coupling reactions was substituent release (decarboxylation, demethoxylation and dehalogenation) from phenolic substrates (Dec et al., 2001). Similar substituent release was observed for reactions mediated by various oxidoreductases, including laccase (Dec et al., 2001).

To date, functional interrelationships of soil components have mostly been tested for binary systems (enzyme–clay mineral, enzyme–organic substrate, or clay mineral–organic

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substrate). Ternary systems were studied by Claus and Filip (1988); Gianfreda and Bollag (1996), and Naidja et al. (1997), who investigated the activity of various oxidoreductases immobilized on clay surfaces and clay with hydroxyaluminum coatings.

The objective of this study was to investigate interactions of a fungal laccase (from *Trametes villosa),* birnessite, and a naturally occurring humic monomer (catechol) as interactive components of a model ternary soil system. Catechol (1,2-dihydroxybenzene) is a soil humic constituent, a plant polyphenol, and an intermediate of microbial metabolism of anthropogenic organic compounds (Majcher et al., 2000). Manganese oxides are common in soils and sediments, and birnessite is one of the most common forms (McKenzie, 1971; Sparks, 1995). The investigation of a model ternary system involving the above components was expected to reveal possible inhibitory or synergistic effects related to catalytic actions of birnessite and laccase, as they participate in humification processes.

2. Materials and methods

2.1. Reaction components

Birnessite was prepared according to the method of McKenzie (1971). Briefly, two moles of concentrated HCl was added dropwise to a boiling solution of 1.0 mol of $KMnO_4$ in 2.5 l of deionized water with vigorous stirring. After boiling for an additional 10 min, the precipitate was filtered and washed several times with deionized water, then freeze-dried. Analysis of birnessite, by X-ray diffraction and infrared spectroscopy showed the poorly crystalline structure, with characteristic peaks matching those reported in the literature (McKenzie, 1971; McBride, 1987; Dixon and Weed, 1989). The X-ray powder diffraction patterns were obtained using a PADV X-ray diffractometer with a Co Kα radiation source (Scintag, Inc., Cupertino, CA). Infrared absorption spectra were obtained using a Nexus 670 Fourier Transform Infrared Spectrophotometer (Thermo Electro Co., Waltham, MA), with 1.0 mg samples of the minerals dispersed in KBr (99.0 mg) pellets analyzed at a range of 4000–1000 cm^{-1}. The specific surface areas of birnessite was 27.7 $m^2\ g^{-1}$, as determined by N_2 sorptometry on an ASAP 2021 sorptometer (Micrometrics, Londonberry, NH) from the linear segment of the multi-point N_2 adsorption isotherms according to the Brunauer–Emmett–Teller theory (BET; Brunauer et al., 1938).

Catechol was purchased from Aldrich Chemical Co. (Milwaukee, WI). Uniformly ring-^{14}C-labeled catechol with specific activity of 3.4 mCi mM^{-1} was purchased from Sigma Chemical Co. (St Louis, MO). Extracellular laccase (*T. villosa*, EC 1.10.3.2) was obtained from Novo Nordisk (Danbury, CT).

2.2. Laccase activity

The activity of laccase (in katals) was determined using a biological oxygen monitor (Model 5300, Yellow Spring Instruments Co., Yellow Springs, OH) equipped with a Clark oxygen electrode. A specific amount of enzyme was added under continuous agitation with a Teflon magnetic bar to the thermostatic (25 °C) sample chamber filled with 0.1 M citrate–phosphate buffer (2.7 ml, pH 3.8). The enzymatic reaction was initiated by the addition of 0.1 ml of 10 mM 2,6-dimethoxyphenol (DMP) solution in the same buffer (stored previously at 25 °C) through a side port next to the electrode. Oxygen consumption was monitored for two minutes. One katal of enzymatic activity was defined as the amount of laccase required to consume 0.017 mmol s^{-1} of O_2. The effect of birnessite on laccase activity was tested by adding specific amounts of the mineral into the reaction mixture at a range from 0 (controls) to 37 mg ml^{-1}.

The inhibition of laccase by free Mn^{2+} was determined by amending the reaction mixture with $MnCl_2$. The concentration of metal ions ranged from 0 (control) to 5.0 mM. Negligible oxygen consumption was determined for metal ions in controls without laccase.

The kinetics of laccase in the absence and presence of Mn^{2+} (0–16 mM $MnCl_2$) was evaluated using the Michaelis–Menten model: $V = V_{max}S/(K_m + S)$, where V was the reaction velocity (absorbance change min^{-1}) and S was the substrate concentration (mM). Kinetic parameters (K_m and V_{max}) were calculated by fitting kinetics data to the Michaelis–Menten equation. The reaction velocity was measured colorimetrically on a Model UV-1601 spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD) using DMP in 2.7 ml of 0.1 M citrate–phosphate buffer at pH 3.8, as a substrate at concentrations ranging from 0.023 to 0.375 mM (the respective S^{-1} values ranged from 0 to 50). The reaction velocity (V) was expressed as the increase in absorbance min^{-1} at 468 nm.

2.3. Catechol transformation

To determine substrate transformation in the ternary model system, various combinations of birnessite (0–5 mg ml^{-1}) and/or laccase (0–3800 katal ml^{-1}) were incubated for 24 h at 25 °C with a 0.1 M solution of uniformly ring-labeled ^{14}C-catechol (2.1×10^5 Bq kg^{-1}) in 0.5% NaCl (1 ml) and centrifuged. The supernatants were analyzed by high-performance liquid chromatography (HPLC) for remaining catechol, and radiocounting for soluble transformation products. The pellets (complexes of birnessite with catechol and/or its metabolites) were analyzed by radiocounting.

To determine laccase inhibition by humic-like polymers produced by birnessite a two-stage experiment was carried out. In the first stage, ^{14}C-catechol (25 mM) was incubated with birnessite (2 mg l^{-1}) in 0.5% NaCl to generate catechol transformation products. In the second stage, 0–1-ml samples of the resulting supernatant, containing soluble transformation products (equivalents of 0–2.75 mg ml^{-1}, calculated on the basis of radioactivity remained in the solution) with no unaltered catechol, were added to a fresh binary reaction mixture consisting of ^{14}C-catechol (0.1 M) and laccase (1800 katal ml^{-1}), and incubated for 24 h. After incubation, the samples were centrifuged, and the supernatants were analyzed for remaining catechol by HPLC and radiocounting.

2.4. Adsorption of laccase on birnessite surfaces

To determine laccase adsorption isotherms, 5 mg of birnessite was mixed with 10-ml volumes of 0.5% NaCl aqueous solutions of the enzyme (0–14.5 ± 0.5 μg ml^{-1}) and shaken for 24 h at 25 °C. At the end of the equilibration period, the suspensions were centrifuged at 17,000 × *g* for 10 min and the supernatants were analyzed by a protein assay.

The Langmuir model, $q = X_m QK/(1 + QK)$, where q and Q are the sorbed and solution concentrations, respectively, was used to fit sorption data. The maximum sorbed concentration (X_m) and adsorption equilibrium constant (K) were determined by non-linear regression.

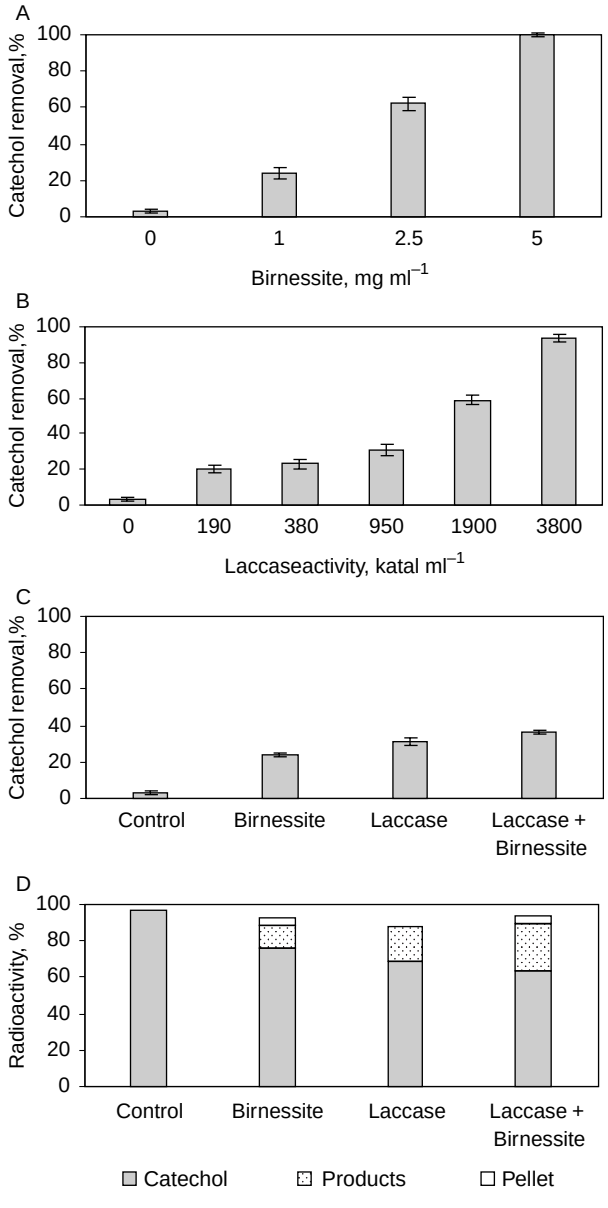

Fig. 1. Transformation of catechol (0.1 M) in binary and ternary systems: (A) Catechol removal by increasing concentrations of birnessite; (B) Catechol removal by increasing activities of *Trametes villosa* laccase; (C) Catechol removal by *T. villosa* laccase (950 katal ml^{-1}) and birnessite (1 mg ml^{-1}) applied together; (D) Distribution of radioactivity after the incubation of ^{14}C-labeled catechol with *T. villosa* laccase (950 katal ml^{-1}) and birnessite (1 mg ml^{-1}). The reactions were carried out in 0.5% NaCl for 24 h at 25 °C.

2.5. HPLC, radiocounting and protein assays

Before HPLC analysis, the supernatants from catechol transformation and adsorption isotherm experiments were filtered through a 0.45-μm nylon membrane. The HPLC analysis was conducted on a Waters HPLC system (Milford, MA) consisting of a 2690 Alliance separation module, a Waters 2487 dual λ absorbance detector operating at 280 nm, and a Supelcosil 15 cm × 4.6 mm LC-18 DB column of 5-μm particle size with a LC-18 DB guard column (Supelco, Bellefonte, PA). The mobile phase, delivered isocratically at a flow rate of 1.0 ml min^{-1}, was composed of an aqueous component A (Milli-Q water) and an organic component B (methanol) mixed at a 80:20 A to B ratio. The detection limit of catechol was 0.1 μg ml^{-1}. Radioactivity measurements were conducted on a Beta Trac 6895 liquid scintillation counter (Tracor Analytic, Elk Grove Village, IL). Aliquots of the supernatant (200 μl) were analyzed in 4.5 ml of Ecoscint (Manville, NJ). Birnessite pellets were combusted in a Biological Oxidizer OX 600 (R.J. Harvey Instruments, Hillsdale, NJ) to $^{14}CO_2$, which was trapped in Harvey Carbon-14 Cocktail for scintillation counting as above.

Protein assays were performed using Bio-Rad reagent to obtain colored protein complexes (Gogstad and Krutnes, 1982). The calibration curve was prepared using bovine serum albumin as the standard protein. The colorimetric measurements were carried out on a Model UV-1601 spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD) at 595 nm. The amount of protein adsorbed on birnessite was calculated as the difference between the amount of protein initially added and the amount remaining in the supernatants after incubation.

3. Results

The effects of birnessite amount and laccase activity on catechol removal are presented in Fig. 1. Based on the HPLC analysis of the binary systems, the percentage of catechol removal increased from 3 to 100% as the amount of birnessite was increasing from 0 to 5 mg ml^{-1} (Fig. 1A). With increasing laccase activity (from 0 to 3800 katal ml^{-1}), catechol removal increased from 3 to 94%. In the ternary system, birnessite (1 mg ml^{-1}) and laccase tested together removed 36% of catechol, as compared to a 24% removal for birnessite tested in the absence of laccase, and to a 31% removal for laccase tested in the absence of birnessite (Fig. 1C). Fig. 1D shows the recovery of radiolabeled carbon from ^{14}C-catechol, as determined by scintillation counting analysis of both the binary and ternary systems. Less than 100% of radioactivity recovery may be due to CO_2 evolution during the catechol transformation. As determined by radiocounting and HPLC of the ternary system (Fig. 1(D)), 90% of the initial radioactivity was present in the supernatant, in the form of unaltered catechol (64%) and soluble reaction products (26%). Only 2%

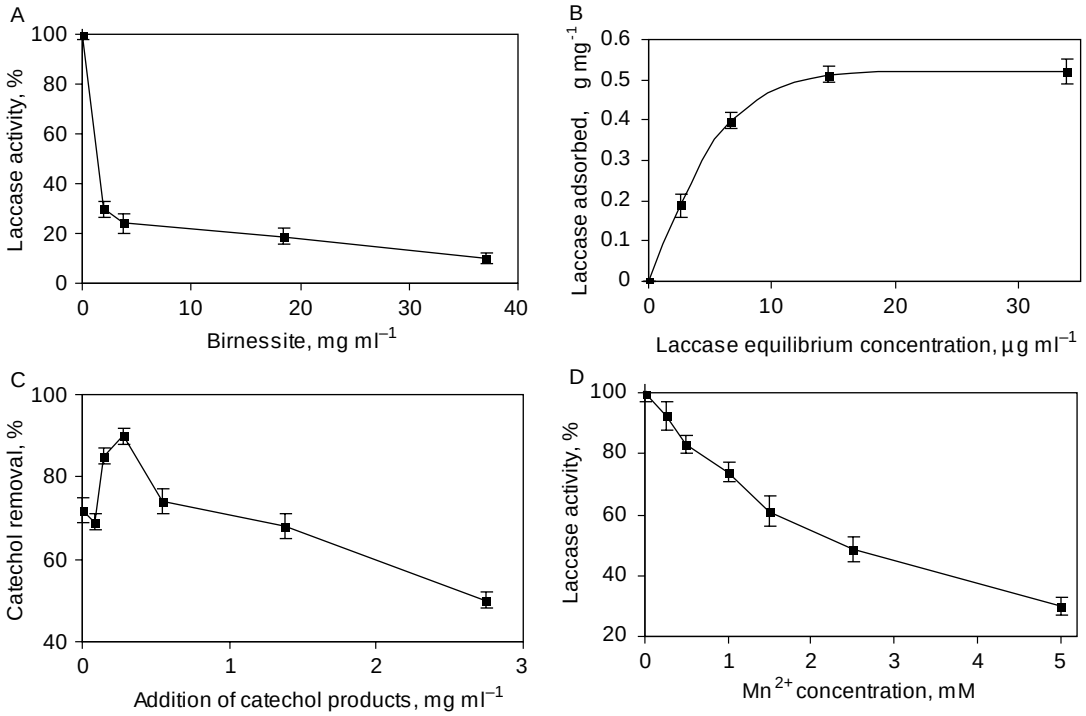

Fig. 2. Effects of various factors on *T. villosa* laccase: (A) Effect of increasing concentrations of birnessite on *T. villosa* laccase activity in pH 3.8 citrate–phosphate buffer at 25 °C; (B) Adsorption of *T. villosa* laccase on birnessite in 0.5% NaCl at 25 °C; (C) Removal of catechol (0.1 M) from 0.5% NaCl in the presence of *T. villosa* laccase (1800 katal ml^{-1}) and different concentrations of catechol products generated previously by birnessite; (D) Effect of increasing concentrations of Mn^{2+} ions on the activity of *T. villosa* laccase in pH 3.8 citrate–phosphate buffer at 25 °C.

was associated with the birnessite pellet, which indicated that adsorption of both catechol and its reaction products on birnessite was very limited.

Laccase activity (measured polarographically) decreased quickly (from 100 to 15%) with increasing amounts of birnessite (from 0 to 37 mg ml^{-1}) (Fig. 2A). As determined by protein assays, small amounts of laccase adsorbed to birnessite ($X_m = 0.52$ µg mg^{-1}) (Fig. 2B). The adsorption data were fit well by the Langmuir equation, showing a low adsorption equilibrium constant ($K = 0.3$ µg ml^{-1}).

Birnessite-generated products of catechol transformation had a mixed effect on laccase-mediated catechol transformation (Fig. 2C). At low concentrations (0–0.08 mg ml^{-1}), catechol transformation products showed no inhibitory effect. At intermediate concentrations of 0.14 and 0.28 mg ml^{-1}, the products had a stimulatory effect, causing an increase in catechol transformation from 72% for the unamended reaction mixture to 85 and 90%, respectively (Fig. 2C). Increasing the concentration of the products to 0.55 and 1.38 mg ml^{-1} resulted in a return to the non-effect level (about 70% catechol transformation), and further increase in the product concentration (to 2.75 mg ml^{-1}) caused a 20% reduction of catechol transformation (to 50%) as compared to the non-effect level.

Laccase activity was severely inhibited by the presence of Mn^{2+} ions, decreasing from 100% in the absence of Mn^{2+} to 30%, as metal ion concentrations were increased to 5 mM (Fig. 2D). The Michaelis–Menten analysis of kinetics data (Table 1) for laccase-mediated substrate transformation showed increasing K_m values (from 0.07 to 1.70 mM) and decreasing V_{max} values (from 3.41 to 1.90 min^{-1}) with increasing concentrations of Mn^{2+} ions (from 0 to 16 mM).

Table 1
Laccase kinetics parameters in the absence and presence of $MnCl_2$

Mn^{2+}	K_m	V_{max}	V_{max}/K_m
mM	mM	Absorbance change (min^{-1})	
0	0.07	3.41	48.71
3.2	0.26	2.83	10.88
6.4	0.56	2.58	4.64
9.6	0.78	2.11	2.71
12.8	1.49	2.28	1.53
16.0	1.70	1.90	1.12

The V_{max} and K_m values represent the maximum reaction velocity and the affinity of laccase for the substrate, respectively, as defined by the Michaelis–Menton equation.

4. Discussion

Since, both metal oxides and enzymes coexist in soils (Stotzky and Burns, 1982; Burns, 1990; Huang, 2000), one can expect the occurrence of synergistic or inhibitory effects on the rate and extent of catalytic reactions leading to humification. This research indicated that birnessite may have an inhibitory effect on soil enzymes. The total catechol transformation in our model ternary system of catechol, laccase, and birnessite, was less than the sum of catechol transformed by laccase and birnessite separately (Fig. 1C).

The inhibition of laccase activity could have occurred through one or more of the following three

mechanisms: (1) enzyme inactivation by birnessite-generated humic-like polymers, (2) enzyme immobilization (adsorption) on the mineral surfaces, and (3) enzyme inactivation by Mn^{2+} ions released from the mineral as the reaction proceeded. Birnessite is very effective in oxidizing phenolic compounds, and it forms dark humic-like polymers with a high degree of humification (Shindo and Huang, 1984; Wang and Huang, 2000b) that may efficiently deactivate enzymes. Birnessite can also mediate ring cleavage in catechol and other polyphenols (Majcher et al., 2000). Therefore, humic-like polymers produced by birnessite tend to have more aliphatic groups than those produced by other minerals. The increased aliphaticity of the reaction products may be a factor in inhibiting enzyme activity. It is well known that soil organic matter can inhibit enzymes (Vuorinen and Saharinen, 1996), and that enzyme activity may be reduced by adsorption on humic polymers (Gianfreda et al., 1998). In this study, relatively low concentrations of humic-like polymers produced by birnessite stimulated laccase-mediated transformation of catechol (Fig. 2C). The stimulation probably resulted from binding of catechol to humic-like polymers formed during the birnessite-mediated reaction. However, at higher concentrations, humic-like polymers inhibited catechol transformation, probably due to complexation of the polymer products with the copper atom present in the active center of laccase, or from conformational changes in enzyme molecules adsorbed on the humic-like polymers. Similar patterns of initially increasing and then inhibited removal of humic components (*o*-methoxyphenol, and syringaldehyde) were observed in the presence of various natural humic acids by Kang et al. (2002).

Enzyme activity may either increase (Stotzky, 1974, 1986) or decrease (Sokolovskii and Kovalenko, 1988; Zimmerman et al., 2004a) after adsorption on inorganic supports as a result of a change in enzyme structure or substrate access to the active site. The amount of birnessite used for the experiment presented in Fig. 1 was only 1.0 mg ml^{-1}, which resulted in a negligible amount of laccase (0.6% of initially added laccase; 0.5 μg mg^{-1}) immobilized on the mineral surfaces, as calculated from the data presented in Fig. 2A. Hence, the adsorption of laccase does not appear to be a factor in the overall inhibitory effect due to birnessite. In addition, it could be estimated that laccase covered about 1.3% of birnessite surface as calculated from using Avogadro's number (6.023×10^{23}), and estimated molecular size (15×4 nm) of laccase (66,000 Dalton) (Zimmerman et al., 2004b). This estimation may exclude the possibility of an inhibitory effect of laccase on birnessite activity due to the laccase adsorption on active sites of birnessite.

Laccase kinetics was considerably affected by the presence of Mn^{2+} ions in solution (Table 1). The K_m value increased with increasing Mn^{2+} concentration, indicating that the affinity of laccase for the substrate decreased in the presence of Mn^{2+}. At the same time, the V_{max} value decreased with increasing Mn^{2+} concentration, resulting in drastically reduced V_{max}/K_m ratios (from 48.7 to 1.1), which is a measure of diminished enzyme performance. According to Rawn (1989); Huang and Shindo (2001); Bisswanger (2002), such kinetic behavior of an enzyme indicates a mixed type of inhibition, which in the case of this study might involve a combination of a non-competitive inactivation of the enzyme by birnessite reaction products (mechanism 1), and a competitive interference of Mn^{2+} ions in the formation of the substrate/enzyme complex (mechanism 3). The release of large quantities of Mn^{2+} ions was observed in previous studies involving birnessite and polyphenols (Majcher et al., 2000; Wang and Huang, 2000a).

To summarize, birnessite tested in a model ternary system had an inhibitory effect on the transformation of catechol by laccase. As one of the most common manganese minerals, it may also affect laccase activity during humification processes in soils. The research using model ternary systems is continued to examine the effects of other common minerals, such as ferrihydrite and aluminum hydroxide, on enzymatic transformation of naturally occurring phenols.

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References

Bisswanger, H., 2002. Enzyme Kinetics: Principles and Methods. Wiley-VCH Verlag GmbH, Weinheim pp. 89–92.

Bollag, J.-M., 1992. Decontaminating soil with enzymes: an in situ method using phenolic and anilinic compounds. Environmental Science and Technology 26, 1876–1881.

Brunauer, S., Emmett, P.H., Teller, E., 1938. Adsorption of gases in multimolecular layers. Journal of American Chemistry Society 60, 309–319.

Burns, R.G., 1990. Microorganisms, enzymes and soil colloid surfaces. In: De Boot, M.F., Hayes, M.H.B., Herbillon, A. (Eds.), Soil Colloids and their Associations in Aggregates. Plenum Press, New York, pp. 337–361.

Claus, H., Filip, Z., 1988. Behaviour of phenoloxidases in the presence of clays and other soil-related adsorbents. Applied Microbiology and Biotechnology 28, 506–511.

Dec, J., Bollag, J.-M., 2000. Phenoloxidase-mediated interactions of phenols and anilines with humic materials. Journal of Environmental Quality 29, 665–676.

Dec, J., Haider, K., Bollag, J.-M., 2001. Decarboxylation and demethoxylation of naturally occurring phenols during coupling reactions and polymerization. Soil Science 166, 660–671.

Dixon, J.B., Weed, S.B., 1989. Minerals in soil environments, SSSA Book Series: 1. Soil Science Society of America, Madison, WI.

Fillazzola, M.T., Sannino, F., Rao, M.A., Gianfreda, L., 1999. Effect of various pollutants and soil-like constituents on laccase from *Cerrena unicolor*. Journal of Environmental Quality 28, 1929–1938.

Gianfreda, L., Bollag, J.-M., 1996. Influence of natural and anthropogenic factors on enzyme activity. In: Stotzky, G., Bollag, J.-M. (Eds.), Soil Biochemistry, vol. 9. Marcel Dekker, New York, pp. 123–193.

Gianfreda, L., Sannino, F., Filazzola, M.T., Leonowicz, A., 1998. Catalytic behavior and detoxifying ability of a laccase from the fungal strain *Cerrena unicolor*. Journal of Molecular Catalysis B—Enzymatic 14, 13–23.

Gogstad, G.O., Krutnes, M.B., 1982. Measurement of protein in cell suspensions using the Coomassie brilliant blue dye-binding assay. Analytical Biochemistry 126, 355–359.

Huang, P.M., 2000. Abiotic catalysis. In: Sumner, M.E. (Ed.), Handbook of Soil Science. CRC Press, Boca Raton, FL.

Huang, Q., Shindo, H., 2001. Comparison of the influence of Cu, Zn, and Cd on the activity and kinetics of free and immobilized acid phosphatase. Soil Science and Plant Nutrition 27, 767–772.

Kang, K.-H., Dec, J., Park, H., Bollag, J.-M., 2002. Transformation of the fungicide cyprodinil by a laccase of *Trametes villosa* in the presence of phenolic mediators and humic acid. Water Research 36, 4907–4915.

Kung, K.-H., McBride, M.B., 1988. Electron transfer processes between hydroquinone and hausmannite (Mn_3O_4). Clays and Clay Minerals 36, 297–302.

Majcher, E.H., Chorover, J., Bollag, J.-M., Huang, P.M., 2000. Evolution of CO_2 during birnessite-induced oxidation of ^{14}C-labeled catechol. Soil Science Society of America Journal 64, 157–163.

McBride, M.B., 1987. Adsorption and oxidation of phenolic compounds by iron and manganese oxides. Soil Science Society of America Journal 51, 1466–1472.

McKenzie, R.M., 1971. The synthesis of birnessite, cryptomelane, and some other oxides and hydroxides of manganese. Mineralogical Magazine 38, 493–502.

Naidja, A., Huang, P.M., Bollag, J.-M., 1997. Activity of tyrosinase immobilized on hydroxyaluminum-montmorillonite complexes. Journal of Molecular Catalysis A–Chemical 115, 305–316.

Naidja, A., Huang, P.M., Dec, J., Bollag, J.-M., 1999. Kinetics of catechol oxidation catalyzed by tyrosinase or δ-MnO_2. In: Berthelin, J., Huang, P.M., Bollag, J.-M., Andreux, F. (Eds.), Effect of Mineral–Organic–Microorganism Interactions on Soil and Freshwater Environments. Kluwer Academic/Plenum Publishers, New York, pp. 181–188.

Rawn, J.D., 1989. Biochemistry. Neil Patterson Publisher, Burlington, NC pp.149–193.

Shindo, H., Huang, P.M., 1984. Catalytic effects of manganese (IV), iron (III), aluminum, and silicon oxides in the formation of phenolic polymers. Soil Science Society of America Journal 48, 927–934.

Sokolovskii, V.D., Kovalenko, G.A., 1988. Immobilization of oxidoreductases on inorganic supports based on alumina: the role of mutual correspondence of enzyme-support hydrophobic-hydrophilic characters. Biotechnology and Bioengineering 32, 916–919.

Sparks, D.L., 1995. Environmental Soil Chemistry. Academic Press, San Diego, CA.

Stotzky, G., 1974. Activity, ecology, and population dynamics of microorganisms in soil. In: Laskin, A.I., Lechevalier, H. (Eds.), Microbial Ecology. Chemical Rubber Co., Cleveland, OH, pp. 57–135.

Stotzky, G., 1986. Influence of soil mineral colloids on metabolic processes, growth, adhesion, and ecology of microbes and viruses. In: Huang, P.M., Schnitzer, M. (Eds.), Interactions of Soil Minerals with Natural Organics and Microbes. Soil Science Society of America, Madison, WI, pp. 305–428.

Stotzky, G., Burns, R.G., 1982. The soil environment: clay–humus–microbe interactions. In: Burns, R.G., Slater, J.H. (Eds.), Experimental Microbial Ecology. Blackwell, Oxford, pp. 105–133.

Vuorinen, A.H., Saharinen, M.H., 1996. Effects of soil organic matter extracted from soil on acid phosphomonoesterase. Soil Biology & Biochemistry 28, 1477–1481.

Wang, M.C., Huang, P.M., 2000a. Ring cleavage and oxidative transformation of pyrogallol catalyzed by Mn, Fe, Al, and Si oxides. Soil Science 165, 934–942.

Wang, M.C., Huang, P.M., 2000b. Characteristics of pyrogallol-derived polymers formed by catalysis of oxides. Soil Science 165, 737–747.

Zhang, H., Huang, C.-H., 2003. Oxidative transformation of triclosan and chlorophene by manganese oxides. Environmental Science & Technology 37, 2421–2430.

Zimmerman, A.R., Chorover, J., Goyne, K.W., Brantley, S.L., 2004a. Protection of mesopore-adsorbed organic matter from enzymatic degradation. Environmental Science & Technology 38, 4542–4548.

Zimmerman, A.R., Goyne, K.W., Komarneni, S., Chorover, J., Kubicki, J., Brantley, S.L., 2004b. Mineral mesopore effects on nitrogenous organic matter adsorption. Organic Geochemistry 35, 335–375.