

Enhancement of Soil Carbon Sequestration: A Catalytic Approach

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Introduction

One of the approaches to minimizing the possible effects of climate change stemming from the recent and significant increases in atmospheric CO₂ levels involves fixing or storing C as biomass in terrestrial ecosystems. Of the possible terrestrial reservoirs for C storage one of the most promising is organic matter in soil.¹ Currently, this soil organic matter, termed humus, contains about twice as much C as is in the atmosphere.²

Historically, many agricultural soils contained as much as 50% more humus before cultivation as they do now. Indeed, before 1970, more C was lost from soils to the atmosphere as a result of land-use changes than was emitted by fossil-fuel combustion.³ In the present context, this inherited soil-C deficit represents a potential reservoir for C, the refilling of which can help buffer the transition to less C-intensive fuels over the next 30-50 years.

Our research has focused on understanding the fundamental process by which humus is created (i.e., humification) and extending this knowledge to enhance the rate of humification. The rate-limiting step in the humification process appears to be the oxidation of polyphenols to quinones.⁴ These quinones then react with peptides and amino acids to form large melanin-like polymers that resist further degradation by microorganisms.

Soil fungi produce enzymes such as polyphenol oxidases and laccases that catalyze the oxidation step.^{5,6} Soil minerals, such as iron and manganese oxides, can also perform this function.^{7,8,9} We have observed a significant synergetic effect when a polyphenol oxidase (tyrosinase) and a mineral phase (e.g., mesoporous silica, manganese oxide, alkaline fly ash) are both present.^{10,11,12} As soil enzyme activity depends on structural conformation, and longevity depends on protection from microbial predation, we are examining the nature of enzyme attachment to soil particles and the impact of physical properties such as pore size on activity and longevity.

In this paper we summarize our results with these co-catalysts and discuss implications regarding reaction mechanisms and management strategies for enhancing soil-C sequestration.

Experimental

Materials. Mushroom polyphenol oxidase (tyrosinase), L-3,4-dihydroxyphenylalanine (L-DOPA), L-serine, L-glycine, monosodium citrate, and vanillic acid were obtained from Sigma Chemical Co. (St. Louis, MO). The tyrosinase had a nominal activity of about 2400 units/mg (oxidation of L-tyrosine to L-DOPA at pH 6.5 and 25°C, 1 unit = ΔA_{280} of 10^{-3} min^{-1} in 3-mL solution). Orcinol, resorcinol, *p*-hydroxybenzoic acid, glycine, silica gel (Davisil, 35-60 mesh, 150Å), and hematite ($\alpha\text{-Fe}_2\text{O}_3$), were obtained from Aldrich Chemical Co. (Milwaukee, WI). Sodium hydroxide was obtained from Fisher Scientific (Hampton, NH). Goethite ($\alpha\text{-FeOOH}$) and birnessite ($\gamma\text{-MnO}_2$) were synthesized in the laboratory. Two alkaline fly ashes were obtained from coal-fired power plants in Texas. One was a Class C ash derived from sub-bituminous coal, and the other was a Class F ash derived from lignitic coal.

Humification Experiments. Several experiments were conducted to determine the impact of fly ash, the Fe and Mn oxides, and pH on a model humification reaction. The basic humification experiment with fly ash was patterned on that of Nelson et al. (1979)

and involved the following steps. A 100 mM NaH₂PO₄ solution buffered at pH 6.5 was used to prepare a 2 mM solution of organic monomers (orcinol, resorcinol, *p*-hydroxybenzoic acid, L-glycine, L-serine, and vanillic acid), and separately, a tyrosinase solution (1 mg tyrosinase mL⁻¹). The pH of the monomer solution was readjusted to 6.5 by addition of NaOH. In sequence, either 1 mg of oxide or 500 mg of fly ash, 1 mL of buffer, 3.5 ml of the buffered monomer solution, and 0.5 ml of the buffered tyrosinase solution were added to a 7.5-ml polystyrene 1-cm pathlength cuvette to yield a final solution volume of 5 mL. Each cuvette was then capped with parafilm and incubated at 22°C. At selected times after mixing, a representative aliquot of the mixture was taken and centrifuged, and the absorbance spectrum of the supernate collected using a UV-Vis spectrophotometer. The supernate and solid were returned to the 7.5-ml cuvette after this analysis to continue the study. Humification progress was measured as increases in absorbance at a wavelength of 486 nm (A_{486}). As humification progressed, extremely absorbing solutions were obtained, and these required ten- or hundred-fold dilution in order to obtain usable data. The diluted specimens were not returned to the cuvette after analysis. To determine the effect of pH on humification, an experiment was conducted at pHs of 5.0, 6.5, 7.5, and 9.0. No fly ash was added and buffers other than phosphate were used at pH 5.0 (disodium citrate) and pH 9.0 (boric acid).

To determine the activity of the dissolved enzyme, 10 μL from an experimental sample or a freshly prepared stock solution [5 mg tyrosinase in 5 ml of 50-mM phosphate buffer (pH 6.5)] was diluted to 1 mL with a 1 mM L-DOPA/50-mM phosphate solution (pH 6.5) in a 1-cm pathlength cuvette. The absorbance at 478 nm (A_{478}) was monitored for up to ten minutes after dilution using a Shimadzu UV-2501PC (Shimadzu, Japan) UV-Vis spectrometer while the solution was maintained at room temperature (22°C). The tyrosinase activity for L-DOPA oxidation was calculated from the rate of increase in A_{478} (i.e., $\Delta A_{478} \text{ min}^{-1}$).

Results and Discussion

Our humification experiments have involved reacting tyrosinase in the presence of three types of "co-catalysts": mesoporous silica particles, metal-oxide minerals, and alkaline fly ashes. With silica particles, the primary effect is to physically protect the enzyme from degradation, thus prolonging its activity and increasing humification. Our experiments (data not shown) suggest that both enzyme and the resulting humic polymers concentrate in the pores of the silica.

With the Fe and Mn oxides, the primary effect is related to the ability of these minerals to act as oxidants. During the first week of reaction, humification enhancement factors (i.e., the amount observed with the co-catalyst present divided by the amount observed when tyrosinase is the only catalyst present), of 1.2 ± 0.1 , 1.7 ± 0.3 , and 2.9 ± 0.3 were observed for experiments conducted with 0.02 wt% suspensions of $\alpha\text{-Fe}_2\text{O}_3$, $\alpha\text{-FeOOH}$, and $\gamma\text{-MnO}_2$, respectively. Thus, $\alpha\text{-Fe}_2\text{O}_3$ had almost no impact on humification, whereas the amount of humification nearly tripled when $\gamma\text{-MnO}_2$ was present. These results are in the same order as the reduction potentials of the three compounds, as one would expect. However, sorption of tyrosinase to these surfaces, particularly the $\gamma\text{-MnO}_2$ which has substantial microporosity, may also be involved as a stabilizing mechanism.

With the alkaline fly ashes, the primary effect could be physical, due to the presence of broken silica cenospheres, directly oxidative, due to the presence of metal oxides, or indirectly oxidative, due to the increase in pH. Our experiments, which were conducted using 10 wt% suspensions of the ash (i.e., 500 times more co-catalyst than with the metal oxides), showed humification enhancement factors of 2.4 ± 0.1 and 11 ± 2.3 , for the lignitic and sub-bituminous ashes, respectively. Characterization of these ashes showed little difference

in their cenosphere or metal oxide contents. However, a large difference in their titrateable alkalinity (pH 6.5) was observed, with that of the sub-bituminous fly ash (13.4 mmol g⁻¹) being about 4.6 times larger than that of the lignitic fly ash (2.9 mmol g⁻¹), the same factor by which their humification enhancement factors differed. Moreover, for the 0.5-g quantities used in the humification experiments, the alkalinity of both fly ashes was substantially greater than the capacity of the phosphate buffer (ca. 0.5 mmol) resulting in substantial pH increases in these experiments as the fly ashes equilibrated with the humic monomer solution. These results led us to investigate the effect of pH alone on the humification reaction.

Humification experiments at pHs of 5, 6.5, 7.5, and 9 clearly showed a substantial effect of alkaline pH on humification (Fig. 1a). Essentially no humification occurred at pH 5, whereas maximal humification occurred at pH 9. Humification occurred primarily during the first 72-96 hours, and thereafter, little change was observed. Measurement of the enzyme activity during the experiment showed that activity held steady for perhaps 48-60 hours and then dropped rapidly to zero by 96 hours (Fig. 1b). The enzyme activity at pH 9, however, was consistently smaller than that for any other pH, even though the same trend with time was observed. In the absence of tyrosinase, negligible amounts of humification were observed in all but the pH-9 treatments (Fig. 1c), where maximum humification was still only about 5% of that observed when tyrosinase was present.

These results confirm the sequential two-step nature of the humification process, i.e., oxidation of phenolic groups followed by condensation of the resulting quinones with amino acids to form melanins. High pH enhances the process primarily through its effect on the condensation step. Thus, maximum humification rates were obtained at pH 9 even though the enzyme activities were relatively low, whereas no humification was observed at pH 5 when enzyme activities were higher than at pH 9 (Fig. 1a,b). Tyrosinase is needed for the reaction to occur at a useful rate (Fig. 1c), but the level of tyrosinase activity seems less important than high pH in determining the yield of humic polymers. The manner by which condensation is enhanced likely relates to the speciation of the reactants at high pH. Quinones are stabilized, and the pK_a of aliphatic amine groups is near 10. It could be that the anionic form of the amino acids (i.e., a neutral amine group) is critical to the condensation step.

Conclusions

We conclude that co-catalysis of humification occurs by three mechanisms involving physical stabilization of tyrosinase, direct oxidation of the monomers, and promotion of the oxidation and condensation steps by alkaline pH. Although tyrosinase activity is greatest at neutral pHs, the large pH dependence of the condensation step drives the overall reaction to maximum rates under alkaline conditions. Liming of soils to slightly alkaline pH should enhance net carbon sequestration. Alkaline fly ash is a potential liming agent for soils provided that the carbon costs associated with transportation from the source are less than the organic carbon that is humified.

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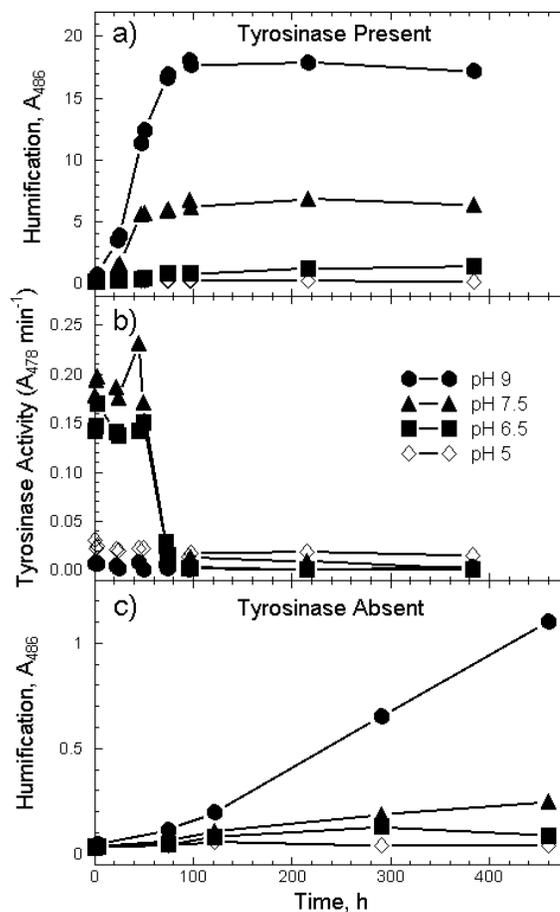


Figure 1. Effect of pH on rates of humification and on tyrosinase activity: a) humification with tyrosinase present; b) tyrosinase activity during the humification experiment; and c) humification in the absence of tyrosinase.

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