Enzymatic Control of Humification

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One of the approaches to minimizing the possible effects of climate change stemming from the recent and significant increases in atmospheric CO_2 levels involves fixing or storing carbon as biomass in terrestrial ecosystems. Of the possible terrestrial reservoirs for carbon storage, one of the most promising is organic matter in soil. Currently, this soil organic matter, called humus, contains about twice as much carbon as is present in the atmosphere. 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. These quinones then react with peptides and amino acids to form large melanin-like polymers that resist further degradation by microorganisms. We have observed a significant synergetic effect when a biological catalyst, such as a polyphenol oxidase (tyrosinase), and an inorganic catalyst, such as a mineral phase (e.g., mesoporous silica, manganese oxide, alkaline fly ash), are both present (Amonette et al. 2000, 2003a, 2003b).

In our experiments with alkaline fly ashes, the primary enhancing effect could be physical because of the presence of broken silica cenospheres, directly oxidative because of the presence of metal oxides, or indirectly oxidative because of 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) being about 4.6 times larger than that of the lignitic fly ash (2.9 mmol/g), the same factor by which their humification enhancement factors differed. Moreover, for the 0.5-g quantities used in the humification experiments, the alkalinities of both fly ashes were 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 (Figure 1a). Essentially no humification occurred at pH 5, whereas maximal humification occurred at pH 9. Humification occurred primarily during the first 72 to 96 hours, and thereafter, little change was observed. Measurement of the enzyme activity during the experiment showed that activity held steady for perhaps 48 to 60 hours and then dropped rapidly to zero by 96 hours (Figure 1b). The enzyme activity at pH 9, however, was consistently smaller than that for any other pH, even though the same trend over time was observed. In the absence of tyrosinase, negligible amounts of humification were observed in all but the pH 9 treatments (Figure 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—that is, 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 (Figure 1a and b). Tyrosinase is needed for the reaction to occur at a useful rate (Figure 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 pKa 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.

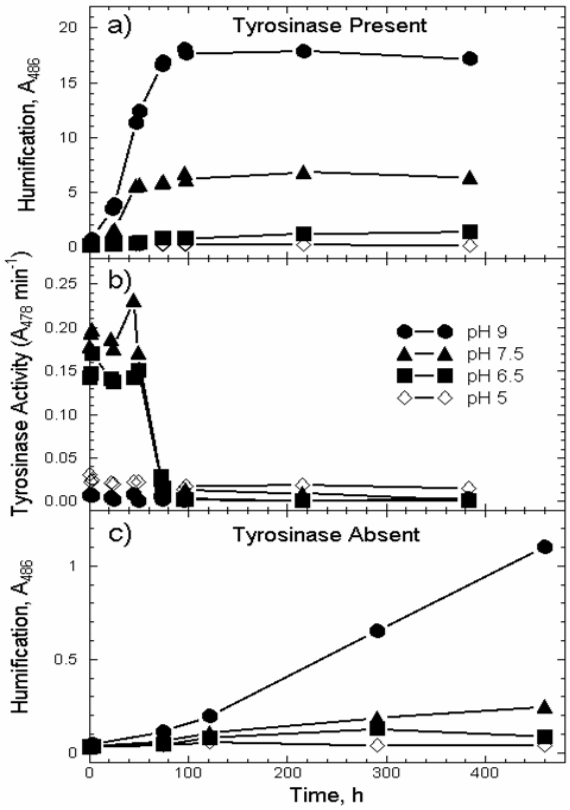

Figure 1. Effect of pH on rates of humification and on tyrosinase activity. (a) Humification with tyrosinase present. (b) Tyrosinase activity during the humification experiment. (c) Humification in the absence of tyrosinase.

References

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