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INCREASED SEQUESTRATION OF AROMATIC CARBON ON THE MINERAL PHASE IN THE PRESENCE OF A BIOCATALYST: THE STUDY UNDER BATCH AND DYNAMIC CONDITIONS

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Presentation Preference: Poster only

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Content: Spontaneous condensation of phenolic and other compounds in the presence of oxidative catalysts (biotic and abiotic) have long time been considered as playing important role in aromatic C accumulation in soils. Currently, organic-mineral interactions are considered to play the dominant role in C stabilization. However, biocatalysts, such as phenoloxidase laccase, are produced extracellularly by fungi and bacteria and are ubiquitous at soil interfaces. The questions are: 1) whether interfacial oxidative reactions can lead to increased sequestration of aromatic C in soils at environmentally relevant concentrations of substrates; 2) what is the effect of mineral phase on the process of enzymatic catalysis?

In order to address these questions we have studied 1) sorption/desorption of phenolic acids on kaolinite modified with aluminum hydroxide in the presence and absence of laccase from *Coprinus comatus*; 2) interactions of laccase with phenolic acids in the absence and presence of mineral. Experiments were conducted in 5 mM Na-acetate buffer (pH 4.5) under batch conditions and in continuous-flow mode resembling leaching regime of soils. In the latter case Teflon microcolumn (total volume 1 ml) equipped with peristaltic pump has been used. Reversed-phase high pressure liquid chromatography was used for acids determination.

The dynamic experiment with an equimolar mixture of phenolic acids (0.01 mM each) have shown the following order of acids sorption: gallic>protocatechuic>>syringic \approx vanillic \approx ferulic \approx p-hydroxybenzoic. Sorption of gallic and protocatechuic acids exceeded sorption of other acids by one order of magnitude. The order of desorption by 5 mM and 50 mM Na-acetate buffer was opposite to the order of sorption. The presence of immobilized laccase substantially increased removal of syringic and ferulic acids from solution in comparison to mineral without laccase (3 and 15 times respectively), while had little effect on other acids. The surface of the mineral became darker, suggesting oxidation/oligomerization reactions. Interactions of individual phenolic acids with free laccase have shown that ferulic and syringic acids are among the best substrates for laccase which can explain their effective sequestration in the presence of the enzyme. Amounts of phenolic acids desorbed from the mineral by 5 mM and 50 mM acetate buffer decreased in the presence of laccase. The batch experiment has shown that the presence of a mineral led to two-times increase in the amounts of gallic, protocatechuic, syringic and ferulic acids metabolized by laccase in comparison to homogeneous system. This is possibly due to removal of the reaction products from the liquid phase which shifts the reaction equilibrium.

The results of the study show the importance of both biotic catalyst and the mineral phase for aromatic carbon sequestration/stabilization in soils. We have proved that oxidative heterogeneous coupling reactions can occur under environmentally relevant conditions and enhance removal of phenolic compounds from the liquid phase. The presence of interfaces makes biotic catalysis more effective.

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