

## Effects of biochar concentration and particle size on electron shuttling in microbial Fe(III) mineral reduction

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Biochar, made by biomass pyrolysis, has been suggested to serve as a soil amendment to lower CO<sub>2</sub>/N<sub>2</sub>O emissions and increase soil fertility. Additionally, biochar is redox-active, functioning as an electron shuttle to influence microbial Fe(III) reduction. The effect of biochar on microbial Fe(III) reduction depends on the redox properties, production pathway, and concentration of the biochar used. However, it is currently unknown i) how biochar particle size influences electron shuttling and microbial Fe(III) reduction, ii) whether the mechanism of electron shuttling by biochar involves diffusion of dissolved biochar leachate molecules, electron hopping between biochar particles, or both and iii) how the aggregation of biochar with cells and Fe(III) minerals influences electron transfer. Therefore, the aims of this research are to investigate microbial Fe(III) mineral reduction in the presence of different concentrations and particle sizes of biochar suspensions as well as after addition of biochar leachate.

Cell suspension experiments with Swiss-biochar pyrolyzed from wood chips at 700°C, ferrihydrite as Fe(III) mineral and *Shewanella oneidensis* MR-1 were used to determine the rates of microbial Fe(III) reduction. Our experiments showed that the smaller the particle size and the higher the concentration of biochar is, the higher the rates and extent of microbial Fe(III) reduction are. At low biochar/Fe(III) minerals ratios, microbial Fe(III) mineral reduction was inhibited. Moreover, we found that dissolved biochar leachates were not responsible for the stimulation of microbial Fe(III) reduction. Finally, fluorescence microscopy and confocal laser scanning microscopy were used to evaluate the effect of aggregation of biochar particles with MR-1 cells and Fe(III) minerals on microbial Fe(III) mineral reduction. These results will allow the evaluation of the impact of biochar on electron transfer processes in environmental systems. Additionally, they will reveal the mechanisms of electron transfer between microbial cells and Fe(III) minerals via redox-active biochar particles.