Enhanced electron transfer by electron mediators via stimulating *Shewanella* biofilm formation

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Microbial extracellular electron transfer (EET) is an important driving force for biogeochemical cycling of elements in soil. Mediation with different electron shuttles is well-recognized as a dominant mechanism facilitating EET, but little is known about the regulation of microorganisms by mediators. This study examined EET in a bioelectrochemical system of *Shewanella oneidensis* MR-1 facilitated with 7 different quinone and phenazine mediators. The results showed that the mediators could enhance the electron transport rates 9 to 86 times greater than the system without mediator and that the biofilms formed in mediator-added systems were much thicker either. Quantification of biofilm total DNA showed that the biomass with mediators was 6 to 36 times higher than that without mediator and that the biofilm total DNA correlates linearly with the maximum current density (R^2=0.92), indicating that biofilm stimulated by mediators plays a key role for EET. We further used the cyclic voltammetry for examining how this biofilm enhances the EET with mediators. The first derivative analysis of CVs revealed that, over the scanned potential range of -459 to 241 mV, two pairs of peaks were observed, representing the mediation process and the biofilm direct contact transport, respectively. While, when the medium with mediators was replaced with mediator-free medium, the current density of the bioelectrochemical system decreased by an average of 75%, demonstrating that mediation process, instead of direct contact, dominated the enhanced EET. We finally investigated how the bacteria growth regulated by mediators by quantifying the rates of mediator reduction process. The results showed that the rate constant of mediator reduction correlates well with the biofilm total DNA, suggesting that rapid consumption of mediators by MR-1 induced higher rate of cell growth, hence enhancing biofilm formation. In conclusion, this study showed that the EET of *Shewanella* was enhanced by a biofilm stimulation mechanism with addition of an electron mediator, and that the mediation process, instead of direct contact, dominated the pathway of electron transfer even after a thick biofilm had formed.

This work was funded by the National Natural Science Foundations of China (41522105 and 41571130052).