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Optimisation of Cu(II) bioreduction and biorecovery by *Shewanella oneidensis*

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Copper is an industrially important metal often present in effluents where Cu recovery can promote the circular economy and valorise waste materials. Cu nanoparticles (Cu-NPs) recovered from waste can be used as catalysts in green click chemistry; used for the production of pharmaceutically important triazoles. A green biosynthesis method for Cu-NPs was previously shown using the metal-reducing bacterium, *Shewanella oneidensis*. In previous work, when lactate was supplied as electron donor, *S. oneidensis* bioreduced $Cu(II)_{(aq)}$ to $Cu(0)_{(s)}$ which was deposited as intracellular, catalytically active Cu-NPs. However, the mechanism of Cu(II) bioreduction was unclear, and cytochrome deletion mutants indicated no involvement of the Mtr pathway often used for the bioreduction of metals by *S. oneidensis*. Understanding Cu bioreduction mechanisms by *S. oneidensis* is crucial for optimising Cu recovery and enhancing Cu-NP yields, necessary steps towards achieving industrially scalable green biosynthesis of Cu-NPs. Here, we explored potential Cu bioreduction pathways in *S. oneidensis,* using various electron donors and deletion mutants. Cu bioreduction experiments were set up using the wild type (WT) strain of *S. oneidensis*-MR1 and deletion mutants with hydrogen or lactate supplied as the sole electron donor. These experiments revealed Cu-NP formation and yield was improved when hydrogen was used as electron donor. Indeed, after 24 h $Cu(II)_{(aq)}$ was removed from solution in WT experiments and in some of the deletion mutant experiments and a visible pink colour change was observed that is characteristic of Cu-NP formation. In these experiments, ICP-MS data showed up to 100 % removal of $Cu_{(aq),}$. In all experiments with lactate Cu-NP formation was limited, and only marginal Cu-removal was observed; comparable to no electron donor controls suggesting biosorption. Cu-NPs were characterised by TEM (with SAED and EELS) and XPS. The catalytic activity of the CuNPs from experiments was shown in a model click reaction and ^{1}H NMR analysis of reaction mixtures indicated a good conversion was achieved. This work has confirmed a role for hydrogenases in Cu bioreduction by *S. oneidensis* and highlights the prospect of enhancing production of Cu-NPs, and potentially other technologically relevant metals (e.g. Au), by optimising the expression/utilisation of hydrogenase systems.