

Paracoccus for Bioremediation: Optimizing Formate Dehydrogenase in Paracoccus denitrificans for Development of a Non-Toxic Bioremediation Strategy for Toxic C1 Compounds in Water

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C1 compounds—methanol, formaldehyde, and formate—are toxic environmental pollutants associated with hazardous spills, water contamination, and severe health effects, including blindness, cancer, and death. An estimated 2–3 million metric tons of C1 waste are produced annually, contributing to \$1.2 billion in losses due to inefficient remediation. Current treatment strategies remain costly and ineffective. To address this, the study investigated *Paracoccus denitrificans*, a non-pathogenic soil bacterium with innate C1-metabolizing capabilities, as a cost-effective, eco-friendly alternative. A major component of *P. denitrificans* is formate dehydrogenase (FDH), the enzyme responsible for degrading C1 compounds. This research analyzed the activity of two FDH isoforms: NAD⁺-linked FDH (NAD-FDH) and membrane-bound FDH (Q-FDH), regulated by transcriptional regulators FlhS, FlhT, and FlhR. Enzyme activity was measured in cells grown on various carbon substrates, including succinate (20 mM) and formate (50–200 mM). Results showed optimal growth and peak FDH activity at 200 mM formate, with FlhS identified as a key regulator of NAD-FDH under high-formate conditions. Building on these findings, FDH activity was enhanced through both chemical and genetic approaches. Supplementing with selenium, a known FDH cofactor, improved activity and reduced formate in water by 75%. *P. denitrificans* genetically engineered to overexpress flhS gene achieved an even greater 85% reduction. Molecular docking predicted FDH-substrate interactions across various conditions. These findings provide a framework for optimizing *P. denitrificans* as a bioengineered solution for industrial C1 compound remediation, offering a cost-effective and eco-friendly alternative to conventional decontamination methods.

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