

Immobilization of *Tth*LPMO9G from *Thermothelomyces thermophilus* on Carbon Felt for Electrochemical Applications

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Lytic Polysaccharide Monooxygenases (LPMOs) belonging to AA9 family are known for their role in oxidative biomass degradation and are being investigated for bioelectrocatalytic applications. Their copper-dependent active sites have attracted interest for direct electron transfer (DET) studies. Previously, electrochemical measurements have been conducted with LPMOs immobilized on glassy carbon electrodes, utilizing techniques such as Fourier-transform alternating current voltammetry (FTacV) to assess electron transfer properties¹. In this study, *Tth*LPMO9G from *Thermothelomyces thermophilus*² was immobilized onto carbon felt (CF), a conductive matrix designed to facilitate electron transfer. The primary challenge was the establishment of a stable covalent bond between the enzyme and the carbon surface, which is a critical step for subsequent electrochemical applications and has not been attempted previously. The immobilization process was optimized by adjusting the covalent attachment method, the buffer composition and the incubation time. Apart from *Tth*LPMO9G which is a C1-specific LPMO, another LPMO with C1/C4 regioselectivity, *Mt*LPMO9H³ was assessed in immobilization tests, and the activity of both enzymes was assessed using established assays, including the quantification of oxidized products from phosphoric acid-swollen cellulose (PASC) and the 2,6-dimethoxyphenol (DMP) peroxidase assay⁴. Improvement in enzyme attachment to conductive surfaces is considered essential for enhancing enzyme-substrate interactions, especially with insoluble cellulose substrates where contact between enzyme and substrate presents a significant challenge. By addressing these foundational issues, progress can be made toward developing advanced applications, including direct electron transfer from electrodes to immobilized, highly potent redox enzymes in bioelectrocatalytic systems. The ultimate goal is to push the state of the art by introducing novel methods to track direct electron transfer and gain new insights into the mechanisms of this enzyme class.

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