Fine-tuning O_2-Modulated Metabolic Fluxes for Enhanced Lignin Bioconversion

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Lignocellulose-derived carbohydrates have been employed for nearly a century in the production of renewable fuels and chemicals. Contemporary trends in lignocellulose valorization emphasize the comprehensive utilization of its three constituents: cellulose, hemicellulose, and lignin. Recent advancements have revealed the abilities of aerobic microorganisms, notably *Pseudomonas putida* KT2440 (hereafter *P. putida*), to catabolize depolymerized lignin and produce intracellular energy storage products, specifically medium-chain-length polyhydroxyalkanoates (*mcl*-PHA), with potential applications in bioplastic manufacturing.[1] However, challenges such as the recalcitrance, heterogeneity, and toxicity of lignin-derived compounds hinder the biological valorization of lignin.

In the biological conversion of lignin to PHA, lignin-derived compounds are assimilated and converted to acetyl-CoA or succinate-CoA in the tricarboxylic acid (TCA) cycle. However, most fluxes tend to diverge toward cell growth rather than the desired industrial products (Fig. 1A). To redirect more carbon fluxes towards PHA synthesis, cell growth is usually restricted through limited nutrient (typically nitrogen) supplement while sustaining PHA synthesis.[1,2] This strategy, commonly employed in the valorization of conventional carbon sources, proved less effective for lignin conversion.[3–5] Depolymerized lignin is a heterogenous mixture comprising classes of linear carboxylic acid, aromatic monomers, dimers, and oligomers.[6] Previous results demonstrated a hierarchical utilization of heterogenous compounds under the growth-limited conditions. This hierarchy is hypothesized to induce the corresponding hierarchy in intracellular enzymatic degradation pathways, known to involve nitrogen. The contradictory nitrogen requirement in growth limitation and lignin degradation is presumed to impede efficient lignin bioconversion. Moreover, the diverse responses of heterogenous lignin-derived compounds to external limitation factors underscore the importance of simplifying the regulation of imposed limitations for scaling up lignin valorization.

This study seeks to regulate the O_2 supplementation to reconfigure the carbon fluxes. In contrast to the conventional nutrient (nitrogen) limitation strategy, dissolved O_2 within the reactor is easier to monitor and regulate, and crucially, facilitates the degradation of concentrated lignin. To test this hypothesis, two lignin monomers (vanillic acid and ferulic acid) were fed to *P. putida* in shaking flasks, supplemented with different volumes of medium to establish discrepancies in O_2 transfer (Fig. 1B and 1C). Results confirmed that dissolved O_2 could reconfigure carbon fluxes towards higher PHA production, and different monomers exhibited diverse O_2 requirements for degradation. Based on the hierarchical utilization of substrates under growth-limited conditions, a two-stage O_2 supplementation was designed, as depicted in Fig. 1D. Specifically, flasks were provided with low O_2 through stirring only for mixing for 96 h, after which they were transferred to shaking mode to increase dissolved O_2 and cleave more complex lignin compounds. Fig. 1E and 1F illustrate lignin fermentation using the nitrogen-limitation strategy and O_2 regulation strategy, respectively. When fed with a low concentration of lignin, O_2 regulation resulted in higher PHA production due to its stronger inhibition in cell growth. When fed with high concentrations of lignin (above 10 g/L), O_2 regulation strategy similarly performed better, primarily attributed to enhanced lignin degradation. Overall, this study demonstrates the feasibility of regulating O_2 supplementation to enhance lignin bioconversion for PHA production, paving the way for the biological valorization of heterogeneous substates.

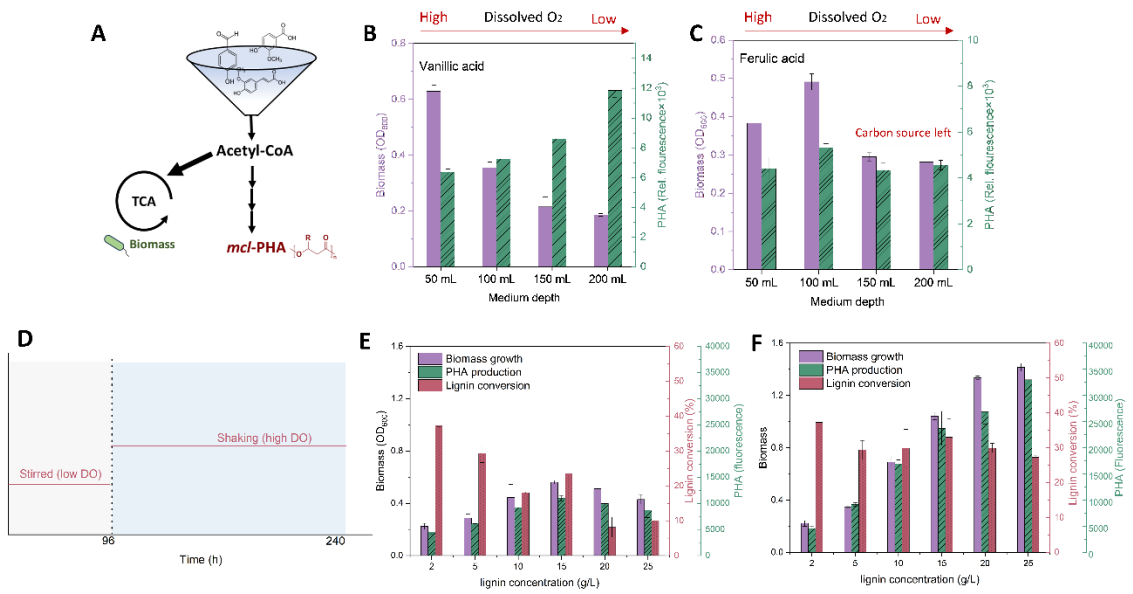

Figure 1 A) Metabolic pathway of lignin via *Pseudomonas putida* KT2440; B and C) Vanillic acid and ferulic acid as carbon sources for PHA production at different volume of mediums in shaking flaks; D) O_2 supplementation strategy for real lignin fermentation; E and F) Lignin degradation, biomass growth, PHA production in lignin fermentation fed with different concentrations through nitrogen-limitation and O_2 regulation approaches.

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