A Convergent Approach for Lignocellulose Valorization Through Integrated Carbohydrates Anaerobic Digestion and Lignin Bioconversion

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Lignocellulosic wastes represent vast resources for biofuels and biochemicals production. The current bioprocessing of lignocellulosic, spanning from pretreatment to bioconversion, had primarily rendered more processible carbohydrates for microbial conversion. Lignin, as the most recalcitrant lignocellulosic component, had been overlooked due to its non-biodegradability in carbohydrates-dominant bioprocessing. Constituting 30% of the lignocellulosic matrix, efficient lignin bioprocessing would facilitate the biorefinery viability and complete lignocellulosic waste valorization. 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]

During PHA biosynthesis, carbon sources were taken up and converted to acetyl-CoA or succinate-CoA at the tricarboxylic acid (TCA) cycle. However, most fluxes tend to diverge towards its preferred cell growth rather than the accumulated products of industrial interest (Fig. 1A).[2,3] To redirect more carbon fluxes towards PHA synthesis, cell growth was usually restricted through limited nutrient (typically nitrogen) supplement while sustaining PHA synthesis. This strategy, commonly employed in the valorization of conventional carbon sources, proved less effective for lignin conversion.[1,4] Alternatively, two-stage operation was implemented to balance the lignin degradation and PHA production. One-pot bioprocessing offers streamlined process, reduced resources consumption, and easy of scale-up. This study aims to achieve one-pot lignin bioconversion under nitrogen-limited conditions, similar to contemporary practices with PHA production from glucose or volatile fatty acids (VFA).

The challenges of one-pot lignin bioconversion stem from the inherent heterogeneity of lignin-derived compounds, intricate intracellular and extracellular enzymatic cleavage, and inhibition from aromatic compounds.[5,6,7] Comparing reported outcomes of lignin bioconversion under different nitrogen conditions, the degradation of lignin was associated with presence of nitrogen and induced high bacterial biomass while PHA production was linked to nitrogen-limited conditions, accompanied by cell growth arrest. Leveraging the strengths of each scenario, it was proposed to make one-pot lignin bioconversion feasible by combining high bacterial biomass with nitrogen-limited conditions.

To test the hypothesis, various biomasses (ranging from OD 0.2 to 2.4) of *P. putida*, cultivated using LB broth, were used to catalyze lignin conversion under nitrogen-limited conditions (1mM $(NH_4)_2SO_4$ supplement). The results presented that initial bacterial biomass enhanced the efficiency of lignin degradation from 22% at initial OD 0.2 to 32.7% at initial OD 2.4, as Fig. 1B showed. Subsequently, higher concentrations of lignin were tested to achieve both high biomass cultivation and lignin conversion in a one-pot operation, albeit the outcome was unsatisfied due to the inhibition from the concentrated lignin. To address this problem, external acetate was introduced into the lignin fermentation, successfully enhancing the lignin degradation and final synergistic PHA production (Fig. 1C). To produce acetate-rich stream at low cost, carbohydrates moiety from pretreatment underwent alkaline methanogenesis-arrested anaerobic digestion, resulting in 4.3$g_{VFA}/g_{yard\ waste}$ with 91% acetate (Fig. 1D). Finally, VFA broth was integrated into lignin fermentation, producing 0.62g/L PHA, a 17% increase compared to the sum of their individual production as sole carbon sources (Fig. 1E). The outcomes of this study contributed to the lignin bioconversion and demonstrated a new route for complete lignocellulose valorization.

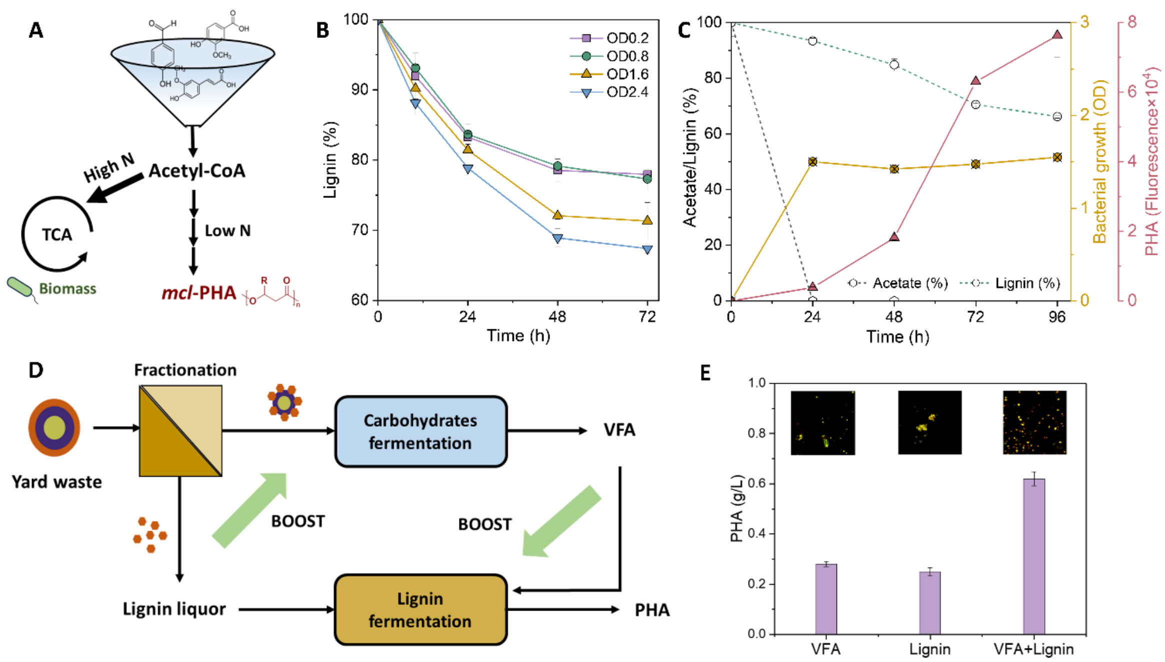

Figure 1 A) Metabolic pathway of lignin via *Pseudomonas putida* KT2440; B) Effect of initial biomass on lignin degradation under nitrogen-limited conditions; C) Effect of acetate addition on lignin bioconversion; D) Scheme of full lignocellulose valorization through fraction-integration route; E) PHA production of VFA and lignin.

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