Lignin valorization to polyhydroxyalkanoates assisted by adding volatile fatty acids

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Lignin is the second most abundant biopolymer on the planet and represents about 30% potential for biofuels. For several decades, lignin is typically underutilized and burned for heat because of its inherent heterogeneity and recalcitrance. It is only recently that the metabolic pathways and products within the microorganisms metabolizing lignin or aromatics had been identified. Regardless of the heterogeneous character, microorganisms can funnel these lignin monomers into the central carbon metabolism, where carbon flux then diverges into two directions - biomass growth and product formation, such as polyhydroxyalkanoates (PHA) (Fig. 1A) [1]. While high-density cells are essential to store products, non-limited carbon flux towards cell growth impedes PHA yield. Therefore, limited nutrition, e.g., nitrogen-limited condition, has been used to reduce carbon loss to cell growth, albeit with only less than 20% lignin degraded [2]. To reconcile lignin degradation efficiency and product yield, the bioprocess has to be performed as a two-stage process to maintain biomass enrichment and nutrition-limited PHA accumulation [3].

Aromatic-catabolic microorganisms like *Pseudomonas putida* KT2440 can grow on fatty acids. We hypothesize that adding an easily degradable carbon source, such as volatile fatty acids (VFA), can improve biomass production in a nitrogen-limited medium, which also supports lignin conversion for PHA production. In our previous work, we have fractionated yard waste into lignin and carbohydrates moieties, and part of the carbohydrates valorized through anaerobic digestion to produce VFAs. In this study, we valorized the fractional lignin stream assisted by supplemented VFAs for PHA using *Pseudomonas putida* KT2440. In a nitrogen-rich medium, although 35% lignin can be catabolized, PHA production was negligible. To intensify the carbon flux towards PHA, we fermented lignin under nitrogen-limited conditions and found that the lignin degradation efficiency was dependent on initial biomass concentration (Fig.1B). To increase biomass concentration under nitrogen-limited condition, we attempted to supplement VFA into the lignin moiety. We examined fermentation using mixed model substrates of VFA (acetate, propionate) and lignin monomers (4-hydroxybenzaldehyde, vanillic acid and ferulic acid). The results showed that *P. putida* KT2440 utilized VFA prior to lignin monomers (Fig. 1C). We then introduced VFA into the fractional lignin stream under nitrogen-limited conditions, successfully improving lignin degradation to 30% and driving more carbon towards PHA. This research demonstrated that it is possible, in a VFA-assisted one-pot bioprocess, to achieve high lignin conversion under nitrogen-limited conditions.

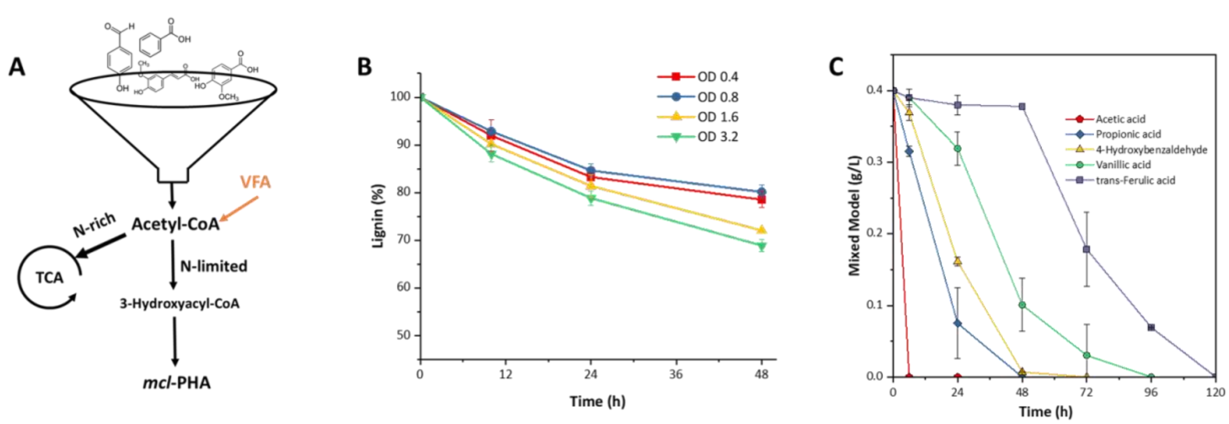

Figure 1 A) metabolic pathway of lignin & VFA using *Pseudomonas putida* KT2440; B) effect of initial biomass on lignin degradation under nitrogen-limited conditions; C) degradation of mixed VFA & lignin monomers.

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