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Spotlight

Tandem chemocatalysis and biological funneling to valorize lignin

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In 2014, Linger *et al.* presented a tandem process for lignin valorization by integrating chemical and biological catalysis. Chemical pretreatment of corn stover generated mixed lignocellulose-derived monomers that were converted to a single product, polyhydroxyalkanoates, by *Pseudomonas putida.* Tandem processes have since been developed for diverse feedstocks to support the bioeconomy.

A sustainable bioeconomy is predicated on valorizing the three major components of biomass [1]. Of these components, cellulose and hemicellulose are industrial feedstocks for a variety of materials and chemicals, but the energy-dense and recalcitrant aromatic polymer that is lignin has only been converted to date on a large scale to heat and power. While the potential of lignin has long been recognized, only recent developments in chemical and biological engineering have set the stage for its conversion at scale.

Decades of effort have elucidated many of the microbial mechanisms for lignin breakdown and mineralization in nature. The polymer is predominantly broken down by enzyme systems secreted by wood-decay fungi, such as white rot [2]. The resulting assortment of lignin-derived aromatic compounds (LDACs) can serve as growth substrates for a wide variety of bacteria, including *Pseudomonas putida*, now a well-studied metabolic engineering chassis. Elegant genetic and biochemical studies have elucidated many of the genes and pathways involved in the catabolism of LDACs [3]. A key organizing principle in the aerobic catabolism of aromatic compounds is convergence: a relatively large number of 'upper' pathways initially transform a wide of range compounds to a small number of shared metabolites, which are then transformed to central metabolites by a smaller number of 'lower' pathways [4]. This convergent architecture lends itself to biological funneling, in which mixtures of aromatic compounds are simultaneously catabolized to target bioproducts. In visionary work, Rojo *et al*. exploited this convergent architecture to engineer a pseudomonad to simultaneously degrade methyl- and chloro-substituted aromatic pollutants [5].

However, industrial application of biological lignin depolymerization is limited by its rate, extent, and scalability. Conversely, many catalytic and thermal approaches to lignin deconstruction have been developed that rapidly and extensively depolymerize and are amenable to scale-up. Catalytic lignin depolymerization usually yields heterogeneous mixtures of chemicals that can be valorized as mixtures for fuel applications, or separated as pure chemicals; yet, the cost and complexity of separations remain key challenges [6].

Linger and colleagues presented two major innovations to overcome the challenge of lignin recalcitrance and heterogeneity: integrating chemical and biological catalysis, and introducing the concept of biological funneling [7]. Their work converted corn stover to medium chain-length polyhydroxyalkanoates (*mcl*-PHAs), a biodegradable polyester, by combining alkaline pretreatment for lignin extraction and partial depolymerization with microbial cultivations (Figure 1A). First, corn stover was treated with sodium hydroxide, releasing LDACs, such as *p*-coumarate, ferulate, and vanillate, from the lignin polymer into an aqueous liquor (termed 'APL'). Polysaccharide-derived compounds, such as glucan and xylan, were largely retained in the solids along with much of the oligomeric (longer than trimeric) lignin. A 2-day treatment of the solids with commercial enzymes released monomeric sugars, which were then catalytically upgraded. To valorize the APL, the authors used a strain of the soil bacterium *P. putida* without any genetic engineering. *P. putida* catabolizes four of the primary extractives (*p*-coumarate, ferulate, acetate, and glucose) when provided as individual components or in a mock mixture. In addition, *P. putida* naturally accumulates *mcl*-PHAs under nitrogen-limiting conditions. Thus, the mixed LDACs and other APL-derived extractives were upgraded to *mcl*-PHAs by cultivating *P. putida* in APL as the sole carbon source under nitrogen-limiting conditions. Due to analytical challenges at the time, the LDACs from APL could not be quantified during the cultivation and, thus, incorporation of *p*-coumarate into *mcl*-PHAs was shown using ^{13}C-labeled *p*-coumarate. PHA production was observed at similar yields from mock mixtures [34–39% cell dry weight (cdw)] and corn stover APL (32% cdw). Finally, the authors demonstrated an application of *mcl*-PHAs beyond bioplastics by thermal depolymerization and deoxygenation to fuel-range hydrocarbons.

Since this study was published, similar tandem processes involving biological funneling have been applied to various biomass-derived feedstocks. One important innovation was the expansion and increased sophistication of synthetic biology toolsets, enabling rapid development of improved chassis for biological funneling. Other key innovations include diversification of products beyond PHAs, including many atom-efficient products from LDACs [8]; onboarding of additional microbial chassis with favorable attributes; improved substrate utilization, including for LDACs, dimers, and sugars; and increased titers, rates, and yields for target bioproducts. Biochemical characterization of diverse

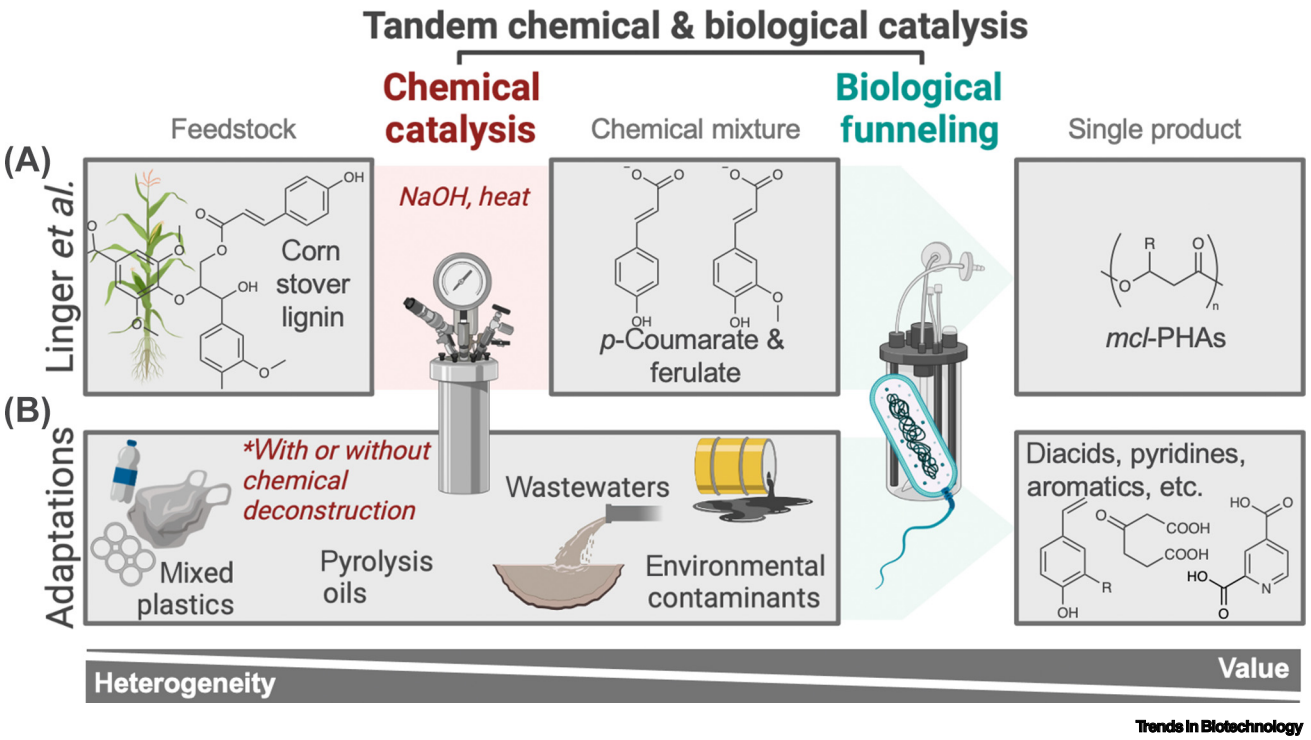

Figure 1. Process overview for realized and possible adaptations of tandem chemical catalysis and biological funneling. (A) Process described by Linger and colleagues [7], wherein corn stover is chemically converted to a mixture of lignin-derived aromatic compounds (LDACs) and other extractives, which were then biologically converted to medium chain-length polyhydroxyalkanoates (*mcl*-PHAs). (B) Possible and realized adaptions of this workflow for feedstocks, such as plastics, pyrolysis oils, wastewaters, and environmental contaminants, to products spanning the general categories of diacids, pyridines, aromatics, among others [10,14]. Some but not all feedstocks would require chemical catalysis before biological funneling. In all cases, biological funneling reduces heterogeneity to increase value. Elements of this figure were created with BioRender (BioRender.com).

enzymatic paradigms and enzyme engineering for key LDAC conversions underpins many of these advancements [9]. Weiland, Kohlstedt, and Wittmann's excellent 2022 review provides an extensive analysis of metabolic engineering advances for lignin valorization [10].

Looking ahead toward producing commodity products from lignin, improved methods will be needed for lignin deconstruction and process integration between chemical and biological procedures [11]. A paramount challenge is lignin processing to generate higher yields of bioavailable LDACs because the extent of biologically accessible carbon from polymeric lignin determines the economic viability of coproducts from lignin in the lignocellulosic biorefinery [12]. Additionally, an increased focus on the integration of chemical deconstruction methods with biological funneling platforms will be necessary to identify challenges, such as engineering microbial tolerance to chemical byproducts or expanding the biological funnel to accommodate additional substrates.

Beyond lignin, particularly intriguing opportunities lie in: (i) tandem chemobiological catalysis to valorize other recalcitrant feedstocks; and (ii) biological funneling of other chemical mixtures (Figure 1B). Toward (i), chemical deconstruction methods that generate biologically available substrates are critical. For example, mixed plastic wastes were recently chemically deconstructed to a mixture of bioavailable oxygenated intermediates that were subsequently biologically funneled to a single product [13]. Toward (ii), the possible substrates for biological funneling are expansive, including pyrolysis oils, agricultural and industrial wastewaters, and environmental contaminants, many of which were recently reviewed [14]. Ultimately, only creativity and the catalog of engineerable biochemistries limit the possible products that can be produced from these varied wastes.

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Declaration of interests

None declared by authors.

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