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Integrating Metabolic Engineering and Heterogeneous Chemocatalysis: New Opportunities for Biomass to Chemicals

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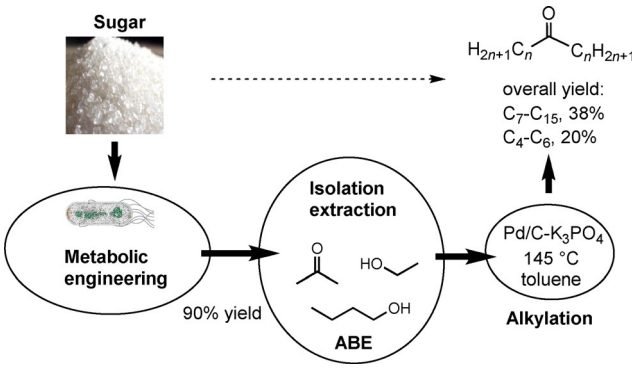
Driven by the constant energy- and resource-saving demand, pursuing high step- and atom-economy is an endless process in chemical synthesis.[1] Recently, several comprehensive reviews involving the combination of biocatalysis and chemocatalysis were published.[2,3] Combining individual reaction steps into one pot, even from the same type of technology such as chemocatalysis or biocatalysis, has great potential in terms of saving solvent, energy, time and space, as well as reducing waste production.[3,4] The integration of reaction steps from different fields will hence be more attractive in unveiling new opportunities and achieving challenging tasks. In biocatalysis, reactions are catalyzed by enzymes and engineered metabolic processes that are typically carried out in water at low concentrations. This system would typically generate biogenic impurities from fermentation. Chemocatalysis using organo- or metal catalysts is generally carried out in various solvents and catalysts that may sometimes be sensitive to impurities. One major challenge in combining biocatalysis and chemocatalysis lies in the compatibility of the two systems, including reaction conditions, conversions, and selectivities.[4,5] Although the groundbreaking pioneering work in this topic was reported by the Bekkum group in 1980 illustrating a heterogenized metal-catalyzed hydrogenation coupled with an enzymatic isomerization of D-glucose for the synthesis of D-mannitol, surprisingly this strategy has not been applied to biomass transformation until very recently.[6]

Biomass and derivatives generally are highly functionalized substrates with high oxygen content. Upgrading biomass to fuel and chemicals generally starts with reducing its oxygen content, which often has less structure versatility when using chemocatalysis.[7] Alternatively, in biocatalysis (especially metabolic processes) biomass and its derivatives serve as substrates and/or nutrition in many natural processes. Various products with different carbon chain structures and different functionalities could be produced.[8] The strategy of combining metabolic engineering and heterogeneous chemocatalysis is particularly suitable for biomass transformation processes. With natural or engineered metabolic processes, biomass or derivatives can be selectively defunctionalized to platform intermediates suitable for chemocatalytic upgrading to value-added chemicals, especially for "drop-in" chemicals.

Logically, there are different levels of combining/integrating biocatalysis and chemocatalysis that represent different levels of step and atom economy. The basic level corresponds to linking two individual processes with one key intermediate, which is generated from the first process but could serve as feed for the second reaction. This type of combined process is similar to the traditional cascade synthesis, and the intermediate compound needs to be isolated and purified before being applied to the second step. Well-designed combinations of metabolic processes and chemocatalysis could be assembled together through engineering efforts to bridge the gap between two fields and realize challenging tasks. Anbarasan et al. reported an excellent example of this strategy for the conversion of sugars to jet fuel precursors in high yield.[9] It is well known that short-chain alcohols, such as ethanol and *n*-butanol, can be produced through natural biological routes at yields approaching theoretical limits. However, to produce longer chain precursors of higher-molecular-mass hydrocarbons, which are commonly used in jet and diesel fuels, is still challenging to date. As shown in Scheme 1, *Clostridium acetobutylicum* (*C. acetobutylicum*) was chosen by Anbarasan et al. to produce acetone–*n*-butanol–ethanol (ABE) from sugars. The key point is the combination of fermentation products in which acetone has a nucleophilic α-carbon, which is a suitable functionality to



Scheme 1. Conversion of sugars to high-molecular-mass hydrocarbons through a combined metabolic engineering and chemocatalysis process.

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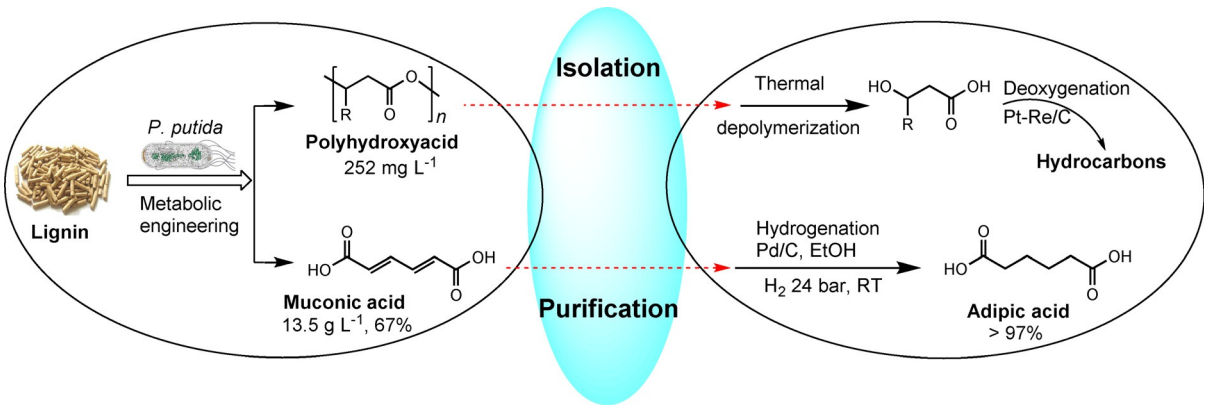
achieve a metal-catalyzed C–C bond formation with electrophilic alcohols present in the ABE fermentation products. Glucose undergoes fermentation with *C. acetobutylicum* to yield acetone, *n*-butanol, and ethanol in 2.3/3.7/1 molar ratio at an overall carbon yield of 90%. Fermentation products went through a two-step extraction, and ABE was then passed through a Pd/C-K_3PO_4 heterogeneous catalyst system. Eventually, this combined system gave C_7–C_{15} products in 38% overall carbon yield from glucose. The yield increased to 58% after inclusion of C_4–C_6 products. Isolation and purification of fermentation products ABE is a critical issue in this combined system. It is energy intensive to extract ABE from water and to remove all impurities that may poison the metal catalyst.

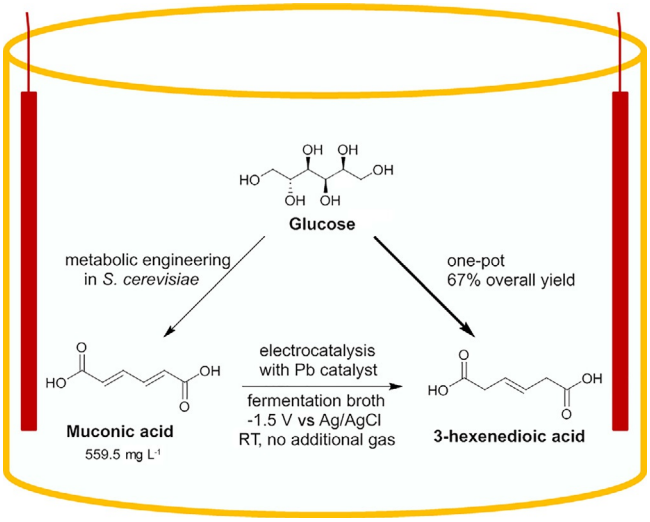
Beckham et al. also demonstrated this strategy recently for the conversion of lignin to chemicals by combining biological and chemical catalysts (Scheme 2).[10,11] Lignin is one of the major sources of biomass, which is a heterogeneous polymer consisting of phenylpropanoid monomers. Due to its aromatics-rich heterogenized structure, lignin is very difficult to be selectively valorized. Chemically, lignin could be depolymerized by metal-catalyzed reduction or oxidation under relatively harsh conditions.[12] However, some organisms use lignin-derived aromatic molecules as carbon sources in their metabolic pathways.[13] Aromatic-catabolizing organisms convert pretreated lignin into central intermediates such as catechol and protocatechuate. Dioxygenase enzymes then cleave C–C bonds in the aromatic ring to produce ring-opened species via the β-ketoadipate pathway. Beckham et al.[10] demonstrated this technology by using *Pseudomonas putida* (*P. putida*) to produce medium-chain-length polyhydroxyalkanoates (mcl-PHAs) from lignin in an integrated biological process. mcl-PHA was extracted from the cell culture system and re-precipitated from ethanol. The purified PHA underwent thermal depolymerization, and then the depolymerized alkenoic acids were catalytically converted to hydrocarbons over a platinum–rhenium solid catalyst (300 °C, 27.5 bar H_2). In another work from the same group,[11] metabolic engineering of *P. putida* selectively produced muconate (molar yield 67%) from lignin-derived monomers. Muconic acid was isolated and purified from culture media through activated carbon absorption and recrystallization at low pH. Purified muconic acid then underwent metal-catalyzed hydrogenation to produce adipic acid (Pd/C, 24 bar H_2, 24 °C).[14] These two examples opened a new window for upgrading lignin, a relatively inert biomass, by ring-opening of aromatic ring through biocatalytic and then further upgrading using chemocatalytic methods, although there are some difficulties in the connection between biocatalysis and chemocatalysis.

For this two-step biocatalysis–chemocatalysis process one major drawback is that the intermediate substrates need to be isolated and purified to fulfill the requirement of the metal-catalyst system. This drawback will apparently affect the overall energy, mass, and economic efficiency of the combined system. Schwartz et al. worked on improving catalyst tolerance toward biogenic impurities by manipulating microenvironments of catalytically active sites of heterogeneous catalysts.[15] Triacetic acid lactone (TAL) is a platform molecule derived from sugars by metabolic processes.[16] In the process of further upgrading to sorbic acid via palladium- or ruthenium-catalyzed hydrogenation reactions, metal catalysts are very sensitive to the trace biogenic impurities even after the TAL went through an intensive purification process. It was found that a poly(vinyl alcohol) (PVA) coating could create a microenvironment that is unfavorable for biogenic impurities. Therefore, a PVA-overcoated metal catalyst was an order of magnitude more stable than catalyst without PVA coating in the hydrogenation of TAL. Efforts to minimize the gap between bio- and chemocatalysis are definitely important. However, development of a new strategy allowing biocatalysis and chemocatalysis to work in one pot is more attractive and appreciable.

Due to the different natures of bio- and chemocatalysis, combining two processes into one pot is very challenging. Recently, Suastegui et al.[17] made a breakthrough in this field by introducing electro-chemocatalysis into this strategy and realized the combination of bio- and chemocatalysis in one pot without any additional work-up in between (Scheme 3). The process started from fermentation of glucose with engineered strain of *Saccharomyces cerevisiae* to muconic acid, a well-known metabolic process. High muconic acid titer of 559.5 mg L^{-1} was achieved using this new engineered strain. Muconic acid was then directly electrocatalytically hydrogenated to 3-hexenedioic acid (3-HAD) in a three-electrode electro-



Scheme 2. Combination of metabolic engineering and chemocatalysis for lignin upgrading to hydrocarbons and adipic acid.



Scheme 3. Sequential fermentation/electrocatalytic reduction of glucose into 3-hexenedioic acid through a combined metabolic engineering-and-eletrocatalysis process in one pot. The selected electrocatalysis step allows using fermentation broth as electrolyte and hydrogen source.

chemical cell without any further work up process. In this step, hydrogen as a reducing agent was produced in situ due to water splitting on the electrode, and the reaction occurred at ambient temperature and pressure. 94% yield of 3-HAD (overall yield of 67% from glucose) was obtained in the system in the presence of all the biogenic impurities. It was claimed that the charge on the electrode surface could help with the mitigation of poisoning. Although lead, a toxic metal, was used in this system to increase the resistance of the electrode to biogenic impurities, the system did demonstrate an exciting potential in integrating biocatalysis and chemocatalysis in one pot for biomass upgrading to chemicals.

It is clear that there is great potential of applying integrated metabolic-process and chemocatalysis systems in biomass transformation. Due to the great versatility of metabolic systems, different biomass resources could be converted into a variety of intermediate compounds. Chemical processes can further turn these intermediates to various desired targets. Moving from a two-step procedure to a one-pot reaction is an important step forward as it means tremendous savings in energy, mass, time, and space. Further efforts may direct to develop real integrated system where biocatalysis and chemocatalysis work concurrently and synergistically in one system.

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