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Editorial: Cell Free Biocatalysis for the Production of Bioproducts

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Editorial on the Research Topic

Cell Free Biocatalysis for the Production of Bioproducts

Biological approaches show promise for the sustainable and cost competitive production of commodity fuels and chemicals. A lot of efforts have been devoted to engineering microbial chassis to produce select fuels and chemicals. These approaches have had some level of success exemplified, of course, by the production of ethanol at high yield and titer but also recent successes with commodity chemicals such as 1,4-butanediol, 1,3-propanediol, and even to some extent with sustainable aviation fuel intermediates such as farnesene. However, many of these processes still suffer from low yields, usually below the standard needed to drive a sustainable and atom efficient bioeconomy. Additionally, for many other chemicals and fuel precursors, titers and yields are only a fraction of what they are for ethanol. Indeed, there exist many limiting factors associated with the use of microbial biocatalysts. Some of these factors include the diversion of carbon from products to sustain growth and biomass formation, the production of byproducts or regulatory mechanisms, the toxicity of end products or intermediates, and the difficulty of separating products from growth media.

Cell free biocatalysis, essentially operating metabolic enzyme cascades outside of the microbial cell, has emerged as an attractive alternative to circumvent many of these limitations. A lot of progress has been made in the last 15 years in the field of cell free biocatalysis for the production of bioproducts. These include the production of several key molecules, e.g., 2,3 butanediol, isobutanol, terpenes, with titers, rates, and yields rivaling or far exceeding those achieved with microbial biocatalysts. Despite promise, key challenges of existing cell-free platforms still include: enzyme production costs, catalyst/enzyme stability, enzyme inhibition, inactivation, or regulation by reaction intermediates and products, cost of reagents (e.g., cofactors), and the fact that current optimizations are not focused on processes that could proceed to large-scale economic production.

In this research topic, the review on cell free biocatalysis (Bergquist et al.) offers a comprehensive survey of the field including examples of cell free enzyme pathways recently explored but also points out areas that need significant improvements to enable the scale up and commercialization of these technologies. These areas include scaling up of protein production, increasing protein operating lifetime, and of course achieving better cofactor management. The cost and management of cofactors is especially crucial if one considers the production of commodity chemicals. Recent studies have shown that cofactors can be recycled very efficiently during the production of several biochemicals for many days. However, the cost of "priming" the production system with expensive natural cofactors remains more than prohibitive for commodities. Therefore, recent efforts have tried to use biomimetic cofactors (semi synthetic or synthetic cofactors) that can be chemically synthesized and can be more than 100-fold cheaper than natural cofactors. Some redox enzymes are able to use these cofactors natively, however, the vast majority of these enzymes will require significant engineering.

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As mentioned above, enzyme stability and longer operating lifetimes are also key to develop viable cell free processes, able to operate at higher temperatures or in desired solvents. This can be achieved through enzyme engineering, enzyme immobilization, or by exploring natural diversity, especially in thermophilic microbes. The paper on the characterization and engineering of a hyperthermophilic laccase (Stevens et al.) offers an approach for the engineering of a lignolytic enzyme to increase its activity in ionic liquids. In this study, Stevens et al. demonstrate that, even though solvent tolerance and thermophilicity are often considered interchangeable, a hyperthermophilic enzyme can be used as a template and still benefit from engineering to increase its enzymatic activity in solvents.

When considering the commercialization of cell free based processes, the cost of enzyme production becomes an important factor that depends on protein production titers but also on post processing steps, such as protein purification. A new study by Xu et al. on self-assembling metabolons describes an approach relying on an arraying strategy, used by some cellulolytic bacteria in nature, to tether and organize metabolic enzymes for the conversion of glycerol to 1,3 propanediol. In this approach, the metabolic pathway enzymes are coexpressed with a protein scaffold, able to assemble in a functional metabolon *in vivo*, and can be purified, at once, directly from a crude cell lysate on a cheap and sustainable cellulose matrix.

Finally, cell free approaches can also be used to prototype and troubleshoot metabolic pathways for *in vivo* or *in vitro* applications. In their paper, Cui et al., developed a cell-free extract reaction system in *C. thermocellum* that can operate at both mesophilic and thermophilic temperatures. These systems are essential to directly observe the utilization/build-up of intermediates. They also allow the perturbation of the system by added exogeneous enzymes to determine their effect on metabolism.

The idea of using cell free biocalatysis for the production of biochemicals is not new and has been around for more than a 100 years. However, applications in industry are still rare, comprised of a few enzymatic steps, or limited to the production of high value chemicals. Nevertheless, this field has received renewed attention in the last 20 years due, in part, to the advances in enzyme production and engineering but also due to the advent of cell free protein synthesis. Given these advances and recent successes in the production of several key biochemicals at high yields, titers, and productivities, cell free biocatalysis could become the disruptive technology that the biochemical industry desperately needs.

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