ACS Sustainable Chemistry & Engineering

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Editorial

New Insights on Enzyme Stabilization for Industrial Biocatalysis

Cite This: *ACS Sustainable Chem. Eng.* 2021, 9, 15073−15074

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Due to their versatility and ability to provide tunable chemo-, regio-, and enantio-selectivitites, enzyme-mediated transformations offer an effective and eco-friendly alternative to the traditional multistep chemical synthesis methods, which often require organic solvents, hazardous reagents, and tedious purification processes.

While enzymes provide high catalytic and specific activity at mild conditions, several operational drawbacks hamper the application of enzymes as industrial biocatalysts. These include poor performance when confronting non-natural substrates, inhibition by certain components in the reaction media, short lifetime, lack of reusability, and recovery postreaction. Among these, achieving enzyme stabilization is most challenging from a practical standpoint.

To enhance enzyme stability, many strategies have been pursued including either random or rational immobilization of the biocatalysts onto different suitable materials, use of extremophiles, implementation of protein engineering methodologies and structural bioinformatics in biocatalyst design, and reaction medium engineering.

This Virtual Special Issue (VSI) on "New Insights on Enzyme Stabilization for Industrial Biocatalysis" aims to highlight the recent trends in enzyme stabilization. To this end, a total of nine articles, including perspectives, are compiled in this VSI.

In their Perspective, Sheldon and Brady (https://pubs.acs.org/doi/10.1021/acssuschemeng.1c01742) highlight the importance of biocatalyst design and process engineering in bioprocess development. Emphasis is placed on recent advances in molecular biology for designing versatile biocatalysts. The authors also discuss recent advances in enzyme immobilization and applications of such enzymes in flow biocatalysis.

Biocatalyst immobilization is a preferred attribute for industrial implementation. Several articles provide new advances on this topic. Ölçücü et al. (https://pubs.acs.org/doi/10.1021/acssuschemeng.1c02045) discuss solutions for *in vivo* biocatalyst immobilization, highlighting a vast repertoire of increasing complexity, including entrapment of proteins in inclusion bodies and protein crystals, designed synthetic organelles, polyhydroxyalkanoate-based systems, and virus-like particles.

In a similar vein, Gkantzou et al. (https://pubs.acs.org/doi/10.1021/acssuschemeng.1c02557) display a novel β-glucosidase/ZnO nanowire continuous flow microreactor for the enzymatic glycosylation of diverse natural products. Interestingly, the immobilized enzyme did not suffer any loss of hydrolytic activity after 1000 reaction cycles and also maintained 70% residual activity when exposed to different organic solvents for 24 h. Additionally, the productivity was enhanced by up to 30 fold compared to the free enzyme and the batch immobilized system, respectively. Finally, as a proof of concept, the authors demonstrated the effectiveness of this novel enzyme microbioreactor for the continuous glycosylation of alcohol and tyrosol.

Morellon-Sterling et al. (https://pubs.acs.org/doi/10.1021/acssuschemeng.1c01065) report on the application of divinyl sulfone-activated supports in enzyme coimmobilization. Two different model combi-biocatalysts are presented. The first model involves the multipoint covalent coimmobilization of trypsin and chymotrypsin onto vinyl sulfone (VS)−agarose particles, followed by ionic adsorption of β-galactosidase from *Aspergillus oryzae*. The second involves the coimmobilization of trypsin and ficin, with trypsin being covalently linked to the (VS)−agarose particles and ficin coimmobilized by cation exchange. The ionic adsorption of the less stable catalysts allows facile turnover rates by incubating the combi-catalysts at high concentrations of ammonium sulfate.

The VSI also features thermostable enzymes. Bosch et al. (https://pubs.acs.org/doi/10.1021/acssuschemeng.1c00699) developed an activity-independent method for the selection of thermostable enzymes using *Thermus thermophilus* as the host strain. This method is based on the folding interference at high temperature of a thermostable antibiotic reporter protein at the C-terminus of a fusion protein between a thermosensitive target protein (located in the N-term) and a thermostable kanamycin nucleotidyltransferase (located in the C-term). Interestingly, this method allows a simple and rapid method for *in vivo* screening of thermostable enzyme variants from directed evolution experiments.

In a related theme, Geiss et al. (https://pubs.acs.org/doi/10.1021/acssuschemeng.1c01165) report improved turnover stability of cellobiose dehydrogenase by high-throughput enzyme engineering. As a result, 11 thermostable variants (from a total of 13,736 screened colonies) were selected, produced, and biochemically characterized. Moreover, the authors also provide new insights into two different mechanisms contributing to turnover stability. These insights will help the scientific community to design oxidoreductases with enhanced turnover stability through protein engineering.

Received: October 5, 2021
Published: November 15, 2021

The article by Mateljak and Alcalde (https://pubs.acs.org/doi/10.1021/acssuschemeng.1c00622) reports the most thermostable high-redox potential laccase (HRPL) engineered to date ($t_{1/2}$ 75 °C = 225 min; up to 32-fold superior to the parental enzyme), by introducing 27 stabilizing mutations identified in previous evolved fungal HRPLs.

The VSI also features articles on the effects of reaction medium engineering strategies on enzyme stability. Petermeir et al. (https://pubs.acs.org/doi/10.1021/acssuschemeng.1c01536) report a detailed study of process engineering variables for enzyme-mediated glycosylation of indoxyl (a key step for the industrial production of indican). The authors characterized soluble and immobilized uridine diphosphate (UDP)-glycosyltransferase from *Polygonum tinctorium*, exploring the effect of diverse operating parameters on the catalyst activity and stability. In another contribution, Gran-Scheuch et al. (https://pubs.acs.org/doi/10.1021/acssuschemeng.1c02012) explored the formation of the different reactive oxygen species (ROS) during the flavin-mediated reduction of dioxygen catalyzed by diverse oxidoreductases (flavoprotein oxidases and monooxygenases) under different experimental conditions (different substrate concentrations, pH values, and cosolvents).

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Notes

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ACKNOWLEDGMENTS

We hope that the advances presented in this VSI will be of interest to the readers. We thank all the authors, reviewers, and the Editorial Board members of *ACS Sustainable Chemistry & Engineering*, whose contributions and support were vital to the publication of this VSI.

https://doi.org/10.1021/acssuschemeng.1c06785
ACS Sustainable Chem. Eng. 2021, 9, 15073−15074