

ENZYMES IN DEEP EUTECTIC SOLVENTS: BALANCING ENZYME ACTIVITY AND STABILITY

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Deep Eutectic Solvents (DES), neoteric systems that emulate the natural environment for various biomacromolecules, are under extensive study as non-toxic and highly versatile solvents applicable across diverse fields, including synthesis, (bio)catalysis and biomedicine.^[1] Originally, the term DES was coined to describe a physical mixture of two or more components that solidifies at a single temperature lower than the crystallization point of any individual component. Over time, these solvents/systems have come to encompass mixtures comprising two or more components that exhibit characteristics similar to a eutectic system, allowing them to maintain a liquid state at a specific desired temperature.^[2]

The synergistic combination of DES and biotechnological methods, employing enzymes as catalysts, aligns naturally with the efficient and sustainable production of various organic compounds. Biocatalysis, as a biotechnological approach, enables the catalysis of otherwise challenging transformations with high regio-, chemo-, and enantioselectivity under mild and cost-effective conditions, while DES serve as a robust green medium for modulating and directing reaction pathways to attain the desired product.^[3]

Here, we present our extensive experience in the field of biocatalysis in DES, emphasizing the interplay between enzymatic activity and stability in these solvents, and how this interplay influences the fate of DES ability to enhance biocatalytic processes. Specifically, we will focus on our recent research on behavior of oxidoreductive (various dehydrogenases) and hydrolytic enzymes (lipase and lysozyme) in DES and developing Quantitative Structure-Activity Relationship (QSAR) models to predict enzyme's behavior in DES.

[1] Hansen, B.B.; Spittle, S.; Chen, B.; Poe, D.; Zhang, Y.; Klein, J.M. et al, Deep Eutectic Solvents: A Review of Fundamentals and Applications. *Chemical Reviews* **2021**, *10*, 1232-1285.

[2] Abranches, D.O. and Coutinho J.A.P., Everything You Wanted to Know about Deep Eutectic Solvents but Were Afraid to Be Told. *Annual Review of Chemical and Biomolecular Engineering* **2023**, *14*, 141-163.

[3] Panić, M.; Cvjetko Bubalo, M.; Radojčić Redovniković, I., Designing a Biocatalytic Process Involving Deep Eutectic Solvents. *Journal of chemical technology and biotechnology* **2021**, *96*, 14-30.