

# THE EFFECT OF ORGANIC SOLVENTS, IONIC LIQUIDS AND MICRO-AQUEOUS REACTION SYSTEMS ON BIOTRANSFORMATIONS: THREE CASE STUDIES

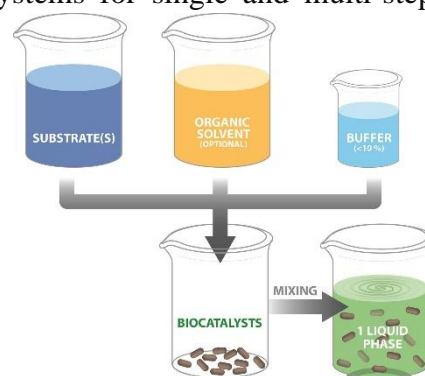
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In nature, enzymes typically function under aqueous conditions. Consequently, aqueous buffers are often used as reaction media in transformations involving enzymes. However, for industrial applications, the poor water solubility of many compounds of industrial relevance means that aqueous systems frequently fail to achieve sufficient substrate and therewith product concentrations. Switching to a non-aqueous solvent system can provide a solution, a practice already common with lipases but more challenging for biocatalysts from other enzyme classes. Recently, several potent green solvents come to market, showing an interesting alternative to classical, environmentally less benign solvents. Some are well suited to biotransformations, when an appropriate formulation is found.

In this presentation, three examples will be focused on. In case studies one and two, the effect of additives on the activity, stereoselectivity, and chemoselectivity of carbonylation reactions with thiamine diphosphate-dependent enzymes is demonstrated. Since the chosen reaction is very sensitive to additives in a mono liquid-phasic buffered system, it can be clearly shown how some organic solvents as well as ionic liquids directly interact with areas of the active site, partially competing with the substrates. This affects the selectivity of the enzymes, which can, in the optimal case, lead to increased or inverted selectivity.<sup>[1]</sup> Thus, solvents can be an interesting parameter for engineering.

As a third example, the use of micro-aqueous reaction systems for single and multi-step enzyme-catalyzed processes will be demonstrated.<sup>[2]</sup> Due to significantly increased substrate concentrations in these non-buffered systems, high product concentrations and space-time yields can be achieved, while stereoselectivities are maintained. Additionally, the use of an economical catalyst formulation and simplified downstream processing makes its application advantageous.<sup>[3]</sup> It also offers great potential when biotransformations and chemical transformations are combined in multi-step processes.<sup>[4]</sup>



**Figure 1:** Micro-aqueous reaction system.

[1] Gerhards T, Mackfeld U, Bocola M, von Lieres E, Wiechert W, Pohl M, Rother D. Influence of organic solvents on enzymatic asymmetric carbonylations. *Adv. Synth. Catal.* **2012**, 354: 2805-2820.

[2] Van Schie M M C H., Spöring J-D, Bocola M, Domínguez de María P, Rother D. Applied biocatalysis beyond just buffers – from aqueous to unconventional media. Options and guidelines. *Green Chem.* **2021**, 3: 3191-3206.

[3] Oeggel R, Maßmann T, Jupke A, Rother D. Four Atom Efficient Enzyme Cascades for All 4-Methoxyphenyl-1, 2-propanediol Isomers Including Product Crystallization Targeting High Product Concentrations and Excellent E-Factors, *ACS Sus. Chem. Eng.* **2018**, 6: 11819–26.

[4] Graf von Westarp W, Wiesenthal J, Spöring J D, Mengers H G, Kasterke M, Koß H J, Blank L, Rother D, Klankermayer J, Jupke A. Interdisciplinary development of an overall process concept from glucose to 4, 5-dimethyl-1, 3-dioxolane via 2, 3-butanediol. *Commun. Chem.* **2023**, 6 (1): 253.