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DOI
10.1016/j.mcat.2021.112035

Publication date
2022

Document Version
Final published version

Published in
Molecular Catalysis

Citation (APA)
Holtmann, D., & Hollmann, F. (2022). Is water the best solvent for biocatalysis? *Molecular Catalysis*, *517*, Article 112035. https://doi.org/10.1016/j.mcat.2021.112035

Molecular Catalysis 517 (2022) 112035

Contents lists available at ScienceDirect

Molecular Catalysis

journal homepage: www.journals.elsevier.com/molecular-catalysis

Is water the best solvent for biocatalysis?

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At first glance, the answer is quite obvious: water is the perfect solvent for biocatalytic reactions. Life depends on water, living cells contain approx. 75% of water, most enzymes and many essential cofactors are exclusively soluble in water-based solvents. Moreover, water is a nontoxic, and non-hazardous solvent [1]. So, it appears just natural to perform biocatalytic reactions in aqueous media.

The flaw of this argumentation however is that the majority of reagents of interest in organic synthesis are rather hydrophobic and therefore poorly soluble in aqueous media. A direct consequence of this apparent discrepancy in polarities (between solvent and reagents) can be seen in Fig. 1. Analysing the starting material concentrations of biocatalytic and chemocatalytic transformations reported in the journals Molecular Catalysis and ChemCatChem in the year 2020 reveals that 90% of all biocatalytic reactions utilise less than 100 mM of starting material (corresponding to roughly 20 $g \times L^{-1}$ of substrate loading). In chemocatalysis only a bit more than 30% of the reports fall into this range. In fact one quarter of all chemocatalytic reactions report no solvent use (which in biocatalysis still represents an exception). *Chemists use the most suitable solvent for a given reaction while biotechnologists usually use water.*

We believe that low substrate (and product) concentrations in biocatalysis largely consume the benefits of water as unproblematic solvent: A stirred tank reactor containing approx. 20 $g \times L^{-1}$ of reagent also contains approx. 980 $g \times L^{-1}$ of water. In other words, energy used to stir, heat and cool the reaction mixture is predominantly (to approx. 98%) used for the solvent and not the product. In this respect it is worth noting that the heat capacity of water (ca. 4.2 $kJ \times (kg \times K)^{-1}$) is amongst the highest of the common solvents used translating in higher energy demands to achieve temperature changes for water than for most solvents. The issue of the rather high boiling point of water can to some extend be alleviated by using water-soluble cosolvents forming azeotropic mixtures with water and thereby lowering the temperature for destillative solvent recovery [2].

It should, however, also be taken into account (vide infra) that water represents a very safe solvent especially if compared with volatile and flammable solvents.

Increasing the reagent concentration represents a major current challenge of biocatalysis to fulfil its green promise and to make it an attractive synthetic alternative for preparative chemists [3,4]. Of course this argumentation does not apply to biocatalytic reactions involving highly water-soluble reagents.

Non-aqueous of biphasic reactions

Replacing water by other, more suitable solvents in biocatalysis is easier than thought: 'The best solvent is no solvent' [5]. This statement is particularly true if all reagents are liquid and soluble in each other in the temperature range of biocatalysis. For example, the enzymatic esterification of fatty acids with fatty alcohols to synthesise so-called emollient esters has been established on industrial scale [6,7]. Other 'neat' hydrolase reactions are reported frequently.

If not all reagents are liquid, hydrophobic solvents often represent an alternative to aqueous reaction media. As early as the 1980s Klibanov and coworkers pointed out the benefits of biocatalytic reaction in non-aqueous media [8,9] such as enzyme stability at temperatures above 100 °C. Other benefits of non-aqueous reaction media comprise the absence of undesired hydrolytic side reactions such as hydrolysis of ester or epoxide functional groups or the water-related racemisation of α-substituted carbonyl products. For example, hydroxynitrile lyase-catalysed formation of cyanohydrins profits from nonaqueous conditions as here the spontaneous, non-enantioselective cyanohydrin formation can be largely eliminated resulting in enantiomerically pure products [10]. Also alcohol dehydrogenase-catalysed redox reactions can be performed in non-aqueous or microaqueous media [11–13]. Next to the drastically increased reagent solubility, this approach minimises the water-related degradation of the nicotinamide cofactor, which due to the non-aqueous environment stays bound to the enzymes' active sites. Also transaminase- [14] or imine reductase-catalysed [15] reductive aminations, lyase-catalysed aldehyde coupling reactions (Umpolungs reactions) [16] or peroxygenase-catalysed oxyfunctionalisation reactions [17,18] have been reported.

Of course there are reactions where a liquid aqueous layer cannot be

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https://doi.org/10.1016/j.mcat.2021.112035
Received 20 October 2021; Received in revised form 27 November 2021; Accepted 27 November 2021
Available online 3 December 2021

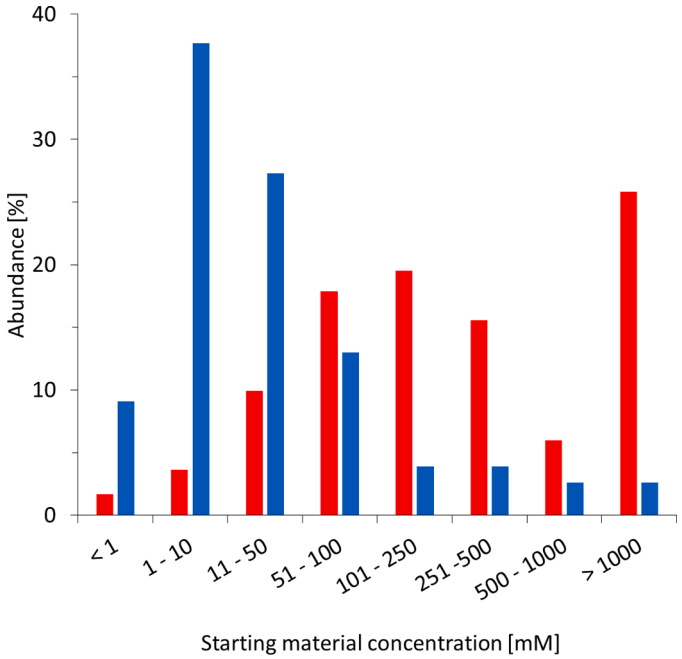

Fig. 1. Starting material concentrations reported in biocatalytic reactions (blue) and chemocatalytic (red) reactions in 2020 in the journals Molecular Catalysis and ChemCatChem. Analysed were reactions performed in liquid phase. Total of reactions: 379 (77 biocatalytic and 302 chemocatalytic reactions) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

omitted for example if diffusible, highly charged cofactors are required. Multi-phase reactions represent a doable solution here to increase the overall substrate loading. This approach, in fact is biomimetic as the lipid membrane of (microbial) cells represents a hydrophobic, organic solvent. The principle of these reactions is that the majority of the reagents is not present in the aqueous (biocatalyst containing) reaction phase but partitions between both phases. This may result in very low aqueous concentrations of the starting material which at first sight may seem like a limitation for the reaction. Enzyme catalysts, however, are principally well suited to operate with low substrate concentrations. While many 'chemical' reactions follow first order rate equations, enzymatic reactions usually follow Michaelis-Menten kinetics (Fig. 2). As a consequence, provided the biocatalyst exhibits a sufficiently high affinity (i.e. a low K_M value), maximal conversion rates can already be achieved at low aqueous concentrations of the staring material.

The main challenge then will be to ensure sufficiently high phase transfer rates to avoid substrate limitation; increasing the interphase area e.g. by vigorous mixing is the most widely chosen strategy here. Interfacial inactivation of the biocatalyst represents a limitation frequently encountered. Though we are still far away from a molecular understanding of the mechanisms underlying interfacial inactivation, already now protein engineering [19] or reaction engineering [20] help to solve this issue. Micellar reaction systems may represent a promising approach. On the one hand a surfactant-stabilised emulsion requires less energy input (e.g. via stirring) to maintain a high interfacial surface area. On the other hand, the polar or even ionic masking of the hydrophobic layer may reduce its interaction with the biocatalyst and thereby also its inactivation. Possible challenges include efficient methods to break the emulsions after the biotransformation step.

Neoteric solvents

Since long, the world of non-aqueous media is not limited to classical organic solvents anymore. Novel solvents continuously enter (and leave) the stage of biocatalysis. Supercritical solvents were amongst the first ones to receive considerable attention in biocatalysis. The number of publications dealing with supercritical solvents peaked between 2002 and 2006. Shortly afterwards, ionic liquids came into focus (peaking between 2004 and 2018) and are now succeeded by deep eutectic solvents (currently increasing exponentially). It is interesting to note that many contributions dealing with neoteric solvents do not primarily aim at increasing reagent concentrations but rather explore the 'proof-of-concept' for a greener alternative to established solvents.

The question 'are we looking at the right thing?' [21] persists.

Nevertheless, some interesting applications of neoteric solvents have been reported. For example, a tailored ionic liquid could be used as second phase to selectively extract the coproduct of an alcohol-

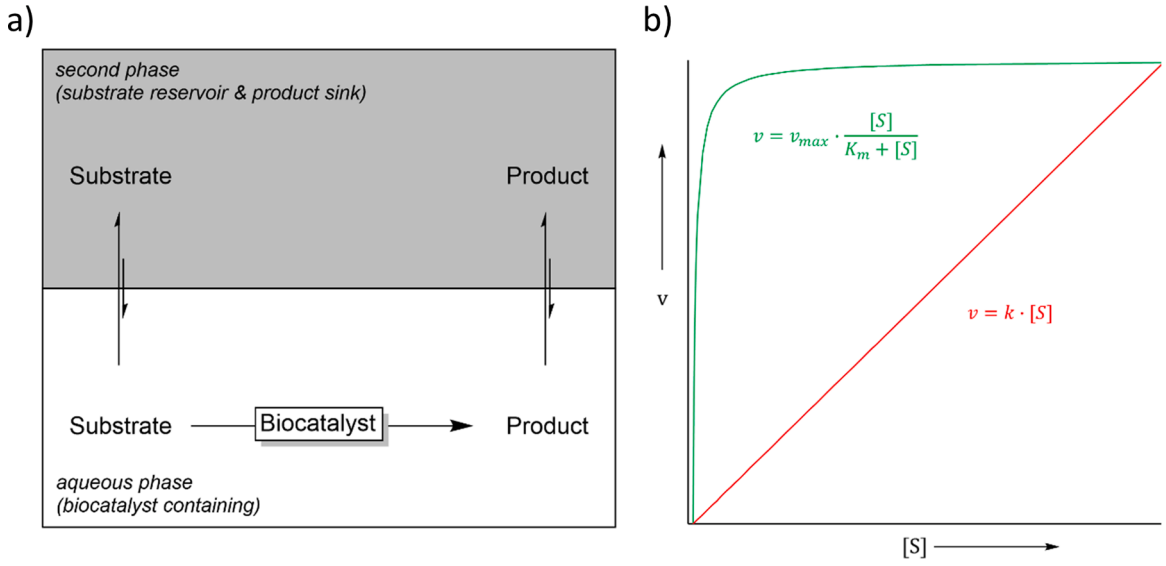

Fig. 2. (a) Multiphase biocatalytic reactions utilise an aqueous, biocatalyst-containing reaction phase in contact with a second phase (solid, liquid or gaseous) between which the reagents can diffuse. Often, the aqueous concentration of the reagents is low, which not necessarily represents a major issue provided the biocatalyst exhibits a sufficiently high affinity to the substrate (S). (b) While in many chemical reactions the reaction rate linearly correlates with the substrate concentration (red), enzymatic reactions often already exhibit high rates (green) in the presence of low substrate concentrations (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

dehydrogenase catalysed reduction reaction thereby shifting the unfavourable thermodynamic equilibrium of the reaction [22]. Another interesting application of deep eutectic solvents is if they are (partially) composed out of the reagents of the reactions as exemplified in the synthesis of mentholesters [23] or as cosubstrate for biocatalytic redox reactions [24,25]. An intriguing application of carbohydrate-based DES was reported for the synthesis of carbohydrate fatty acid esters (Fig. 3) [26]. The poor solubility of carbohydrates in solvents other than water complicates the (bio)catalytic synthesis of carbohydrate-based surfactants via (trans)esterification due to the competing hydrolysis reaction. Here, carbohydrate-ChCl-DES represent an interesting solution to this challenge.

How to choose the most suitable solvent?

There is no universal solvent (class). Identification of the most suitable solvent for a given (bio)catalytic conversion will depend on various, interconnected parameters such as its solvent properties, influence on the catalyst, ease of recycling, environmental impact etc.). Decision trees such as the one recently proposed by van Schie et al. [3] simplified Life Cycle assessments [21] or solvent guides [27] can certainly assist in the decision-making. Yet, the solvent plays a central role in various aspects of the overall process (Everything is connected). Solvents standing out with one desirable property may at the same time fail in others. For example, a solvent enabling high enzyme activity and–stability may pose considerable challenges for the reaction due to its high viscosity or to the product isolation.

Solvent selection comprises weighing multiple objectives, which generally cannot be quantified in the same physical or monetary unit. A Value-Benefit Analysis (VBA) or weighted sum model (WSM) may help to rank alternative solvents based on a framework of weighted parameters [28]. It allows a systematic assessment of the consequences of different alternatives. The method leads to a ranking of alternatives based upon weighting of the objectives and evaluation of the contribution of each alternative to these objectives. Using VBA, it is not necessary to have absolute measured values. Table 1 shows an example of a VBA were 2 solvents are compared (the SI contains an Excel® file that can be used for advanced analysis of given questions). We believe that the prime criterion for the solvent choice should be the solubility of the reagents used in the transformation. Usually, this results in a selection of solvents from which those, enabling the highest activity/stability of the biocatalyst, should be singled out. From these, the environmentally least demanding should be the solvent of choice for the reaction.

For the deeper knowledge-based weighting, however, a variety of

Table 1
Exemplarily Value-benefit-analysis of two different reaction media for an enzyme catalysed synthesis. Reaction medium options are water or an organic solvent. The rating columns represent the average rating of maximum five scores given by an expert team (working groups of the authors). Values are calculated by multiplying the rating with the weight of each criterion. The final score of each option is the sum of the criteria values and is highlighted in bold.

		Option 1 Water		Option 2 Organic solvent	
	Weight	Rating	Value	Rating	Value
Physicochemical aspects					
Toxicity/environmental impact	5%	5	0.25	2	0.1
Substrate solubilizing capacity	30%	1	0.3	5	1.5
Safety aspects/hazards (flammability, volatility)	5%	5	0.25	2	0.1
Reaction engineering aspects					
Enzyme activity (v_0)	10%	3	0.3	3	0.3
Enzyme stability ($t_{1/2}$)	15%	4	0.6	2	0.3
Productivity/yield/selectivity	25%	1	0.25	4	1
Other aspects					
Cost	10%	5	0.5	3	0.3
Sum	100%		**2.45**		**3.6**

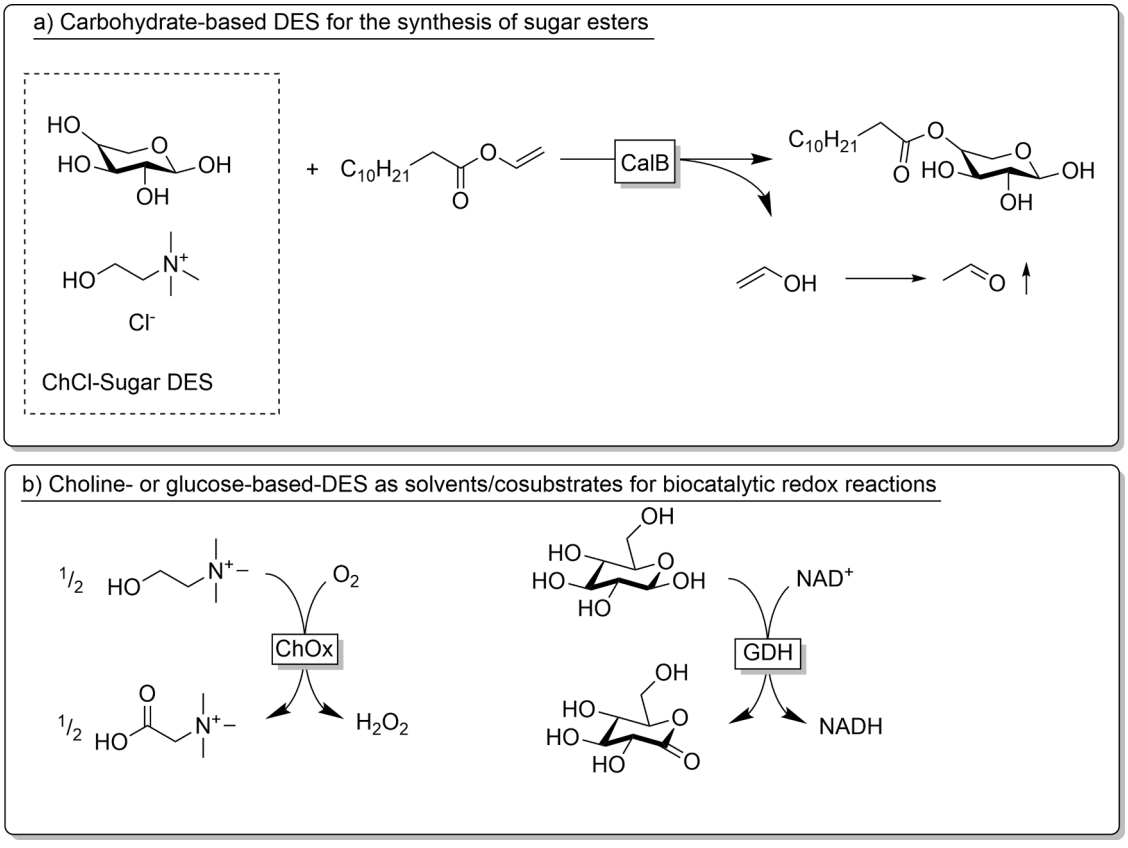

Fig. 3. Smart applications of DES utilising one component as more than just a solvent.

information is often needed. These are often not available at the beginning of an investigation. Here, models and simulations can be used to estimate the parameters. In the future, these computational tools should be increasingly used to select solvents in regard of the overall process. For example, questions about the interactions between the solvent and the biocatalyst, the needed energy input during the reaction and also the product downstream can be addressed. These models can also lead to a holistic understanding of the process, because the phrase "Everything is connected to everything" also applies for the selection of the 'best' solvent. May be an enzyme works in a solvent with high activity, stability and selectivity, but the product cannot be re-extracted from the solvent. Or impurities in the preparation of a solvent have no influence on the performance of the biocatalyst, but cause high costs in the final separation. These effects can only be identified in a holistic view of scientists and engineers on the complete process.

Overall, solvents play a critical role in biocatalysis. Water is not necessarily the solvent of choice aiming a practical and environmentally acceptable transformations. Non aqueous reactions are now moving into the focus of biocatalysis research but further intensification of the research efforts are needed.

CRediT authorship contribution statement

Dirk Holtmann: Writing – original draft. **Frank Hollmann:** Writing – original draft.

Declaration of Competing Interest

The authors declare no Conflict of Interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mcat.2021.112035.

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