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Unveiling the Hidden Performance of Whole Cells in the Asymmetric Bioreduction of Aryl-containing Ketones in Aqueous Deep Eutectic Solvents

Paola Vitale,[a,*] Vincenzo Mirco Abbinante,[a] Filippo Maria Perna,[a] Antonio Salomone,[b] Cosimo Cardellicchio,[c] and Vito Capriati[a,*]

[a] Dipartimento di Farmacia–Scienze del Farmaco, Università degli Studi di Bari "Aldo Moro", Consorzio C.I.N.M.P.I.S., Via E. Orabona, 4, I-70125 Bari, Italy
Fax: (+39) 080 5442539
phone: (+39) 080 5442174; e-mail: paola.vitale@uniba.it vito.capriati@uniba.it

[b] Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, Prov.le Lecce-Monteroni, I-73100 Lecce, Italy

[c] CNR-ICCOM, Dipartimento di Chimica, Via E. Orabona 4, I-70125 Bari, Italy

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Dedicated to Professor Herbert Mayr on the occasion of his retirement

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Abstract: In this contribution, we report the first successful baker's yeast reduction of arylpropanones using deep eutectic solvents (DESs) as biodegradable and non-hazardous co-solvents. The nature of DES [*e.g.* choline chloride/glycerol (2:1)] and the percentage of water in the mixture proved to be critical for both the reversal of selectivity and to achieve high enantioselectivity on going from pure water (up to 98:2 er in favour of the *S*-enantiomer) to DES/aqueous mixtures (up to 98:2 er in favour of the *R*-enantiomer). As a result, both enantiomers of valuable chiral alcohols of pharmaceutical interest were prepared from the same biocatalyst by simply switching the solvent. The possible inhibition of some (*S*)-oxidoreductases making part of the genome of such a wild-type whole cell biocatalyst when DESs are used as co-solvents may pave the way for an *anti*-Prelog reduction. The scope and limitations of this kind of biotransformations for a range of aryl-containing ketones are also discussed.

Keywords: Deep Eutectic Solvents; Green chemistry; Whole-cell catalysis; Ketones; Reduction; Alcohols

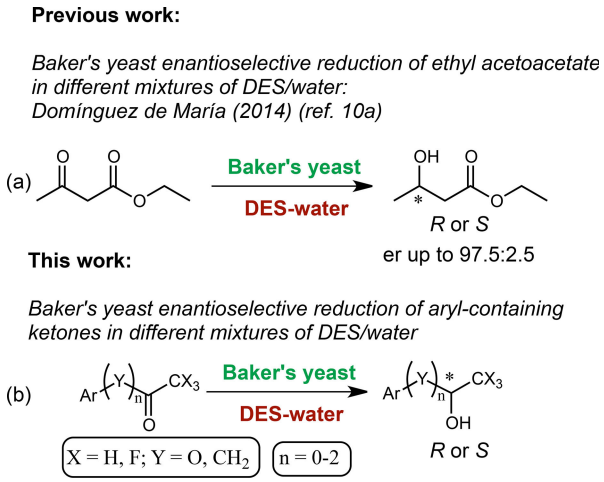
Asymmetric catalysis (AC) is a type of catalysis in which a chiral catalyst addresses the formation of a particular stereoisomer of a chiral compound. This is in particular central to the pharmaceutical industry considering that more than half of the drug molecules currently in use are chiral and contain at least one stereogenic center.[1] Despite the tremendous advances in AC, however, developments of environmentally friendly synthetic protocols especially in chiral drug product manufacturing represent a challenge, and toxic and hazardous volatile organic solvents are still the solvents of choice in medicinal chemistry. Enzyme-based biocatalysis represents a formidable tool for asymmetric green chemistry development. Thus, not surprisingly, it has received a great deal of attention for the industrial production of biologically active molecules.[2]

Wild-type whole-cell biocatalysts are often chosen as biocatalysts because they are cheaper than isolated and purified enzymes,[3] easy to handle, and come with a continuous source of enzymes and efficient internal cofactor regeneration systems (e.g. NAD(P)H). Their disadvantages are sometimes represented by lower yields and/or selectivities because of competing reactions catalyzed by other enzymes present in the whole cells (e.g. hydrolysis processes), the low solubility of organic reactants in water, low substrate loadings, and low volumetric and catalyst productivities. To overcome these drawbacks, many research groups have focused their attention on the discovery of new strain microorganisms (wild-type or recombinants), and on the metabolic and evolutionary engineering of known biocatalysts.[4] After the pioneering work by Zaks and Klibanov in 1984 describing enzymatic catalysis in conventional organic media,[5] biocatalysis in unconventional, nonaqueous reaction media sparked a surge

of interest among practitioners, particularly after the introduction of second-generation ionic liquids (ILs) derived from readily available, renewable natural products, and of so-called deep eutectic solvents (DESs).[6]

The latter are the result of the correct combination of two or three safe and inexpensive high-melting-point hydrogen bond donors (HBDs) and acceptors able to undergo self-association so as to achieve a significant depression of the freezing point. DESs are generally obtained by mixing and gently warming a quaternary ammonium halide salt [e.g. choline chloride (ChCl)] with metal salts or a HBD [e.g. urea, glycerol (Gly)], but a combination of a carbohydrate, a urea derivative, and an ammonium salt in varying ratios is also known. Thanks to their shallow ecological footprint, attractive low prices, non-flammability, biodegradability, ease of preparation with no further purification, low volatility, low toxicity, and recyclability, DESs nowadays represent a nascent class of sustainable solvents with ever-increasing applications in several fields spanning organic synthesis,[7a–i,o–q] metal polishing,[7e] extraction and separation processes,[7j,k] polymerization and material sciences,[7l,m] and biomass processing.[7n] Applications of DESs in organocatalysis,[8a–c] organometallic chemistry,[8d–i] metal-catalyzed reactions,[8j–o] solar technology,[8p] and biotransformations[8q,r] are still somewhat unexplored fields, but with encouraging and particularly interesting developments in the last few years. As per biocatalytic reactions, not only have DES-aqueous-media mixtures been shown to be excellent solvents for biotransformations catalyzed by a variety of enzymes including lipases, epoxide hydrolases, proteases, and peroxidases, but they have also significantly contributed to enhancing the activity and stability of these enzymes, and to facilitating high substrate loadings, which is crucial for industrial applications.[6,9] The use of DESs as effective reaction media for whole-cell biocatalysis is still in its infancy, and only a very few examples have been reported to date. These include: the enantioselective reduction of ethyl acetoacetate by whole cells of *Saccharomyces cerevisiae* (baker's yeast) in ChCl-based eutectic mixtures with variable amounts of water,[10a,b] the enantioselective reduction of a variety of aromatic ketones in a DES (ChCl/Gly):buffer 80:20 v/v catalyzed by whole cells of *Escherichia coli* overexpressing specific oxidoreductases as biocatalysts,[10c] the dehydrogenation of cortisone acetate in ChCl/urea-water mixtures catalyzed by immobilized whole cells of *Arthrobacter simplex*,[10d] the asymmetric reduction of 3-chloropropiophenone with immobilized *Acetobacter sp.* CCTCC M209061 cells in a ChCl/urea DES as the most suitable co-solvent,[10e] and the combination of a biphasic system consisting of a DES and a water immiscible IL for both the efficient synthesis of (*R*)-2-octanol by the biocatalytic reduction of 2-octanone with *Acetobacter pasteurianum* GIM1.158 cells[10f] and the asymmetric oxidation of 1-(4-methoxyphenyl) ethanol with *Acetobacter sp.* CCTCC M209061 cells.[10g,h]

Genomes of wild-type whole cells often comprise a complex mixture of enzymes with different, sometimes opposite, enantioselectivities. Whole cells of baker's yeast have also been traditionally used in preparative/industrial scale conditions for the enantioselective reduction of prochiral ketones to enantiomerically enriched chiral secondary alcohols, usually with *S*-stereo-preference (Prelog's rule), the latter being valuable synthons for the synthesis of chemicals, pharmaceuticals, and flavours.[11] We questioned whether the innate disadvantages of whole cells such as baker's yeast (*vide supra*) could be turned into an inherent advantage offering the possibility of synthesizing the opposite enantiomers of a chiral building block simply starting from the same whole-cell biocatalyst. Inspired by the recent report by Maugeri and Domínguez de María on the use of different DES-aqueous mixtures to control the enantioselective reduction of ethyl acetoacetate,[10a] (Scheme 1a) and building on our recent findings in using non-conventional yeasts in whole cells biocatalytic processes[12] and DESs for exploring novel paradigms,[7o,8b,d,f,i,o,p] herein we investigate baker's yeast bioreduction of a range of aryl-containing ketones using DESs as co-solvents (Scheme 1b). The scope and limitation of this methodology in terms of a successful tuning of stereo-preference are also discussed.

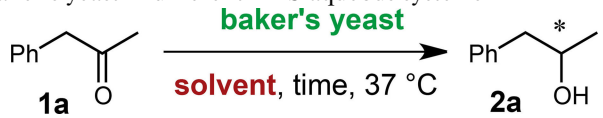


Scheme 1. Enantioselective reduction reactions catalyzed by baker's yeast in different DES–water mixtures.

As a bench reaction, we set out to investigate the enantioselective reduction of phenylacetone **1a** to 1-phenylpropan-2-ol **2a** in DES systems catalyzed by

one of the multiple alcohol dehydrogenases (ADHs) present in the cheap and commercially available baker's yeast (Table 1). Alcohol **2a** is a crucial synthon in the preparation of L-deprenyl, a neuro-protective drug currently used in the treatment of neurodegenerative disorders such as Alzheimer's and Parkinson's disease,[13] as well as amphetamine and its analogues, which are known to be potent central nervous system stimulants used in the treatment of attention deficit hyperactivity disorder, narcolepsy, and obesity.[14]

Table 1. Stereoselective reduction of ketone **1a** catalyzed by baker's yeast in different DES-aqueous systems[a]



Entry	Solvent	Time [d]	Yield (%)[b]	Abs. config.[c]	er[d]
1	water	1	88	*S*	98:2
2	DES A[e]	5	0	–	–
3	DES A + 40 w% water[e]	5	31	*S*	89:11
4	DES A + 20 w% water[e]	5	20	*R*	67:33
5	DES A + 10 w% water[e]	5	14	*R*	80:20
6	DES B + 50 w% water[f]	6	88	*S*	94:6
7	DES B + 40 w% water[f]	6	53	*R*	52:48
8	DES B + 20 w% water[f]	6	44	*R*	80:20
9	DES B + 10 w% water[f]	6	36	*R*	98:2
10	DES B[f]	6	0	–	–
11	DES C + 10 w% water [g]	6	5	*S*	81:19
12	DES D + 10 w% water [h]	6	–	–	–

[a] *Reaction conditions*: ketone **1a** (1.5 mM), baker's yeast (230 mg mL^{-1}) at 37 °C.
[b] Calculated by ^1H NMR of the crude reaction mixture; no other products were detected.
[c] Absolute configuration of the major enantiomer.
[d] Enantiomeric ratio (er) determined by HPLC.
[e] DES A: ChCl/D-fructose (3:2 w/w) (50 g).
[f] DES B: ChCl/Gly (1:2 mol/mol) (50 g).
[g] DES C: ChCl/D-glucose (2:1 mol/mol) (50 g).
[h] DES D: ChCl/urea (1:2 mol/mol) (50 g).

A preliminary reaction run in tap water was done for comparison. After incubating a mixture of ketone **1a**, baker's yeast, and water (see Supporting Information) at 37 °C for 24 h, and centrifuging the slurry to get the supernatant, extraction with EtOAc was followed by purification of the crude by column chromatography on silica gel, furnishing the expected alcohol **2a** in 88% yield and with an enantiomeric ratio (er) of 98:2 in favour of the *S*-enantiomer (Table 1, entry 1). Under these conditions, the stereoselectivity gained proved to be higher than that observed in the reduction of **1a** by *Kluyveromyces marxianus* CBS 6556 growing cells.[12a] The replacement of water by neat ChCl/D-fructose DES mixture (3:2 w/w) did not lead to any conversion of **1a** to **2a** for up to 5 days (Table 1, entry 2), whereas the employment of a ChCl/D-fructose eutectic mixture with 40 w% water provided **2a** with an er as high as 89:11, the yield being 31% after 5 days incubation at 37 °C (Table 1, entry 3).

These results are consistent with the fact that a certain amount of water is an absolute necessity for baker's yeast whole cells in order for them to display their catalytic activity which is, however, also preserved at long reaction times. On reducing the amount of water to 20w%, the yield dropped to 20% but, interestingly, an inversion of stereoselectivity was observed this time in favour of the *R*-enantiomer (er = 67:33) (Table 1, entry 4). Finally, alcohol (*R*)-**2a** could be recovered (14% yield) with an er of 80:20 using DES as a co-solvent with the addition of 10 w% water only (Table 1, entry 5). Since the nature of DES is known to affect the cells' viability and metabolism, whose variation, in turn, influences both the activity and selectivity of the enzymes involved, as well as the regeneration of the coenzyme present in the whole cells,[10b] we also focused on different DES systems in order to even further improve the *R*-enantioselectivity. Satisfyingly, a second set of experiments performed in the prototypical ChCl/Gly eutectic mixture (1:2 mol/mol) and in the presence of different amounts of water revealed a similar trend. As can be seen from the results reported in Table 1, after 6 days incubation at 37 °C, and by reducing the percentage of water from 50 w% to 10 w%, the enantioselectivity shifted from an er of 94:6 for the *S*-enantiomer of **2a** (88% yield) to an er of 98:2 in favour of (*R*)-**2a** (36% yield) (Table 1, entries 6–9). An almost racemic mixture was obtained with 40 w% water (Table 1, entry 7), whereas, again, no bioconversion occurred in the absence of water even after 6 days incubation at 37 °C (Table 1, entry 10). Two additional ChCl-based DESs were also investigated: ChCl/D-glucose (2:1 mol/mol) and ChCl/urea (1:2 mol/mol), each one with 10 w% water. In the former case, under the best experimental conditions previously set up, the conversion dropped drastically (up to 5%) and **2a** was recovered with an er of 81:19 but in favour of the *S*-enantiomer (Table 1, entry 11). Therefore, the inversion of enantioselectivity did not take place. On the other hand, upon switching to ChCl/urea with 10 w% water no enzymatic activity was noticed after 6 days incubation at 37 °C (Table 1, entry 12). Two aspects of these results are worth noting. The first is that both the opposite stereoselective bioreductions of phenylacetone (**1a**) can be

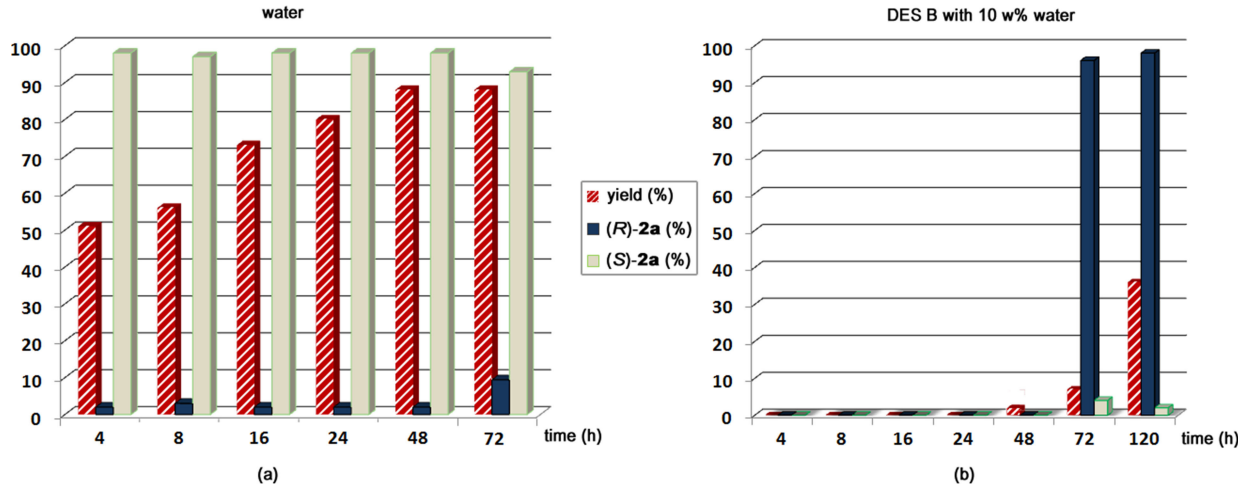


Figure 1. Time course of the biocatalytic reduction of ketone **1a** by baker's yeast at 37 °C in water (a) and in a ChCl-Gly-based mixture (DES B) with 10 w% water (b). (Each value reported represents the mean of three experiments. One experiment is shown as an example. Deviation of repetitions never exceeded 10%. The experimental error associated with ^{1}H NMR and HPLC measurements has been calculated to be within the range of 2–3% and 0.5–1%, respectively).

successfully carried out using the inexpensive and commercially available baker's yeast as a wild-type whole-cell biocatalyst. The second noteworthy aspect is that an important chiral building block such as 1-phenyl-2-propanol (**2a**) can be obtained with opposite and high enantioselectivity simply on switching from water to an appropriate DES-water-based mixture as a non-hazardous, unconventional medium.

To date, the chiral inversion of alcohol (*S*)-**1a** to (*R*)-**1a**, *en route* to both enantiomers of amphetamine, has been achieved by exploiting a two-sequence pathway including a nucleophilic substitution with a mixture of Et_3N/AcOH followed by hydrolysis of the corresponding ester, although with partial racemization.[15] ■■Where is Figure 1???■■

In general, in AC and when enantio-control is dominated by central chirality, a reversal in the stereoselectivity takes place by changing the configuration of the stereocenter(s) of the chiral catalyst.[16] Up to now, strategies for enhancing the enantioselectivity of biocatalytic systems have made use of medium-engineering tactics such as the modification of substrates[17a–c] and/or the use of organic additives (e.g. esters, chiral amines, organic solvents, ILs) mainly applied in the bioreduction of β-ketoesters, in the kinetic resolution and hydrolysis of esters, and in transesterification and asymmetric carboligation processes.[17d–j]

ChCl-based DESs used as solvents or co-solvents in aqueous solution have recently been shown to be effective in enhancing the activity and stability of *Candida rugosa* lipase,[9e] horseradish peroxidase,[9f] and *Penicillium expansum* lipase,[9g] the properties as a whole of the extensive H-bonding network typical of DES mixtures most probably being the key to understanding the beneficial effects of these neoteric solvents on enzyme activity. On the other hand, no certain interpretations have been made regarding the influence of DESs on the catalytic performance of whole cells, and a multitude of factors (e.g. protein denaturation, modification of cell membranes, enzyme inhibition, etc.) may be playing a role.[10f] As transpires from the data reported in Table 1, different results are obtained simply by changing the nature of DES components. Thus, while many isolated enzymes were shown to retain their activity in urea-based DESs,[6,9] the presence of such a strong hydrogen-bond donor in an eutectic mixture exerted detrimental effect on baker's yeast catalysis (Table 1, entry 12). To the best of our knowledge, the baker's yeast-promoted reversal of enantioselectivity in DES-aqueous mixtures has only been reported in the bioreduction of prochiral β-ketoesters.[10a,b] One of the possible explanations advanced for this phenomen involves the enantioselective inhibition of some ADHs with *S*-stereopreference present in baker's yeast, whereas ADHs with *R*-selectivity remain active.[10a]

From this perspective, the bioreduction of prochiral ketone **1a** was further investigated by evaluating the kinetics of yeast ADHs both in water and in a ChCl-Gly-based (DES B) mixture with 10 w% water at different reaction times. In pure water, the expected *S*-configured alcohol **2a** was isolated after only 4 h in 51% yield with an er value of 98:2, which suffers no erosion up to 48 h. After 72 h incubation in water, however, a slight erosion in the er of (*S*)-**2a** was detected: 91:8 (yield: 88%) (Figure 3a; see also Supporting Information). On the other hand, in the presence of the above DES-water mixture, it took up to 72 h to detect (*R*)-**2a** in the crude in 7% yield (^{1}H

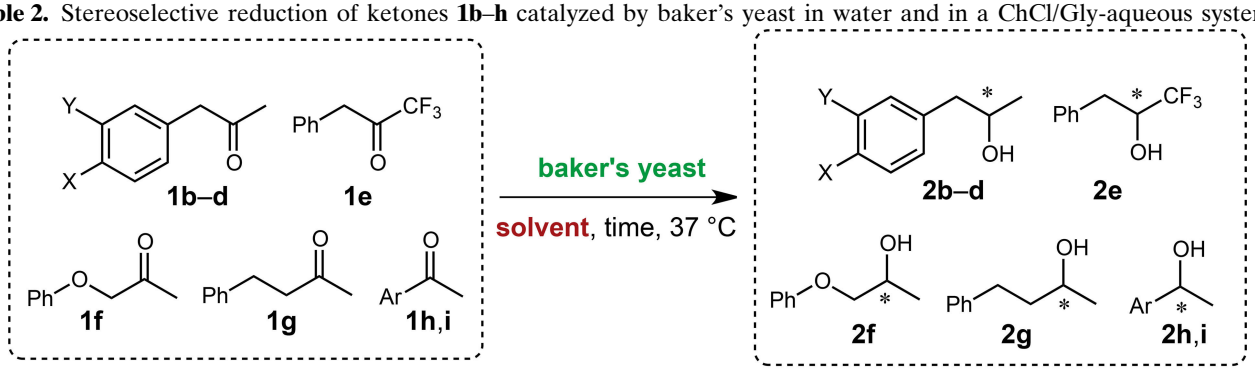
NMR analysis) but enantiomerically enriched up to 96:4 er. After 120 h, the baker's yeast-catalyzed bioreduction of **1a** in DES-water as a medium afforded (*R*)-**2a** in 36% yield and with an er value of 98:2. (Figure 3b; see also Supporting Information). These observations strongly suggest the following: (a) bioreduction catalyzed by *S*-ADHs (normally overexpressed in baker's yeast) takes place at a higher reaction rate in pure water and with no competition with the reaction catalyzed by ADHs with *R*-stereopreference up to 48 h; (b) ChCl-Gly eutectic mixture acts as an efficient inhibitor of *S*-ADHs, thereby paving the way for catalysis promoted by *pro R*-ADHs, which is, however, effective only at much longer reaction times. The low conversion rates observed may be related either to the low concentrations of *R*-ADHs in the whole cells of *Saccharomyces cerevisiae* or to their low affinity towards the substrate. Catalytic activity of *R*-oxidoreductases, however, competitively affected the final enantiomeric enrichment of (*S*)-**2a** also in pure water starting from 72 h. Similarly, the enantioselective reduction of ethyl acetoacetate to the corresponding ethyl (*R*)-3-hydroxybutanoate catalyzed by baker's yeast in mixtures of water with the ChCl/Gly DES, was observed only for long reaction times (>200 h).[10a] The role played by water in tuning the conversion and enantioselectivity is intriguing. Recent investigations established that the dilution of DESs in water results in a progressive rupture of the hydrogen-bond network between the starting material. The supermolecular structure of DESs, however, is still preserved up to 50% water dilution. Further dilution produces an aqueous solution containing the "free" forms of the solvated individual components.[18] In this respect, the switch in stereoselectivity sometimes observed in the biocatalytic reduction of prochiral ketones may be due to a subtle interplay of "solvation" of whole cells and the selective "inhibition" of some enzymes according to the percentage of water in the mixture, with the more diluted solution exhibiting physico-chemical properties quite close to that of pure water.

The biocatalytic *anti*-Prelog reduction of phenylacetone (**1a**) to (*R*)-1-phenyl-2-propanol (**2a**) successfully conducted in DES-water mixtures led us to examine the scope and limitations of this kind of transformation in order to prepare other optically active secondary alcohols. 4-Fluorophenylacetone (**1b**) provided, after 3 days incubation at 37 °C in water with baker's yeast, the corresponding chiral alcohol (*S*)-**2b** in 82% yield and with an er of up to 97:3 er (Table 2, entry 1). When this substrate was incubated in DES B with 20 w% water, an inversion of absolute configuration for the major enantiomer again took place, and alcohol (*R*)-**2b** was formed with an er of 91:9 in 14% yield (Table 2, entry 2). By reducing the amount of water to 10 w%, the er value increased to up to 95:5, the conversion being 10% (Table 2, entry 3). The 3-(trifluomethyl) phenylacetone (**1c**), which has a strong electron-withdrawing group on the phenyl ring, was reduced to (*S*)-**2c** by baker's yeast in water both in high yield (90%) and high er (99:1), whereas the enantiomer (*R*)-**2c** could be recovered from DES B with 10 w% with an er of 70:30 and in 15% yield (Table 2, entries 4,5). On the other hand, in the presence of a strong-electron-donating group on the phenyl ring (**1d**), reduction was seriously hampered, and the corresponding alcohol (**2d**) did not form in either the water or the eutectic mixture (Table 2, entries 6,7). The replacement of the terminal methyl group in **1a** by CF_3 gave an almost racemic mixture of alcohol **2e** in water (49:51) whose er, however, incresed to up to 62:38 (12% yield) again in favour of the *R*-enantiomer after 6 days incubation at 37 °C in DES B with 10 w% (Table 2, entries 8,9).

We next evaluated the effect on the enantioselectivity when the spacer length between the carbonyl moiety and the phenyl group was extended to two atoms. 1-Phenoxy-2-propanone (**1f**) successfully underwent reduction by baker's yeast in water delivering optically active (*S*)-1-phenoxy-2-propanol (**2f**) in 89% yield and 91:9 er after 24 h incubation at 37 °C (Table 2, entry 10). Moving on DES B with 10 w% water as the reaction medium, the enantioselectivity dropped to 88:12 (33% yield) but still in favour of the *S*-enantiomer, after 5 days incubation at 37 °C with a yield of 33% (Table 2, entry 11). Similarly, baker's yeast-catalyzed reduction of 4-phenyl-2-butanone (**1g**) provided optically active (*S*)-4-phenyl-2-butanol (**2g**) in 50% yield and 91:9 er after 24 h incubation in water, and in 38% yield and 62:38 er after 6 days incubation at 37 °C in DES B with 10 w% water (Table 2, entries 12,13). Hence, a switch in stereopreference was not observed in the case of ketones **1f** and **1g**. It is worth noting that the bioreduction of **1g** to **2g** was proven to proceed in 74% yield, but with complete racemization when performed by *Kluyveromyces marxianus* CBS 6556 yeast growing cells (96 h incubation in water at 30 °C).[12a] Therefore, the longer the spacer between the aromatic portion and the carbonyl group, the lower the *S*-selectivity in the reduction of prochiral ketones. Similarly, using recombinant short-chain RasADH from *Ralstonia* sp. DSM 6428 overexpressed in *Escherichia coli*, the stereoselective reduction of small-bulky ketones proved to be highly substrate-dependent with somewhat contrasting results in terms of stereoselectivity.[19]

As per aromatic ketones, the baker's yeast-mediated acetophenone (1 h) reduction was relatively low also in water providing the corresponding alcohol 2 h in only 31% yield after 6 days incubation at 37 °C and in 83:17 er according to the Prelog's rule (Table 2, entry 14). Conversely, on replacing the water with a ChCl/Gly–water mixture, the bioreduction of 1 h did

Table 2. Stereoselective reduction of ketones **1b–h** catalyzed by baker's yeast in water and in a ChCl/Gly-aqueous system[a]



b: X = F, Y = H; **c**: X = H, Y = CF_3; **d**: X = MeO, Y = H; **h**: Ar = Ph; **i**: Ar = 2-furanyl

Entry	Compound	Solvent	Time [d]	Product [yield %][b]	Abs. config.[c]	er[d]
1	**1b**	water	3	**2b** (82)	*S*	97:3
2	**1b**	DES B + 20 w% water[e]	5	**2b** (14)	*R*	91:9
3	**1b**	DES B + 10 w% water[e]	5	**2b** (10)	*R*	95:5
4	**1c**	water	1	**2c** (90)	*S*	99:1
5	**1c**	DES B + 10 w% water[e]	6	**2c** (15)	*R*	70:30
6	**1d**	water	1	**2d**[f]	–	–
7	**1d**	DES B + 10 w% water[e]	6	**2d**[g]	–	–
8	**1e**	water	1	**2e** (>95)	*S*	49:51
9	**1e**	DES B + 10 w% water[e]	6	**2e** (12)	*R*	62:38
10	**1f**	water	1	**2f** (89)	*S*	91:9
11	**1f**	DES B + 10 w% water[e]	5	**2f** (33)	*S*	88:12
12	**1g**	water	1	**2g** (50)	*S*	91:9
13	**1g**	DES B + 10 w% water[e]	6	**2g** (38)	*S*	62:38
14	**1h**	water	1	**2h** (31)	*S*	83:17
15	**1h**	DES B + 50 w% water[e]	7	**2h**[g]	–	–
16	**1i**	water	1	**2i**[g]	–	–
17	**1i**	DES B + 10 w% water[e]	6	**2i**[g]	–	–

[a] *Reaction conditions*: ketone **1b–i** (1.5 mM), baker's yeast (200 mg mL^{-1}) at 37 °C.
[b] Calculated by ^1H NMR of the crude reaction mixture; no other products were detected.
[c] Absolute configuration of the major enantiomer.
[d] Enantiomeric ratio (er) determined by HPLC.
[e] DES B: ChCl/Gly (1:2 mol/mol) (50 g).
[f] Not determined because of the trace content.
[g] No reaction.

not occur at all even in the presence of 50 w% water and after 7 days incubation at 37 °C (Table 2, entry 15). In general, the bioreduction of aromatic ketones is notoriously more difficult with conventional yeasts, and a higher stereoselectivity has only been observed when using recombinant whole cells overexpressing oxidoreductases as biocatalysts even in unconventional media.[10c,19] A lower enantioselectivity (74:26 er, *S*:*R*) was instead achieved with *Kluyveromyces marxianus* CBS 6556 growing cells.[12a] Finally, upon switching to an heteroaromatic ketone such as 1i, which has a 2-furanyl moiety, baker's yeast reduction to the corresponding secondary alcohol 2i took place neither in water not in the eutectic mixture (Table 2, entries 16,17).

In summary, we first reported that baker's yeast exhibits a fascinating switch in the rate of reaction and enantioselectivity in the reduction of arylpropanones by simply changing the solvent from water to DES-water mixtures, which is not a trivial matter. In particular, the *R*-selectivity was surprisingly high (er up to 98:2), even if at rather long reaction times (up to 6 days), when a ChCl/Gly (1:2) eutectic mixture was used and the reaction medium, in addition, contained up to 10 w% water. The obtained chiral secondary alcohols serve as precursors for valuable products; e.g., enantioenriched (*S*)- and (*R*)-1-phenylpropan-2-ol 2a, which are key synthons for the preparation of neuroprotective drugs. Our results are consistent with a selective inhibition of *S*-oxidoreductases in aqueous

ChCl/Gly eutectic mixtures, which paves the way for an *R*-ADH-mediated bioreduction catalyzed by enzymes present at low concentrations.

The final outcome in DES mixtures, however, has also proven to depend on the nature of DES components. Thus, the replacement of D-fructose by D-glucose did not similarly produce a change in stereochemistry, the resulting metabolic microenvironment, most probably, being as a whole still quite similar to that of water. Glucose-based DESs are known to act as solvents and substrates when isolated enzymes are used.[20] On the other hand, upon switching to ChCl/urea with 10 w% water, a complete knock out of whole-cell biocatalyst took place as no enzymatic activity was detected. Thus, the final stereoselectivity achieved may be the result of a subtle interplay of "solvation" of whole cells and the selective "inhibition" of some enzymes according to the nature of DES components and the percentage of water in the mixture.

Chiral enantioenriched *R*-configured alcohols can still be obtained in aqueous ChCl/Gly eutectic mixtures starting from arylpropanone derivatives functionalized with strong electron-withdrawing groups (*e.g.* CF_3) either in the aryl moiety or at the position next to the carbonyl group. On the other hand, by increasing the spacer length between the aryl and the carbonyl groups to two atoms a change in the stereopreference did not take place in eutectic mixtures, and aromatic and heteroaromatic ketones could not even be reduced. Up to now, the baker's yeast-catalyzed bioreduction with reversal of stereoselectivity in unconventional media has only been reported in the case of β-ketoesters.[10a,b] Thus, these results enlarge even further the horizons of whole-cell biocatalysis in these neoteric mixtures. Although under optimized conditions for the enantioselective *anti*-Prelog reduction of arylpropanones *R*-ADHs showed a narrow substrate pattern, physico-chemical properties of DES mixtures can be fine-tuned by the correct selection of specific partners within certain chemical classes. Thus, by using enzymes of *cheap* and *commercially available* whole cells in novel biodegradable and environmentally friendly DESs, not only may their notoriously hidden performance be revealed and exploited, but it is very promising and of great interest for developing a sustainable biocatalytic chemistry, thereby boosting its application in industry.[21] Further investigations into related processes for other substrates using custom-tailored DES mixtures are ongoing in our laboratories.

Experimental Section

General Procedure

Baker's yeast bioreduction in ChCl-Gly (1:2 mol/mol, DES B)–water mixtures: DES B was prepared by gently heating and stirring at 60 °C for 5 min the corresponding individual components (28.5 g of Gly and 21.5 g of ChCl kept in an Erlenmeyer flask) until a clear solution was obtained. Then water [5 mL (10% w/w)] was added to the DES kept at 37 °C, and the baker's yeast[22] (12.5 g) was dispersed to give a smooth paste in the mixture. Ketone (1 a–g) (100 mg) was added, and the mixture was stirred at 37 °C in an orbital shaker (250 rpm), monitoring the reaction's progress by 1H NMR. After a fixed time (Table 1), the reaction was stopped by the addition of EtOAc followed by centrifugation, decantation, and extraction with EtOAc. The extracts were dried over anhydrous Na2SO4, and the solvent evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane/EtOAc (90:10 or 60:40) as an eluent to yield the desired alcohol (**2a–g).** Spectral data are in agreement with those previously reported (see Supporting Information).

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Unveiling the Hidden Performance of Whole Cells in the Asymmetric Bioreduction of Aryl-containing Ketones in Aqueous Deep Eutectic Solvents

Adv. Synth. Catal. **2016**, *358*, 1–10

P. Vitale*, V. M. Abbinante, F. M. Perna, A. Salomone, C. Cardellicchio, V. Capriati*