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Baker's yeast catalyzed asymmetric reduction of prochiral ketones in different reaction mediums

Adi Wolfson*, Christina Dlugy and Dorith Tavor

Green Processes Center, Chemical Engineering Department, Sami Shamoon College of Engineering, Bialik/Basel Sts. Beer-Sheva, 84100 Israel

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Abstract: Baker's yeast catalyzes the asymmetric reduction of prochiral ketones in water and in various organic solvents. The reaction in water, which is the first solvent of choice for bio-reactions, led to a high product yield and enantiomeric excess, but the low miscibility of organic molecules in water resulted in lower conversions when more hydrophobic ketones were used. Petroleum-based solvents such as hexane and petroleum ether were also successfully employed as reaction mediums, but the viability of the yeast in these solvents was negligible, and they have severe environmental impacts due to their high toxicity levels. Performing the reaction in green solvents, like ionic liquids, fluorous media, and glycerol-based solvents, which have low volatilities and can be recycled, enabled dissolution of the substrates and of the energy source and also promoted isolation of the product. Among all tested green solvents, glycerol-based solvents are preferable due to their biodegradable natures and their origins from renewable sources.

Keywords: Asymmetric reduction; β-keto esters; green solvents; glycerol.

1. Introduction

Green or sustainable chemistry is a relatively new paradigm in chemical design and production. Its overarching goal is to ameliorate the environmental impact of chemical production processes by reducing the number and quantities of natural resources used and by exploiting those resources more efficiently, discharging less pollution, and producing chemicals that are less hazardous to human health and the environment.[1,2] The fundamentals of green chemistry are summarized in twelve principles[1] that consider the life-cycle of the reaction from the raw material used through process engineering to the desired and unwanted products.[3,4] The objective of this goal, in many cases, is the replacement of traditional organic syntheses by novel catalytic processes, as catalysis combines several transformations in one step and may enable the substitution of toxic reagents with less toxic ones.[5] Furthermore, the improvement of chemo- and stereoselectivity in catalytic processes lead to the reduced formation of by-products, thereby facilitating simpler, cleaner, and more effective separation processes.

Chiral compounds are important building blocks in the synthesis of fine chemicals for pharmaceuticals, agrochemicals, and food ingredients.[6-8] Though pure enantiomers can be separated from readily available natural compounds, i.e. the "chiral pool," the generation of asymmetry through

* Corresponding author:E-Mail: adiw@sce.ac.il

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asymmetric synthesis as a much more versatile and efficient process was extensively studied in the past 40 years.[9-22]

The *creation* of chirality via asymmetric synthesis requires the use of chiral homogeneous or heterogeneous bio- or chemo-catalysts. Biosynthesis presents an excellent example of natural, efficient, green, low energy and compatible multistep concerted synthesis. Besides their more environmentally benign production and disposal due to their natural origin, biocatalysts, which include enzymes and microorganisms, possess high activity and enantioselectivity, though they are usually compound specific. In addition, water, the greenest and cheapest solvent, is the solvent of choice for many bio-transformations. But the low miscibility of many organic compounds in aqueous solution motivated the research and development of non-aqueous bio-catalytic synthesis. Chiral modified metal catalysts were also introduced within the framework of appealing heterogeneous systems, yet only two well-characterized systems are known to achieve high enantiomeric excess: nickel catalyst modified with tartaric acid as a chirality inducer and sodium bromide as a co-modifier (Ni/TA/NaBr) for the enantioselective hydrogenation of β-keto esters and ketones[18-20], and cinchona alkaloid modified platinum catalysts in the asymmetric hydrogenation of activated ketones.[21,22]

Alternatively, chiral transition metal complexes (TMCs) were successfully employed as active and enantioselective homogeneous catalysts for asymmetric synthesis.[2-5] Despite their excellent catalytic performance, they are limited in their applicability due to their tedious synthesis processes, high prices, and the difficulties associated with their separation from the reaction medium and their reuse. Moreover, most chiral TMCs require large amounts of volatile organic solvents during their production process and to assist in catalytic cycle generation.

If a biocatalyst is used, employing the whole cell is preferable to using pure enzymes. The use of the former precludes the tedious process of separating the enzymes from the reaction mixture, and it also supports cofactor regeneration. Moreover, microorganisms usually tolerate harsher reaction conditions than do purified enzymes. Yet substrate and product diffusion through the cell membrane may decrease the reaction rate, and the existence of other enzymes can influence selectivity. Sheldon and coworkers compared the enantioselective reduction of methyl and ethyl acetoacetate (MAA and EAA, respectively), as representative β-keto esters, with baker's yeast (*Saccharomyces cerevisiae*), heterogeneous Ni/TA/NaBr, and homogenous chiral TMC of the type Ru-BINAP. They concluded that although yeast reduction is the much cheaper and more environmentally benign route, with no need for high hydrogen pressure, its low volume yield and productivity render it less attractive.[23]

Productivity in yeast reduction systems is lower still when bulkier β-keto esters are used as substrates due to the negligible solubility of β-keto esters in water. Thus, the enantioselective reduction of various prochiral ketones with baker's yeast was also studied in different organic solvents.[24-29] Nevertheless, although use of the organic reaction medium avoids most of the drawbacks associated with water, the organic solvents damage the cells and have severe environmental impacts. In addition, the glucose usually added to the reaction mixture as hydrogen source and electron donor in the regeneration of the cofactor is negligibly soluble in certain organic solvents.[27]

Finally, research investigating green solvents that can dissolve the organic substrate, allow the yeast cell or its enzymes to catalyze the reaction, and assist in the product separation and catalyst recycling procedures has also been reported in the literature.[30-33] In addition, immobilized baker's yeast (IBY) was also tested for its abilities to ease cell separation and to retain water, around the cells, essential to good yeast performance.[34] Yeast beads with alginate constitute the most frequently used immobilization technique due not only to its easy preparation method and low price, but also to the relatively high affinity of alginate for water and to its ability to form gel under mild conditions.[35,36]

In this review we will explore the scope and limitations of baker's yeast catalyzed asymmetric reductions of prochiral β-ketoesters and ketones in different reaction mediums (Fig. 1).

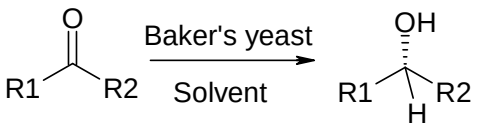

Figure 1. Baker's yeast catalyzed asymmetric reduction of prochiral ketones

1.1. Water

Pure, optically active β-hydroxy esters are used in the syntheses of enantiomerically pure pharmaceuticals, pesticides, and flavors and as monomers for biodegradable polymers.[37] The reduction of β-keto esters using baker's yeast was first described in 1918, and since then the reaction in water has been extensively studied. The reaction is typically run for 1-5 days under very moderate conditions, usually temperatures of 30-37 °C and atmospheric pressure, where mainly glucose or sucrose is used as the hydrogen source, yielding high enantiomeric excess (*ee* > 90%). Generally, the reaction involves the consumption of relatively large amounts of yeast and sucrose, and it yields enormous quantities of biomass that both require cumbersome downstream processing and hinder product separation.

Yeast cells dictate the formation of only one enantiomer at a time, and in either MAA or EAA reduction, the product is (*S*)-methyl/ethyl hydroxybuterate (*S*)-MHB/EHB. However, it was found that the growth conditions of baker's yeast affect its stereoselectivity.[38,39] Growing baker's yeast under oxygen-limited conditions, for example, resulted in shift of stereoselectivity towards the *R*-enantiomer formation.[40]

The heterogenization of yeast cells greatly facilitates their separation. The most frequently used heterogenization method is the encapsulation of yeast in calcium alginate[34, 41-44],but other immobilization matrices, such as polyurethane[41], carrageenan[43], and chrysotile fibers[45], have also been used. The occurrence of the reaction inside the beads, however, usually results in a lower reaction rate due to mass transfer limitations imposed on the substrate, the hydrogen source, and the product.[34] Nevertheless, it was found that by controlling the mean particle diameters of the beads, comparable specific reduction rates were achieved in the asymmetric reduction of EAA as in reduction with freely suspended cells.

1.2. Petroleum based organic solvents

Despite the ideal environmental profile of water, its use in organic reactions is limited due to the low dissolving ability of most organic substrates, a characteristic that reduces the reaction rate. Moreover, as the enantioselectivity in water could be reduced by the side effects of the different enzymes in the yeast cell, organic solvents can alter the enantioselectivity to an even greater degree when compared to the corresponding transformations in water.[45]

Haag et al. reported the first successful implementation of non-aqueous yeast reduction using a mixture of isopropyl hexadecanoate and soybean phospholipids as the reaction medium.[46] Later, Jayasinghe et al. reported the asymmetric reduction of EAA with free baker's yeast in organic solvents.[47] They used ordinary organic solvents, such as petroleum ether, diethyl ether, toluene, and carbon tetrachloride, in addition to a small amount of water (0.8 ml $(\text{g-yeast})^{-1}$) that hydrates the enzyme and protects it from the detrimental effects of the bulk organic solvent.[48]

North employed petrol as the reaction medium with the addition of water and studied the effect of the EAA/yeast/water ratio on product yield and enantioselectivity (Table 1[24]). He generally found that an increase in the amount of substrate increased reaction productivity while the enantioselectivity was high in all reactions (entries 1-4). Yet employing a high concentration of substrate resulted in lower activity and enantioselectivity (entry 5). In addition, increasing the water or yeast contents also increased reaction conversion, which was comparable to that obtained solely in water under similar conditions (entries 6-9). But decreasing the amount of petrol and increasing the concentration of the substrate while keeping the yeast/water/EAA ratio constant resulted in lower enantioselectivities (entries 7 and 10, respectively). Finally, the reaction was both successful and enantioselective when more bulky β-ketoesters were employed.

Table 1. Asymmetric reduction of ethyl acetoacetate in petrol[24]

Entry	Yeast/water/EAA (g/g/g)	Conversion[a] (%)	ee[a] (%)
1	7.5/6/1	45	>98
2	7.5/6/2	40	>98
3	7.5/6/3	40	>98
4	7.5/4/6	10	>98
5	7.5/5/6	33	36
6	15/24/2	100	>98
7	22.5/18/2	100	>98
8	22.5/15/2	50	>98
9	30/24/2	100	>98
10[b]	22.5/18/2	100	87

[a]Reaction conditions: 250 mL petrol, room temperature, 18 h.
[b]125 mL petrol.

Medson et al. also studied the enantioselective reduction of several β-keto esters in light petroleum, which resulted in high yields of 56-96% and very high enantioselectivities (94-99%) in favor of the *S*-enantiomer that was even higher than in pure water (Table 2[26]). As can be seen from the results listed in Table 2, increasing the size of the ester group required greater amounts of yeast to achieve high product yields, an outcome due possibly to increased steric interactions with the enzyme binding site or to lower mass transport. It was also found that yeast activity in the reaction system gradually decreased after exposure to the solvent system for 24 h. The use of time lapse ^{13}C NMR spectroscopy for examining the deactivation of the reductase enzymes showed that it began after about 12 h in the organic solvent system and that after 24 h little activity remained.[49] The deactivation was highly temperature dependent, such that at 30 °C enzymatic activity had ceased after about 8 h while at 10 °C no observable diminishment in enzymatic activity was apparent, even after 60 h. Performing the asymmetric reduction of EAA at 116 psi in liquefied petroleum gas (LPG) as a cheaper, less toxic, and recyclable organic solvent resulted in full conversion and an isolated yield of ethyl (*S*)-EHB of 74% with enantioselectivity of 95%.[28]

As the exposure to the organic solvent harmed the living cells but preserved the activity of the enzymes for a certain time, the tolerance of free and immobilized baker's yeast in organic solvents was studied.[34] It was found that the tolerance of immobilized baker's yeast varies in different solvents, and as the polarity of the organic solvent decreased, as expressed by an increase of its log P, the logarithm of the partition coefficient of a given solvent in the two-phase octanol/water system, the metabolic activity retentions of both free and immobilized yeast were increased. It was suggested that polar solvent led to dehydration of the cells that was detrimental both to their viability and to the activity of the enzymes, which require water to preserve their active conformation. In addition, besides their ease of separation, immobilized cells are more stable and thus more active in all organic solvents, in which the cells remain in an aqueous environment. Finally, both pre-incubation time and temperature affected the tolerance of immobilized baker's yeast, which reached a maximum at 30 °C.

Table 2. Asymmetric reduction of β-keto esters in light petrol[26]

Entry	Substrate	Yeast (g/mmol)	Isolated yield[a] (%)	ee[a] (*S*) (%)
1	Methyl acetoacetate	1	57	98
2	Ethyl acetoacetate	1	64	99
3	i-Propyl acetoacetate	2	96	97
4	n-Butyl acetoacetate	3	89	>99
5	*t*-Butyl acetoacetate	11	68	98
6	s-Butyl acetoacetate	4	89	97
7	Benzyl acetoacetate	5	72	94

[a]Reaction conditions: l mmol substrate, 0.8 ml water, 50 ml light petroleum, room temperature, 24h.

1.3. Green solvents

Although used daily in organic syntheses to combine reactants and catalysts and to assist in mass, heat, and momentum transfer, solvents are responsible for a large percentage of the waste and pollution generated by chemical processes. Therefore, the quest for a solvent with a minimal impact on the environment, e.g., a green solvent, is of the utmost importance.[50,51]

In the last three decades several green solvents with unique physical properties, and characterized by their recyclability and reusability, were introduced. The main solvent systems that have been studied are ionic liquids (ILs)[52,53], fluorous solvents[54,55], supercritical fluids[56,57], and glycerol[58,59] and its derivatives.[60] Their greenness is attributed mainly to their unique physical properties, such as low volatility and high stability, and to their recyclability and reusability.

Table 3. Asymmetric reduction of ketones in ionic liquid of the type [bmim]PF_6[30]

Entry	Substrate	Isolated yield[a] (%)	ee[a] (%)
1	2-Hexanone	40	79 (*S*)
2	Methyl acetoacetate	22	95 (*S*)
3	Ethyl acetoacetate	70	95 (*S*)
4	Ethyl 2-oxocyclopentanecarboxylate	75	84 (*S*,*S*)
5	Ethyl pyruvate	60	79 (*R*)

[a]Reaction conditions: l0 mmol substrate, 10 ml water, 100 ml [bmim]PF_6, 33 °C, 72 h.

Some of the aforementioned green solvents were also tested in the baker's yeast asymmetric reduction of β-keto esters and ketones.[30-34,60] Howarth and co-authors employed a mixture of IL of the type 1-butyl-3-methylimidazolium hexafluorophosphate [bmim]PF_6 and water (volumetric ratio of 10:1) in the bio-reduction of several prochiral ketones with immobilized baker's yeast (Table 3[30]). The reactions resulted in moderate alcohol yields and enantioselectivity when compared with the same reduction in water or simple organic solvents. It was suggested that since the co-enzyme, NADPH, cannot be recycled in non aqueous solutions, the extent of the reaction was limited by its initial

concentration within the yeast.[30,47] Product separation was successfully done at the end of the reaction by extraction with diethyl ether after filtration of the yeast beads, but it was also found that since [bmim]PF_6 has negligible volatility and a high boiling point, products distillation under high vacuum is also possible.

The asymmetric reduction of several prochiral ketones was also performed in perfluorooctane as the representative fluorous media using immobilized yeast (Table 4[31]). The authors tested glucose and methanol separately as the energy sources[61] and found that although enantioselectivities were comparable with either, the reactions with methanol proceeded more slowly than those with glucose. Although most of the reactions reached full conversion, isolating the product by extraction with methanol, which is immiscible in perfluoros solvents, after the yeast beads were filtrated, resulted in very low yields. Yet the fluorous phase was easily recovered after product separation by extraction with methanol for a yield of 90-94% and was successfully reused in the asymmetric reduction of EAA, yielding the same conversion and enantioselectivity (Table 4, entry 2).

Table 4. Asymmetric reduction of ketones in fluorous medium[31]

Entry	Substrate	Energy source	Time (h)	Conv.[a] (%)	Isolated yield (%)[a]	ee (%)
1	Ethyl acetoacetate	Glucose	41	100	25	95
2[b]	Ethyl acetoacetate	Glucose	41	100	26	95
3[b]	Ethyl 2-oxocyclopentanecarboxylate	Glucose	29	100	28	99 (*S*,*S*)
4[b]	Benzyl acetoacetate	Glucose	21	100	66	94 (*S*)
5[b]	Ethyl acetoacetate	Methanol	212	93	35	87
6[b]	Ethyl 2-oxocyclopentanecarboxylate	Methanol	118	100	19	99 (*S*,*S*)
7[b]	Benzyl acetoacetate	Methanol	168	84	54	93

[a]Reaction conditions: 0.25 g substrate, 15 g of immobilized baker's yeast prepared from 2.5 g of dry baker's yeast, 50 ml perfluorooctane, 1.0 g glucose/0.25 g methanol, 30 °C
[b] Solvent was re-used after distillation

We recently reported that glycerol, which is a polar, non toxic, biodegradable, and recyclable liquid manufactured from renewable sources, was used as an alternative green organic solvent for several organic reactions.[58,59] However, as glycerol has relatively high polarity and viscosity, other glycerol derivatives, which are also non toxic, biodegradable, and recyclable, can also be employed as green reaction mediums.[60] The asymmetric reductions of EAA and 2-heptanone as the representative β-keto ester and ketone, respectively, with immobilized baker's yeast were thus tested in glycerol, glycerol triacetate (triacetin), or glycerol tributyrate and compared to the reaction in water under similar conditions (Table 5[60]). The results in Table 5 illustrate that the asymmetric reduction of EAA in the three glycerol-based solvents yielded comparable conversions, which were lower than the conversion in water, and also high enantioselectivity as in water. A low conversion in the organic solvents can be attributed to the lower viability of yeast cells in these solvents and to the lack of cofactor regeneration. On the other hand, the reduction of 2-heptanone, which has negligible solubility in either water or glycerol, was tremendously increased in glycerol triacetate, where solubility of 2-heptanone is high.

Table 5. Asymmetric reduction of ethyl acetoacetate and 2-heptanone in representative glycerol-based solvents[60]

Entry	Solvent	EAA[a]		2-Heptanone[b]	
		Conv. (%)	ee (%)	Conv. (%)	ee (%)
1	Water	74	>99	20	>99
2	Glycerol	45	>99	8.8	97
3	Glycerol triacetate	52	97	30	97
4	Glycerol tributyrate	50	98	-	-

[a]Reaction conditions: 35 mL solvent, 16 g IBY, 5 mmol ethyl acetoacetate, 3.5 g glucose, 37 °C, 48 h.
[b]5 mmol 2-heptanone, 72 h.

Testing free cell viability showed that cell growth was significantly affected by exposure to glycerol, where a correspondingly dramatic decrease in viability (about 100%) was observed after several minutes while the more hydrophobic glycerol derivatives preserved cell viability somewhat: up to 48 h in triacetin and up to 4 h in glycerol tributyrate (Fig. 2). The negligible viability in glycerol after short times can be explained by the high osmotic pressure that was imposed on the cell by the solvents, which caused water to diffuse out of the cells, a process that may dry the cells, thereby affecting their viability.[62] However, although yeast viability in glycerol was very low, the reduction progressed with time after one day, implying that enzymatic activity was preserved (Fig. 3[33]).

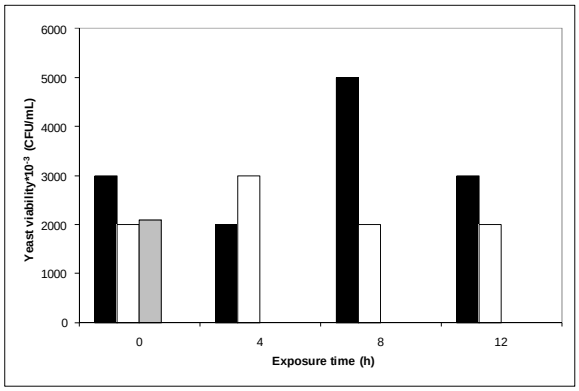

Figure 2. Comparison of the viability of free baker's yeast in glycerol-based solvents and water: water – black; glycerol triacetate – white; glycerol tributyrate – gray.[60]

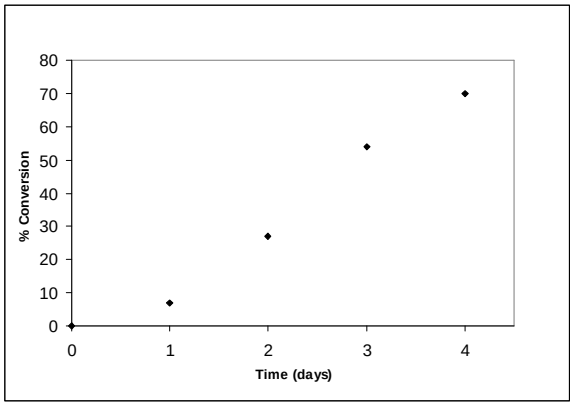

Figure 3. Conversion progress in baker's yeast catalyzed asymmetric reduction of MAA. Reaction conditions: 50 mL glycerol, 10 g free baker's yeast, 1 g MAA, 5 g sucrose, 37 °C.[33]

Although it is clear that water is the natural and most suitable solvent for biocatalysis from the perspectives of viability and activity, glycerol is preferable in terms of substrate solubility and product separation procedure. Therefore, to exploit the specific advantages of the two solvents, the asymmetric reduction of MAA was also tested with free baker's yeast in glycerol-water mixtures (Fig. 4[33]). As expected, the results show that increasing the glycerol content up to 50 wt% in water did not change the conversion. Yet a further increase in the amount of glycerol gradually decreased the conversion. Nevertheless, product enantioselectivity reached 99% in all the reactions and was not affected by the addition of glycerol.

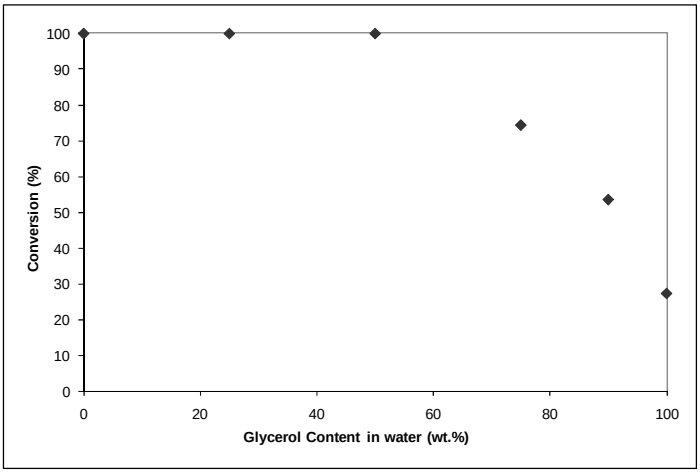

Figure 4. MAA conversions in baker's yeast catalyzed asymmetric reduction in water and glycerol mixtures. Reaction conditions: 50 mL solvent, 10 g free baker's yeast, 1 g MAA, 5 g sucrose, 37 °C, 48 h.[33]

Furthermore, glycerol-based solvents also allowed easy product separation by extraction.[32,33,60] Both the solvent type used in the extraction and the extraction procedure affected product extraction yields, as illustrated in Table 6, of (*S*)-MHB from glycerol and water by representative glycerol and water immiscible solvents.[60] Slightly higher extraction yields from glycerol were detected than those in water for all tested solvents, and in contrast to that from water, extraction from glycerol did not lead to the formation of an emulsion with the extracting solvents, and hence, the separation was simpler.

Table 6. MHB recovery from glycerol[33]

Entry	Extracting solvent	Dielectric constant	Steps	Extraction yield (%)[a]	
				water	glycerol
1	n-Hexane	1.89	2	-	23
2	Diethyl ether	4.34	2	32	37
3	Dichloromethane	9.08	1	21	24
4	Dichloromethane	9.08	2	30	35
5	Dichloromethane	9.08	3	65	70

[a] RT, 40 mL of solvent in each extraction step, 50 mL glycerol, 1 g (*S*)-MHB

Finally, triacetin was also employed as solvent and acyl donor in one-pot synthesis of cinnamyl acetate from cinnamaldehyde, yielding full conversion and 91% selectivity for cinnamyl acetate after 96 h at room temperature (Fig. 5[63]). The reaction comprises of two sequential steps, reduction of cinnamaldehyde to cinnamyl alcohol using immobilized baker's yeast as catalyst in the first step and

free or immobilized acid as catalysts in the transesterification of cinnamyl alcohol to cinnamyl acetate in the second step.

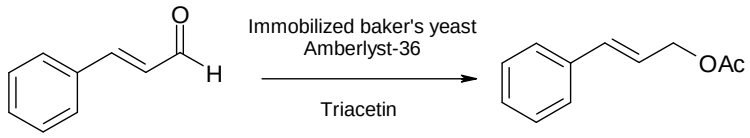

Figure 5. One-pot synthesis of cinnamyl acetate from cinnamaldehyde.[63]

2. Conclusions and perspectives

Asymmetric bio reduction of prochiral ketones has definite advantages as it requires neither high hydrogen pressure nor the utilization of expensive chemocatalysts. Using the whole cell instead of purified enzymes also has economic, environmental, and operational benefits. However, although water is the first solvent of choice for enzymatic reactions, the low miscibility of organic substrates in water may limit its application as a reaction medium. Although organic petroleum-based solvents can be successfully employed as reaction mediums for biocatalytic synthesis, performing the reactions in green organic solvents, which enables substrate dissolution and easy product separation and has a low environmental impact, is a viable alternative.

The enantioselective reduction of various prochiral ketones with baker's yeast was studied over the years in water and in different organic solvents. Though employing an organic reaction medium suppresses most of the drawbacks associated with using water and preserves the activity and enantioselectivity of yeast, the main disadvantages of using such solvents are their toxicities, which damage the yeast cells and have severe environmental impacts. Using immobilized yeast cells keeps the yeast cells surrounded by water, an essential feature for their performance, and also improves separation of the yeast from the reaction mixture.

Performing the reaction in a green organic solvent that dissolves the substrate and the energy source well but that also allows the product to be easily isolated is preferred, yet the life cycle of the solvent and its preparation, purification, and disposal procedures should also be considered. It seems that glycerol-based solvents that are prepared from a renewable source and are also biodegradable are the most attractive solvents for asymmetric reduction with baker’s yeast, as they allow high catalytic performance and simple product separation.

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