ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Green techniques for reduction of α, β- unsaturated carbonyl compounds

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ABSTRACT

The present work describes the asymmetric reduction of α-bromo cinnamaldehyde and benzylidene acetone catalyzed by baker's yeast in free as well as immobilized form. Electrochemical Reduction of the above compounds was also employed in the present work using Stainless Steel (SS-316) under galvanostatic conditions at pH = 9.0. Prior to electrochemical reduction cyclic voltammograms of α-bromo cinnamaldehyde and benzylidene acetone were recorded at different pH (5.0, 7.0, & 9.0) to establish the optimum conditions of the reduction. In both of the above reduction methods applied, selective reduction of C=O moiety was observed. Optically active products, were obtained in good yields (75-90 %) with high enantiomeric excess (ee > 81 %) were then isolated, purified and characterized by combined application of chromatographic and spectroscopic techniques.

Keywords: Baker's yeast (Saccharomyces cerevisae) (Free, Immobilized), asymmetric reduction, α-bromo cinnamaldehyde, benzylidene acetone, spectroscopic techniques.

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ISSN: 0975-8585

INTRODUCTION

Biotransformation:

Stereoselective reduction of carbonyl moieties is a very useful tool for the introduction of stereogenic centres into chiral synthons, which are necessary for the preparation of natural products and pharmaceuticals.The enantioselective reduction of cinnamaldehyde derivatives is an attractive strategy to prepare various optically active multifunctional molecules that can be used as chiral building blocks for the synthesis of some HIV- protease inhibitors. Chemical methodologies such as metal hydride reduction, catalytic hydrogenation or hydrogen-transfer reactions, as well as biocatalytic approaches using either isolated enzymes or whole-cell systems have been optimized for transformations of this kind. The prerequisites for the ideal reagent or catalyst should be the following: broad substrate acceptance, predictability of access to enantiomers and /or diastereomers, minimal environmental burden, and possibly at the lowest cost. The whole cell catalyst such as baker's yeast (Saccharomyces cerevisiae) is very interesting candidate as choice of appropriate reducing agents having all the above mentioned qualities and this has recently prompted a renewal of interest in the application of baker's yeast.

Biotransformations by Baker Yeast in aqueous media are currently receiving significant attention as synthetic tools for accomplishing efficient organic manipulations [1]. The chemical industry is turning more and more to enzymatic and fermentation processes in order to obtain enantiomerically pure amino acids, amino alcohols, amines, alcohols and epoxides as intermediates for pharmaceutical industry and agro chemistry where both a high degree of purity and large quantities of compounds are required. Use of biocatalysts in chemical synthesis exploits important properties of enzymes, namely their regio- and stereo-specificity and their capacity to catalyze reactions under mild conditions. Exploitation of regiospecificity has applications in the synthesis or modification of complex chemical structures with multiple recative groups that may not be distinguished in a chemical reaction.Stereospecificity of enzymes is especially important in pharmaceutical production of single enantiomeric forms[2-4].

The asymmetric reduction of carbonyl compounds to their corresponding chiral alcohols was extensively studied with both free and immobilized Baker's yeast cells (FBY and IBY correspondingly). It was found that employing both FBY and IBY resulted in high enantioselectivity (ee > 81 %) and reasonable activity for the carbonyl compound. In this paper we report on the electrochemical as well as baker's yeast mediated asymmetric reduction of α-bromo cinnamaldehyde as representative of cinnamaldehyde derivative and benzylidene acetone in aqueous media as well as in 10% v/v DMSO [5-8].

Electrochemical reduction

Aldehydes and ketones have been found to undergo electrochemical reduction easily to the corresponding alcohols (9-10). The course of reduction of aldehydes and ketones is strongly dependent upon the pH of the medium. Cyclic voltammograms of α-bromo cinnamaldehyde and

benzylidene acetone were recorded at different pH (5.0, 7.0, & 9.0) to establish the optimum conditions of the reduction. The electrochemical reduction of α-bromo cinnamaldehyde and benzylidene acetone was thereafter carried out galvanostatically at pH = 9.0 using Stainless Steel (SS-316) as a working electrode.

The reduction products thus obtained were isolated and purified by chromatographic techniques and characterized on the basis of spectral analysis.

EXPERIMENTAL [9,10]

Reduction using free Baker's Yeast

The biotransformation of α-bromo cinnamaldehyde and benzylidene acetone was carried out as follows:

In a one litre round bottom flask, equipped with magnetic stirrer (Remi-2MLH make), water(200 ml), fresh baker's yeast (10 g) and isopropanol (25 ml) were placed and the suspension was stirred for 30 minutes for activation of BY. The reactant (1 mM) was separately dissolved into alcohol and was poured into baker's yeast suspension. The resulting solution was magnetically stirred for suitable period (Table 1). The suspension changed its color during the course of the reaction.After completion of the reaction,the product was filtered using celite (HIMEDIA grade), the filterate was saturated with sodium chloride and extracted with diethyl ether in three steps (3 x 40ml). Ether extracts were combined and dried over sodium sulphate.After evaporation,the product thus obtained was then purified and characterized using different spectral techniques like IR; NMR etc.

Reduction using Immobilized Baker's Yeast

The experiment was performed under similar conditions with Immobilized Baker's Yeast insitu immobilization of 10g Baker's Yeast in polyacrylamide gel. The details of immobilization are given below

Immobilization of BY in polyacrylamide gel

The gel was prepared using the following solutions.

Solution A: - Acrylamide (10 g) and N, N' – methylene bisacrylamide (2.5 g) in DDW (100 ml),
Solution B: - Tris (5.98 g) and TEMED (0.46 g) and 1N HCl (48 ml) solution to 100 ml,
Solution C: - APS (560 mg) in DDW (100 ml),
Solution D: - Isopropanol (25 ml),

Where- **TRIS** = Trihydroxy Methyl Amino Methane, **TEMED**= N, N, N', N"-tetramethyl Ethylenediamine, **APS**=Ammonium Persulphate, **DDW**= doubly distilled water.

Then solutions were mixed in following way-

Sol.A(10 ml) +Sol.B(5 ml) + BY(5 g) + Sol.C (5 ml). And the solution D was added and deaerated for 30 min.

Procedure of electrochemical study employed in present work

All chemical used viz. sodium acetate, sodium hydroxide, á-bromo cinnamaldehyde and benzylidene acetone etc. were of AR grade. The solutions were prepared in triply distilled water. Cyclic voltammograms were recorded at the scan rate of 100 to 500 mV/sec. using a computer based ECDA-001 instrument, supplied by Con-serv Enterprises, Mumbai (India). The voltammographic curves of á-bromo cinnamaldehyde and benzylidene acetone 0.1M in acetonitrile(1ml), Sodium perclorate 0.4M (2.5 ml) as supporting electrolyte and BR buffer (5ml) (pH= 5.0, 7.0 and 9.0) were recorded using a three electrode cell assembly with 1mm diameter glassy carbon as working electrode, Ag/AgCl as reference electrode and Pt wire as counter electrode (shown in fig.1,2).

Preparative electro-organic synthesis, utilizing the optimum conditions derived from cyclic voltammetric studies was then carried out at pH = 9.0. The conventional H-type cell with two limbs separated by G-4 disc was used for electrolysis. The supporting electrolyte sodium acetate (250 ml, 2M) was filled equally in both the limbs. á, â- unsaturated carbonyl compound (0.1M) was dissolved in the DMSO and placed in the cathodic chamber. The Stainless Steel (SS 316) electrodes having an area 2 õ 3 cm2 were used as cathode as well as anode. The constant current (1 amp.) was passed through the electrolyte for 8 hours with the help of Galvanostat (Prepared by Centre for Development of Physics Education (CDPE) University of Rajasthan Jaipur) The worked up involved extracting the catholyte three times with diethyl ether (40ml each).

Characterization of the product

The purity of the product was checked by Thin Layer Chromatography (TLC) and then characterization of products was done on the basis of IR, NMR (H1).NMR spectra were recorded in $CDCl_3$ solution on Joel (Japan) 300 MHz Spectrophotometer and IR spectra by using Nicolet (USA)FTIR Spectrophotometer. Optical activity of products was measured by using a JASCO P-2000 polarimeter and enantiomeric excess (ee) was calculated. The results of microbial and electrochemical reduction are shown in Table – 1.

ISSN: 0975-8585

Table 1: Current potential measurements by Cyclic Voltammetry for α - bromocinnamaldehyde

Condition applied:
Initial potential E_i: 500 mV
Switching potential E_S: -1800 mV
Auxiliary Electrode: Platinum

Electrodes applied:
Working Electrode: Glassy carbon
Reference Electrode: Ag/AgCl

S. No.	Medium pH	Scan rate (mV/s)	Cathodic Wave				Effect of scan rate	Remark (Cathodic wave)
			I PEAK		II PEAK			
			E_{pc} (mV)	I_{pc} (μA)	E_{pc} (mV)	I_{pc} (μA)		
1.	5.0	100	-865	435	-1525	619	With increasing scan rates peak potential shifts towards negative side of potential	Irreversible
2.	5.0	200	-877	676	-1527	763		Irreversible
3.	5.0	300	-886	896	No peak			Irreversible
4.	5.0	400	-899	1027	No peak			Irreversible
5.	5.0	500	-908	1283	No peak			Irreversible
6.	7.0	100	-873	577	No peak		With increasing scan rates peak potential shifts towards negative side of potential	Irreversible
7.	7.0	200	-900	726	No peak			Irreversible
8.	7.0	300	-946	901	No peak			Irreversible
9.	7.0	400	-951	1073	No peak			Irreversible
10.	7.0	500	-965	1175	No peak			Irreversible
11.	9.0	100	-987	431	No peak		Peak potential shifts towards negative side of potential with increasing scan rates	Irreversible
12.	9.0	200	-1022	607	No peak			Irreversible
13.	9.0	300	-1033	748	No peak			Irreversible
14.	9.0	400	-1047	928	No peak			Irreversible
15.	9.0	500	-1049	1062	No peak			Irreversible

□=scan rate (mV /sec.) Ep=peak potential (mV) Ip=peak current □A

RESULTS AND DISCUSSION

Reduction using Baker's Yeast in free and immobilized form

Baker's Yeast (BY) has been widely used as a biocatalyst in free as well as in immobilized form (IMBY) for organic synthesis, primarily because it is inexpensive and readily available. When whole microbial cells are used for the asymmetric reduction of carbonyl compounds, two enzyme systems are mainly involved in the production reaction. One is the enzyme catalyzing the asymmetric reduction of prochiral carbonyl compounds to chiral alcohols, i.e. carbonyl reductases. The other is a cofactor regeneration system, which supplies NADPH through the oxidation of the energy source, such as carbohydrates and alcohols.

Mechanism of Reduction

The actual reducing agent in this system is NADPH (Nicotinamide Adenine Dinucleotide Phosphate Hydride) which donates hydride ion (H^-) to aldehydes and ketones and thereby reduces them.The electron lone pair on nitrogen atom of NADPH pushes out the hydride ion, which is subsequently added to a carbonyl group of another molecule to cause its reduction. The process is completed by addition of proton to the carbonyl oxygen. The scheme of the reduction is depicted in the Fig.1.

ISSN: 0975-8585

Role of Isopropanol

The reducing agent, NADPH (Nicotinamide Adenine Dinucleotide Phosphate Hydride) is present in limited amount. After reducing the substrate it is itself oxidized to $NADP^+$ **.**Therefore, to continue reduction process it is necessary to reduce $NADP^+$ (Nicotinamide Adenine Dinucleotide Phosphate ion) into NADPH. Yeast contains some saccharides in the cell, which reduce $NADP^+$ to NADPH via pentose- phosphate pathway. To activate this pathway isopropanol is added to the reaction mixture, which is oxidized to acetone and regenerates NADPH from $NADP^+$ **.** Thus it results in an increased concentration of NADPH which ultimately ensures an increase in the enantiomeric excess of the product.

Immobilization

While immobilization of yeast cells reduces catalytic activity because of newly created diffusion limitations, immobilization provide several advantages including increased operational stability of the cells, easier isolation of products and reuse of the catalyst. Moreover, product formation rates as well as enantiomeric excess are also comparatively higher.

Electrochemical Reduction

Most cyclic voltammograms were recorded with an initial potential Ei value of 500 mV and final (switching) potential value Es of – 1800 mV at the scan rate of 100, 200, 300, 400 and 500 mV/sec.Cyclic Voltammetric studies led to the conclusion of irreversibility of the reduction process which can be best carried out in basic media due to the following reasons:-

1. In acidic media & neutral media, there were less defined peaks in comparison to basic media. An overall 2e- and 4H+ reduction process is observed. The peaks are sharp and high indicating the ease of reduction of substrates at 9 pH.
2. Stainless steel (SS-316) electrode can be easily used as cathode more conveniently in basic media as it corrodes in acidic media.
3. On the basis of the cost of product and the reactants this electrochemical transformation is economically viable and has potential of scaling up the process for which further studies are in progress.

Effect of scan rate and pH on peak potential: -

With increase of scan rate and pH, peak potential shifts towards negative side of potential (Table1 and Fig.1, 2, 3, 4).

ISSN: 0975-8585

Table 2: Current potential measurements by Cyclic Voltammetry for Benzylidene acetone

Condition applied:
Initial potential Ei: 500 mV
Switching potential ES: - 1800 mV

Electrodes applied:
WorkingElectrode:Glassycarbon
ReferenceElectrode:Ag/AgCl
Auxillary Electrode: Platinum

S. No.	Medium PH	Scan rate (mV/s)	Cathodic Wave				Effect of scan rate	Remark (Cathodic wave)
			I PEAK		II PEAK			
			E_{pc} (mV)	I_{pc} (μA)	E_{pc} (mV)	I_{pc} (μA)		
1.	5.0	100	-651	212	-950	345	With increasing scan rates peak potential shifts towards negative side of potential	Irreversible
2.	5.0	200	-713	269	-960	479		Irreversible
3.	5.0	300	-714	367	-964	650		Irreversible
4.	5.0	500	-735	388	-993	713		Irreversible
5.	7.0	100	-712	173	-984	374	With increasing scan rates peak potential shifts towards negative side of potential	Irreversible
6.	7.0	200	-742	289	-1036	496		Irreversible
7.	7.0	300	-770	313	-1056	605		Irreversible
8.	7.0	400	-783	392	-1063	758		Irreversible
9.	7.0	500	-786	495	-1082	955		Irreversible
10.	9.0	100	No peak		-1075	450	Peak potential shifts towards negative side of potential with increasing scan rates	Irreversible
11.	9.0	200	-736	219	-1089	474		Irreversible
12.	9.0	300	-763	288	-1083	615		Irreversible
13.	9.0	500	-777	322	-1112	704		Irreversible

□=scan rate (mV /sec.) Ep=peak potential (mV) Ip=peak current □A

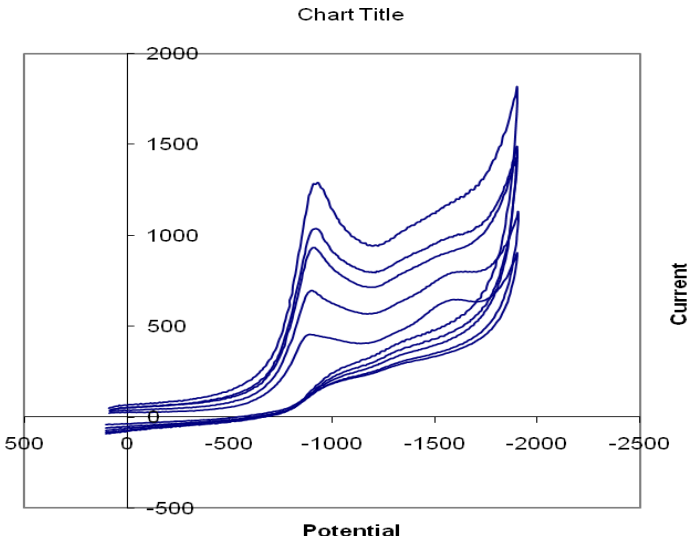
Table – 3 Spectroscopic data for microbial & electrochemical reduction of compounds

S. No	Substrate Name	Reaction medium	Reaction time (in hrs.)	IR Data (cm-1)	NMR Data (δ value)	ee (%) Free BY	ee (%) ImB Y	(% yield)	Expected Product
1.	α - bromo cinnamaldehyde	Alcohol : water (microbial)	72	3417,2924,2359,1495,1454,1076,1041,748,700	7.36-7.20, 4.35, 3.83, 3.27, 3.18, 2.01	87	89	77(FreeBY) 82 (ImBY)	α-bromo cinnamyl alcohol
		10%v/v DMSO (microbial)	72	3417,2924,2359,1495,1454,1076,1041,748,700	7.36-7.20, 4.35, 3.83, 3.27, 3.18, 2.01	87	89	79(FreeBY) 83 (ImBY)	

ISSN: 0975-8585

		AcONa + DMSO (electrochemical)	8	3417,2924,2359,1495,1454,1076,1041,748,700	7.36-7.20, 4.35, 3.83, 3.27, 3.18, 2.01	-	-	88	
2.	Benzylidene acetone	Alcohol: water (microbial)	72	3410, 2930, 1485,1460, 1050, 860	7.51-7.34, 6.23, 6.54, 4.51, 1.35	80	83	78(FreeBY) 81 (ImBY)	4-phenyl-3-buten-2-ol
		10%v/v DMSO (microbial)	72	3410, 2930, 1485,1460, 1050, 860	7.51-7.34, 6.23, 6.54, 4.51, 1.35	80	83	82(FreeBY) 85 (ImBY)	
		AcONa + DMSO (electrochemical)	8	3410, 2930, 1485,1460, 1050, 860	7.51-7.34, 6.23, 6.54, 4.51, 1.35	-	-	89	

Fig 1: Cyclic voltammogramms of α-bromocinnamaldehyde at pH 5.0, 7.0 & 9.0 (At variable scan rates from 100 to 500 mV/sec)



ISSN: 0975-8585

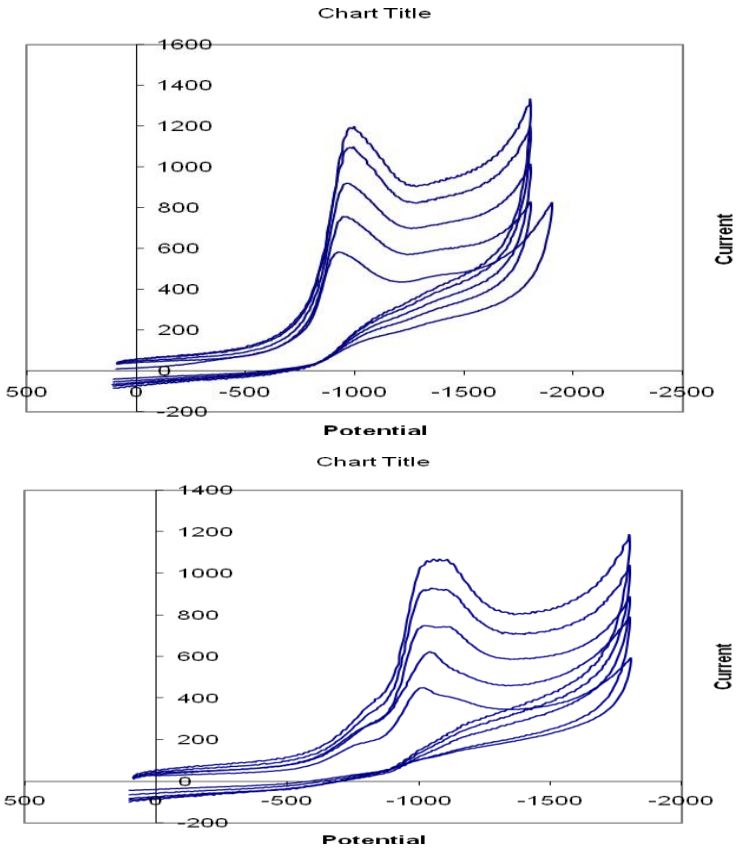
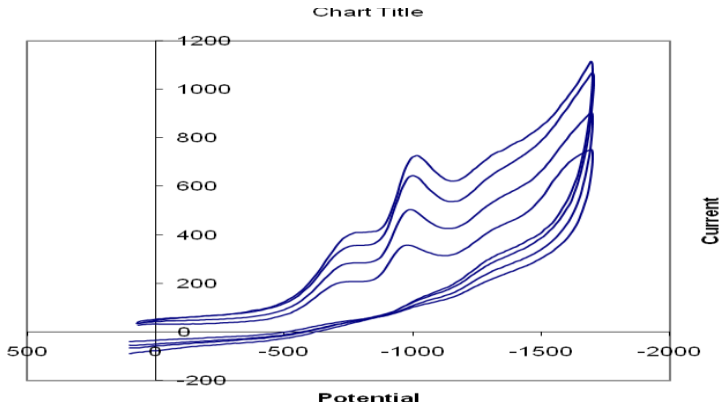


Fig.2. Cyclic voltammogramms of Benzylidene acetone at pH 5.0, 7.0 & 9.0 (At variable scan rates from 100 to 500 mV/sec)



ISSN: 0975-8585

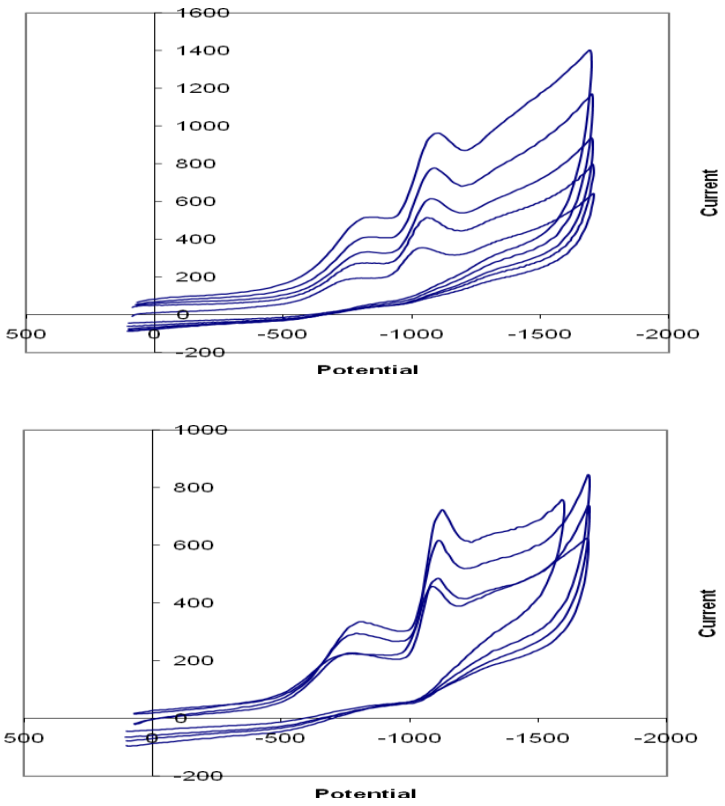
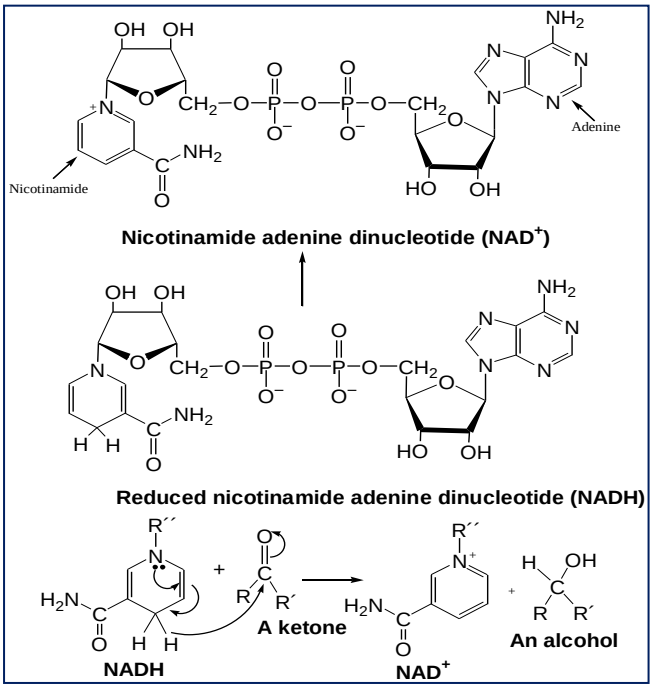


Fig.3 Mechanism of microbial reduction



ISSN: 0975-8585

Use of organic media

Biotransformation processes involving free or immobilized Baker's yeast were carried out in aqueous as well as in non- conventional organic media, provided the enzyme remains surrounded by a monolayer of water. Advantages of biocatalytic reactions carried out in non-conventional organic media are as follows:

1. Enhanced solubility of poor water soluble substrates.
2. Suppression of undesirable side reactions.
3. Decrease of substrate and product inhibition.
4. Enhanced stability of the biocatalyst.
5. Higher product yield, ease of product and catalyst recovery.
6. Shift of thermodynamic equilibria.
7. Manipulation of enantioselectivity in organic solvents.

CONCLUSION

As compared with classical methods which generally involve use of either corrosive reagent or yield product which are burden to the ecosystem, the use of Baker's yeast offers alternative pathway to carry out reduction quite a simple essentially green experimental setup at room temperature with an easy work-up of products and good yields.

ACKNOWLEDGEMENT

Authors thank the Head, Department of Chemistry, University of Rajasthan, Jaipur (India) for providing necessary facilities.

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