Asian J. Exp. Sci., Vol. 19, No. 2, 2005, 135-141

Rapid Publication

New Eco-friendly Synthetic Procedures for the Reduction of Carbonyl Compounds

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Abstract : The bioreduction of some carbonyl compounds *viz*., Acetophenone, Benzalacetophenone, *o*-Aminoacetophenone, Benzil, Cinnamaldehyde, Crotonaldehyde and Salicylaldehyde was carried out with free Baker's yeast (*Saccharomyces cerevisiae*) as well as Immobilized Baker's yeast. The results of the bioreduction of these compounds have been compared. Merits of bioreduction have also been discussed.

Key words : Baker's Yeast (BY), Immobilized Baker's Yeast (ImBY), Bioreduction, Carbonyl Compounds.

Introduction :

In recent years the influence of chemical industry on the environment has been in focus. Now the term "**GREEN CHEMISTRY**" is used for the technology that reduces or eliminates the use or generation of the hazardous substances in the design, manufacture and application of chemical products.

Use of biocatalytic processes (Stewart, 1998; Rodrigues and Moran 2004) can certainly contribute in this respect. The development of biocatalytic methodologies undoubtedly involves a strong need for extend the applications of microbial catalysts and enzyme catalyzed reactions.

Biocatalytic processes often require less expensive equipments and, therefore, the biotransformation is the pioneer for the ecofriendly synthetic processes. They are, therefore, intended to be complimentary to the traditional chemical processes or to eliminate them wherever feasible.

Several important examples of applications of biocatalysts in the chemical processes are available such as :

A. Microbial transformation of acrylonitrile into acrylamide in industrial scale (> 30,000 tons/years).

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B. Enzymatic synthesis of bioactive carbohydrates, glycoconjugates, glycolipids.

C. Enzymatic synthesis of non-protein α- and β-amino acids.

D. Enzyme catalyzed synthesis of peptides, glycosides, and phosphates, β-lactames, etc. under reverse hydrolysis conditions.

It may be attractive to immobilize the biocatalysts. This may be performed under different conditions for organic synthesis; immobilization is often performed by covalently binding the biocatalysts to a solid inert polymer support.

Biotransformations with immobilized Baker's yeast (ImBY) are attractive due to several reasons although the catalytic activity of the cells is generally reduced as compared to that in the same amount of cells in solution. This loss of activity is caused by an additional permeability barrier introduced by the carrier material and due to some cell damage occurring during the immobilization (Simon *et al.*, 1985).

Four major categories for immobilization of microorganisms in general and of the Baker's yeast (FBY) in particular can be recognized in analogy to the immobilization of enzymes (Sundaram and Pye, 1974).

(i) *Immobilization by physical or chemical adsorption:* surface adsorption to a water-insoluble, solid support e.g., a metal oxide, DEAE-cellulose, or an ion-exchange resin.

(ii) *Cell aggregation of the microorganism*: Physical or chemical (e.g. glutaraldehyde) cross-linking.

(iii) *Covalent attachment to a carrier material:* e.g., CMC (carboxyl methylcellulose).

(iv) Micro-organism entrapment in a gel or a membrane or within microcapsules: Applicable for laboratory as well as industrial use (urethane, cellulose, agar, alginate (Kierstan and Buke, 1977). Collagen, chitosan, k-carrageenan (Sato *et al.*, 1979), polyacrylamide and montmorillonite-K10 (Sorrilha *et al.*, 1992), have been used as polymers porous networks for entrapment)

Materials and Methods :

Material Used :

The chemical Cinnemaldehyde, Crotonaldehyde, Benzal Acetophenone, *O*-Aminoactophenone, Benzil, Acetophenone, Salicyaldehyde and all chemicals required were of AR grade. The solvents and before in use water were doubly distilled. All the reagents and products were stored in corning glasswares.

Experimental :

In a one-liter round-bottom flask, equipped with a magnetic stirrer, 200 ml water, and 50 gm fresh Baker's yeast, and 4 gm glucose were placed and the suspension was stirred for 30 minutes. The carbonyl compound (2 mM) was separately dissolved in ethanol (50 ml) and its ethanolic solution poured in to Baker's yeast suspension. The resulting mixture was made-up with water to one liter and magnetically stirred for a suitable period. The suspension changes its colour from orange to yellow.

After the reaction was over, the product was separated from mixture by filtering the solution. The filtrate was extracted with methylene chloride, the methylene chloride extract was dried over sodium sulphate and on evaporating it, and the product is obtained. The product was then characterized by combined application of techniques *viz*., TLC, Mass, NMR and IR.

Immobilization of Baker's yeast by carrageenan :

Baker's yeast cells (8 gm) were suspended in 8 ml of 0.9% NaCl at 40^{o}C. Carrageenan (2.07 gm) was dissolved in 45 ml 0.9% NaCl at 80^{o}C, and then the temperature of the solution was brought to 45^{o}C. Both solutions were then mixed, and mixture was cooled at 10^{o}C for 30 minutes. In order to increase the gel strength, the gel was soaked in cold 0.3 m KCl. After this treatment, the resultant stiff gel was cut in the smaller cubic gels of 3 × 3 × 3 cm. Immobilized gel was used as such for reduction by the procedure similar to the one used in case of FBY. The carbonyl compounds

viz., Acetophenone, Benzal acetophenone, o-Amino acetophenone, Benzil, Cinnamaldehyde, Crotonaldehyde and Salicylaldehyde were reduced by methods given below.

(i) By using free Baker's yeast (FBY) in water.

(ii) By using immobilized Baker's yeast (ImBY) in water.

Results of the above reductions are summarized in Table 1 and Table 2, respectively.

Table 1 : Reactions of Baker's yeast in water

S. No.	Substrate Name	Reaction Time (In Hours)	FBY Yield (%)	Mass Spectra (m/z)	IR Data (cm^{-1})	NMR Data (δ- Value) —CH-OH
1.	Salicylaldehyde	48	77.07	113,95,81	3320, 1030,1530	3.1
2.	Benzil	48	76.07	190,65,171	3330, 1050,1540	3.5
3.	*O*-Amino Acetophenone	24	73.58	126,80,107	3330, 1030,1530	3.3
4.	Cinnemaldehyde	48	78.01	134,116,65	3310, 1610,1030	2.7
5.	Benzal Acetophenone	48	76.01	187,169,95	3300, 1620,1040	3.7
6.	Acetophenone	24	79.67	122,103,65	3310, 1010,1570	3.1
7.	Crotonaldehyde	24	78.10	73,55,58	3330, 1610,1030	2.3

Table 2 : Reactions of Immobilized Baker's yeast in water

S No.	Substrate Name	Reaction Time (In Hours)	ImBY Yield (%)	Mass Spectra (m/z)	IR Data (cm^{-1})	NMR Data (δ-value) | —CH-OH |
1.	Salicylaldehyde	48	84.71	113,95,81	3320, 1030, 1530	3.1
2.	Benzil	48	82.67	190,65,171	3330, 1050, 1540	3.5
3.	*O*-Amino Acetophenone	24	77.85	126,80,107	3330, 1030, 1530	3.3
4.	Cinnamaldehyde	48	83.67	134,116,65	3310, 1610, 1030	2.7
5.	Benzal Acetophenone	48	85.77	187,169,95	3300, 1620, 1040	3.7
6.	Acetophenone	24	81.13	122,103,65	3310, 1010, 1570	3.1
7.	Crotonaldehyde	24	82.17	73,55,58	3330, 1610, 1030	2.3

Results and Discussion :

The actual reducing agent in present system is NADPH (Nicotinamide Adenine Dinucleotide Phosphate Hydrate) and its amount in yeast cell is limited to a quite low level. In order to allow the reduction

continuously, it is therefore necessary to activate another biological pathway 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. The addition of glucose to the reaction mixture activates the pentose-phosphate pathway and therefore, ensures high concentration of NADPH, which ultimately results in an increase in the enantiomeric excess(es) of the product.

Immobilization enhances the operational stability of FBY and isolation of the products becomes easier. Under these conditions, the product formation rates are usually high (Burg *et al.*, 1988). It also permits easy continuous operation since the immobilized cells can be easily removed from the reaction medium and can be reused repeatedly although with decreasing activity of the immobilized cells. In contrast to enzyme immobilization, a required coenzyme is supplied and regenerated with in the intact cell (Leuenberger, 1984). Comparative studies between use of "free" yeast and immobilized yeast cells have been made only occasionally. Some differences in stereo selectivity and yield are, however, expected to be observed depending on the kind of immobilization and this seems reasonable also, since immobilized yeast cells, exhibit altered physiological, morphological and metabolic properties (Margeritas and Merchant, 1984).

Acknowledgement :

Authors thanks to the Head, Department of Chemistry, University of Rajasthan, Jaipur (India) for providing necessary facilities and one of the authors, Dr. I.K. Sharma, thanks U.G.C. for providing minor research project.

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