

## Eco-Friendly Procedure for the Reduction of o-Hydroxy Acetophenone



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**Abstract :** The microbial catalyst reduction of o-amino acetophenone was carried out with free Beaker's yeast (*Saccharomyces Cerevisiae*) as well as immobilized Beaker's yeast in alcoholic medium. The reduction product was isolated and purified by basis of its spectral analysis.

**Key words :** Baker's yeast (BY) Immobilized Baker's yeast (ImBY) microbial catalysed reduction, carbonyl compounds.

### Introduction :

The general chemical procedures of the synthesis of alcohol by the reduction of carbonyl Compounds are generally not eco-friendly. In recent years the influence of chemical industry has been in focus. The term Green Chemistry is used for the technology that reduces or eliminates the use of the hazardous substances in the design manufacture and application of chemical products.

The aim of the present work is to explore a novel eco-friendly method of organic synthesis using Baker's yeast (BY) in free as well as immobilized form i.e. immobilized Baker's Yeast (ImBY). The microbial catalytic methods involving enzyme microorganism as catalysts are well suited for this purpose. Oxidoreductases catalyse reduction/oxidation by removal or addition of hydrogen in specific manner. The Baker's Yeast (*Saccharomyces Cerevisiae*) is a common microorganism that can be used for this purpose i.e. to bring about oxidation/reduction of substrates utilizing the enzymes

Oxidoreductases present in it. Since it is more easily available than the purified enzymes which have additional disadvantage that they need expensive co-factors like  $\text{NAD}^+$ ,  $\text{NADH}$ , etc.

### Material and Methods :

All the chemicals used in present investigation viz. o-hydroxy acetophenone, Baker's Yeast, isopropyl alcohol, absolute alcohol, methylene chloride for extract, were of AR grade. The solvents and water before in use were doubly distilled. All the reagents and products after proper sealing were stored in covering glass wares in refrigerator where ever necessary

### Experimental Protocol :

In a one liter round-bottom flask, equipped with magnetic stirrer (Remi Make) 200 ml water, 5 gm fresh Baker's Yeast and 25 ml of isopropyl alcohol were placed and the suspension was stirred for 30 minutes. The o- hydroxy acetophenone (2 mM) was separately dissolved in to ethanol (2 ml) and ethanolic solution was poured into Baker's

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Yeast Suspension. The resulting mixture was filled in with water made up to one liter and magnetically stirred for a suitable period.

The experiment was performed under similar conditions with Immobilized Baker's Yeast, obtained by immobilization of 2 gm Baker's Yeast in polyacrylamide gel.

After the completion of the reaction over, the product was separated from the mixture by filtering the solution. The filtrate was extracted with methylene chloride and the methylene chloride extract was dried over sodium sulphate and on evaporating it, the product was obtained. The product was then purified by chromatography and characterized by spectral analysis.

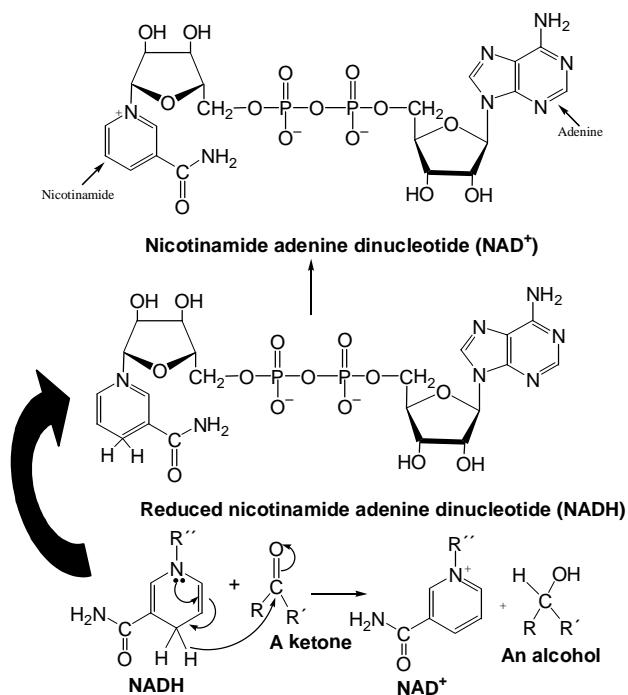
### Immobilization of Baker's Yeast by Polyacrylamide Gel :

The microorganisms and isolated enzymes can be immobilized using various

carrier materials such as polyurethane (Lerens *et al.*, 2004), cellulose, agar, alginate (Kierstan and Buke, 1977). Collagen, chitosan (Zhang *et al.*, 2002), k-carragenan (Sato *et al.*, 1979) and Kieselguhr (Stadler, 2002) as porous networks for entrapment. In present work Baker's Yeast was immobilized using polyacrylamide gel by the methods reported by us earlier (Yadav *et al.*, 2005; Nainawat, *et al.* 2006).

The immobilized Baker's Yeast in polyacrylamide gel was prepared using following solutions.

- 10.0 ml of solution E
- 5.0 ml of solution F
- 5.0 ml of solution G
- 25.0 ml of solution H



**Solution E :** 10 gm Acrylamide and 2.5 gm N,N'-Methylene bis acrylamide in 100 ml double distilled water.

**Solution F:** 5.98 gm Tris\*, 0.46 ml TEMED\*\* and 48 ml 1N HCl to 100 ml solution.

**Solution G:** 560 mg APS (Ammonium per sulphate) in 100 ml DDW).

**Solution H:** Isopropyl alcohol

After preparation of above solutions add in such manner.

E + F (B. Yeast 2 gm) + G

\*Tris = Trihydroxy methyl amino methane.

\*TEMED = N, N, N', N'' - tetra methyl ethylenediamine

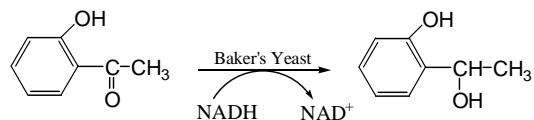
For 5% gel the above solution mix and add solution H deaerate for half an hour.

### 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  $\text{NADP}^+$  (Nicotinamide Adenine Dinucleotide Phosphate ion) into NADPH. Yeast contains some saccharides in the cell, which reduce  $\text{NADP}^+$  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 (ee) of the product. However in the present case

the regeneration of NADPH from  $\text{NADP}^+$  was achieved by using isopropyl alcohol which itself is oxidised to acetone in the process.

The bioreduction of o-hydroxy acetophenone can be depicted by following reaction scheme.



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. Its only limitation is that the cofactors NADPH/ $\text{NADP}^+$  have to be regenerated. This problem we have partially overcome by regenerating NADPH from  $\text{NADP}^+$  by externally added isopropyl alcohol. This helps in making the process continuous without any requirement of glucose which was otherwise essential.

Immobilization enhances the operational stability of FBY and isolation of the products becomes easier. Under these conditions, the product formation rates are usually high. 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 present in the cells and is regenerated continuously.

**Table 1: Spectroscopic results of o-Hydroxy Acetophenone)**

Substrate Name	Reaction Time (In hours)	BY Yield (%)	ImBY Yield (%)	Mass Spectra (m/z)	IR Data (cm <sup>-1</sup> )	NMR Data ( $\delta$ - Value)
<i>o</i> -Hydroxy Acetophenone	48	70.45	83.22	138, 137, 93, 45	3431, 3405, 1619,1602 71	6.80-7.22(m, 4H) 4.81-4.75 (bs, 2H) 4.03 (q, 1H) 1.49 (d, 3H)

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