

ECO-FRIENDLY BIOTRANSFORMATION OF BENZOPHENONE USING FREE AND IMMOBILIZED BAKER'S YEAST

Dr. Mohanlal Meena

Associate Professor Govt. R R College Alwar

Department of Chemistry, University of Rajasthan, JAIPUR-302004 (Raj.) INDIA

ABSTRACT

Benzophenone was reduced biocatalytically in an alcoholic medium utilising the free and immobilised forms of the microbial catalyst Baker's yeast (*Saccharomyces cerevisiae*). Using chromatographic methods, such as HPLC, the reduction product was extracted, purified, and then identified using spectrum analysis.

Key words: Biotransformation, Benzophenone, Baker's yeast (BY),

INTRODUCTION

Since the dawn of civilization, yeast-mediated microbial transformations have been extensively exploited to produce bread, dairy products, and alcoholic drinks. All of these early uses of microbes were mixed cultures, and biotechnology activities have mostly focused on agriculture and human nutrition. The first class of enzymes, such as oxidoreduction, remove or add hydrogen in a specific way to carry out the biotransformations [1-2]. Since pure reductases have the drawback of requiring pricey co-factors like NADH, NADPH, etc., the Baker's yeast (*Saccharomyces cerevisiae*) is a common microbe that may be employed for this purpose cheaply. There are a number of reasons why the production of alcohols using immobilised Baker's yeast (ImBY) cells is appealing. Despite the fact that the cells' overall catalytic activity is lower than it would be with the same number of cells in solution. Permeability barrier is the root reason of this decline in activity.

EXPERIMENTAL

Material and methods

All three substances—benzophenone, isopropyl alcohol, and ethanol—were of AR grade. Water that had previously been used as a solvent was double distilled. All of the items and reagents were kept in

Corning glass containers. 200 mL of water, 5 g of fresh Baker's yeast, and 25 mL of isopropyl alcohol were added to a one-litre round-bottom flask with a magnetic stirrer (Remi Make), and the suspension was swirled for 30 minutes. Separately, 2 mM of picolinaldehyde were dissolved in 2 mL of ethanol, and the ethanolic solution was then added to the suspension of Baker's yeast. The resultant mixture was magnetically swirled for a reasonable amount of time before being topped off with water to a volume of one litre. The suspension turns yellow instead of orange. The experiment was conducted using immobilised Baker's yeast under comparable circumstances, 2 g of Baker's yeast were immobilised in polyacrylamide gel to produce. After the reaction was finished, the solution was filtered to separate the product from the mixture. The product was prepared by extracting the filtrate with methylene chloride, drying the methylene chloride extract on sodium sulphate, and then evaporating the methylene chloride extract. After that, the product underwent HPLC purification and spectrum analysis characterization.

Immobilization of Baker's yeast by polyacrylamide gel

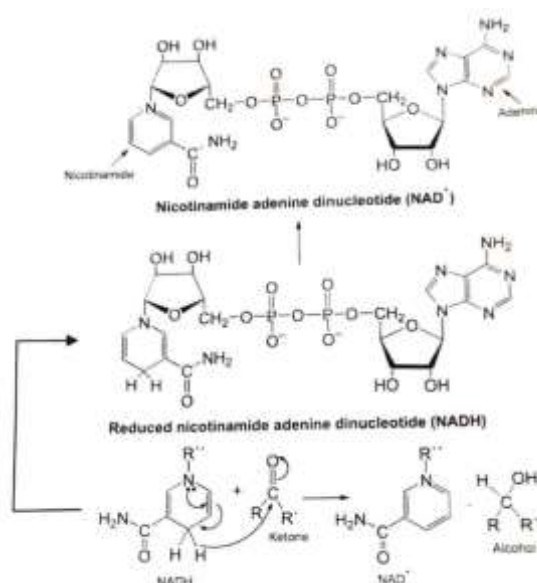
A variety of carrier materials, including urethane, cellulose, agar, alginate (a), collagen, chitosan, k-carrageenan (b), and montmorillonite (c) - K 10, can be used to immobilise the microbe and separated enzymes. In the current study, polyacrylamide gel was used to immobilise Baker's yeast. [3-4] The following solutions were utilised to prepare the Baker's yeast immobilised in polyacrylamide gel..

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 g Acrylamide and 2.5 g N, N' –methylene bisacrylamide in 100 mL double distilled water.

Solution F : 5.98 g Tris*, 0.46 mL TEMED** and 48 mL IN HCl to 100 mL solution.

Solution G : 560 mg APS (Ammonium persulphate) in 100 mL DDW).

Solution H : Isopropyl alcohol

After preparation of above solutions these are added in this manner –

E + F (B. yeast 2 g) + G

*Tris = Trihydroxy methyl amino methane.

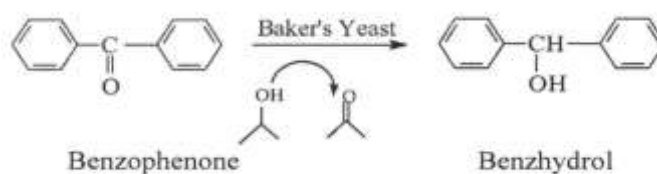
**TEMED = N, N, N', N'' – tetramethyl ethylenediamine

For 5% gel, the above solution was mixed and solution H was added and then deaerated for half an hour.

RESULTS AND DISCUSSION

The real reducing agent in the current system is NADPH[5-6] (Nicotinamide Adenine Dinucleotide Phosphate Hydrate), and yeast cells only contain a very little quantity of it. Therefore, it is important to engage a different biological mechanism to decrease NADP⁺ (Nicotinamide Adenine Dinucleotide Phosphate ion) into NADPH in order to enable the reduction to occur constantly. In yeast cells, there are several saccharides that use the pentose-phosphate route to convert NADP⁺ to NADPH. When glucose is added to the reaction mixture, the pentose-phosphate pathway is activated, ensuring a high concentration of NADPH, which eventually causes the product's enantiomeric excess(es) to rise. However, in this instance, NADH regeneration was accomplished using isopropyl alcohol, which throughout the process is oxidised to acetone.

Biological decrease of The following reaction scheme can be used to visualise picolinadehyde.



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

Immobilization improves FBY's operating stability and makes product separation simpler. The rates of product production are often high under these circumstances. Since the immobilized cells may be simply taken from the reaction media and reused several times, albeit with diminishing activity of the immobilised cells, it also enables simple continuous operation. A necessary coenzyme is supplied[7-8] and regenerated within the intact cell, in contrast to enzyme immobilisation. and in part by isopropyl alcohol injected from outside. Rarely have comparison studies been done between the usage of "free" yeast versus yeast cells that have been immobilised. However, depending on the kind of immobilization, certain variations in stereo selectivity and yield are anticipated to be seen. This expectation appears plausible given that immobilised yeast cells display different physiological, morphological, and metabolic characteristics.

Table 1. Spectroscopic results of Benzophenone)

Substrate name	Reaction time (hrs)	BY yield (%)	ImBY yield (%)	Mass spectra (m/z)	IR data (cm ⁻¹)	NMR data (δ-value)
Benzophenone	48	76.45	83.22.	184, 183, 77, 107	3407, 1623, 1593, 1055	4.98 (S, H)

REFERENCES

1. M. Kierstan and C. Buke, *Biotech. Bioeng.* 19, 387 (1977).
2. T. Sato, Y. Nishida and T. Tosa and I. Chibata, "Immobilization of Escherichia coli Cells Containing Aspartase Activity with k-Carrageenan", *Biochemica at Biophysica Acta*, 570, 179 (1979).
3. A.E. P. M. Sorrilha, M. Marques, I. Joekes, P. J. S. Moran and J. A. R. Rodrigues, *Bioorg. Med. Chem. Lett.*, 2, 191 (1992).
4. K. Burg, O. Mauz, S. Noetzel and K. Sauber, *Anges. Makromol. Chem.*, 157, 105 (1988).
5. H. G. W. Leuenberger, in *Biotransformations*. (Ed). K. Kieslich, Weinheim : Verlag Chemie, 6a, (1984) pp.5-29.
6. A.Margeritas and J. C. R. C. Merchant, *Clin. Rev. Biotechnol.*, 19(1), 339 (1984).
7. S. R. Yadav, A. K. Nainawat, S. Kaushik, A. Sharma and I. K. Sharma, "New Ecofriendly Synthetic Procedures for the Reduction of Carbonyl Compounds", *Asian J. Exp. Sci.*, 19.2, 135 (2005).
8. A.K. Nainawat, G. Wadhvani, P. S. Verma and I. K. Sharma, *Asian J. Exp. Sci.*, 20, 159 (2006).