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Eco-Friendly Biotransformation of Pyridine -2-Carbaldehyde using free and Immobilized Baker's Yeast

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Abstract -The biocatalytic reduction of picolinaldehyde (Pyridine-2- carbaldehyde) In alcoholic medium was carried out using the microbial catalyst Baker's yeast (Saccharomyces cerevisiae) free as well as in its immobilized form. The reduction product was isolated and purified by chromatographic techniques including HPLC and characterized on the basis of spectral analysis.

Keywords - Biotransformation, Picolinaldehyde (Pyridine-2-carbaldehyde), Baker's yeast (BY)

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INTRODUCTION

The biotransformations are achieved by the first class of enzymes e. g. oxidoreduction of removal or addition of hydrogen in specific manner. The Baker's yeast (Saccharomyces cerevisiae) is a common microorganism [1] that can be used for this purpose conveniently as purified reductases have disadvantages that they need expensive co-factors like NADH, NADPH, etc.

Microbial transformations with yeast-mediated transformations have been widely used, since the early days of mankind for the production of bread, dairy products and alcoholic beverages. All of these early applications used mixed cultures of microorganisms and biotechnological operations have primarily been directed in the areas of agriculture and human nutrition.

The syntheses of alcohols by reduction of carbonyl compounds with immobilized Baker's yeast (ImBY) [2-3] cells are attractive due to several reasons although the catalytic activity of the cells is generally reduced when compared to the same amount of cells in solution. This loss of activity is caused by permeability barrier

EXPERIMENTAL

Material and methods

The chemical picolinaldehyde, isopropyl alcohol and ethanol; all chemicals were AR grade. The solvents and before in use water were doubly distilled. All the reagents and products were stored in Corning glasswares.

In a one-litre round-bottom flask, equipped with magnetic stirrer (Remi Make) 200 mL water, 5 g fresh Baker's yeast and 25 mL of isopropyl alcohol were placed and the suspension was stirred for 30 minutes. The picolinaldehyde (2 mM) was separately dissolved into ethanol (2 mL) and ethanolic solution was poured into Baker's Yeast suspension. The resulting mixture was filled in with water until one litre and magnetically stirred for a suitable period. The suspension changes its colour from orange to yellow.

The experiment was performed under similar conditions with immobilized Baker's yeast, [4-5] obtained by immobilization of 2 g Baker's yeast in polyacrylamide gel.

After the completion of the reaction, 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 HPLC and characterized by spectral analysis.

Immobilization of Baker's yeast by polyacrylamide gel

The microorganism and isolated enzymes can be immobilized using various carrier materials such as urethane, cellulose, agar, alginate (a), collagen, chitosan, k-carragenan (b) and montmorillonite (c) – K 10, as porous networks for entrapment. In present work, Baker's yeast has been immobilized using polycarylamide gel.

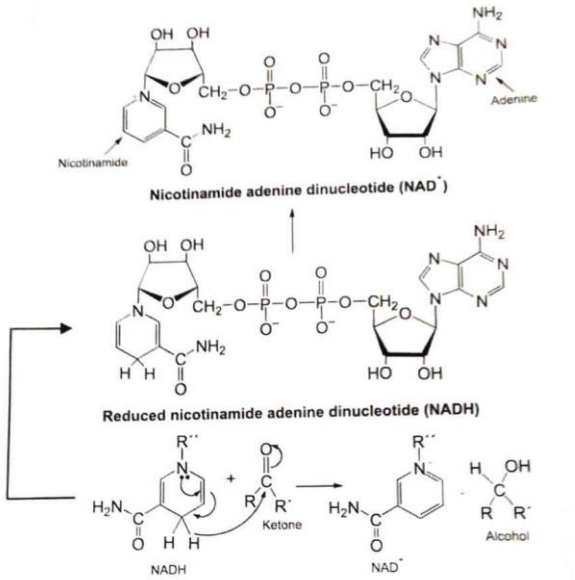
The Baker's yeast immobilized in polyacrylamide gel was prepared using following solutions.

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 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 persulpahte 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.

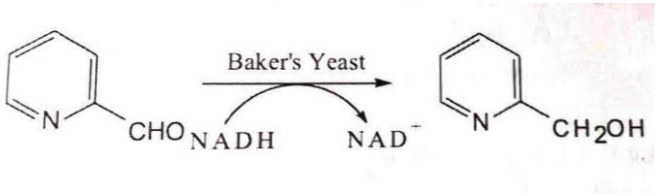
*TEMED = N, N, N'' – tetramethyl ethylenediamine

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

RESULTS AND DISCUSSION

The actual reducing agent in present system in NADPH (Nicotinamide Adenine Dinucleotude 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 Dinucleotude 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. However in the present case, regeneration of NADH was achieved by using isopropyl alcohol, [6-7] which is oxidizel to acetone in the process.

The bioreduction of picolinadehyde 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 in quite a simple essentially green experimental setup at room temperature with an easy work-up of products and good yields.

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 supplied and regenerated within the intact cell and partly by externally added isopropyl alcohol. Comparative studies between use of "free" yeast [8] 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.

Table 1. Spectroscopic results of pyridine 2-carbaldehyde)

Substrate name	Reaction time (hrs)	BY yield (%)	ImBY yield (%)	Mass spectra (m/z)	IR data (cm^{-1})	NMR data (δ-value)
Picolinaldehyde	48	80.25	83.22.	109, 108, 31,78	3417, 1605-1480 3000-3100	7.36-8.50m 4.35 s 4.43 s

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