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Biocatalytic reduction of furfural using free and immobilized Baker's yeast

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Abstract : The biocatalytic reduction of furfural was carried out using the microbial catalyst Baker's Yeast (*Saccharomyces cereviciae*) as well as its immobilized form in aqueous medium. The product was isolated and purified by Chromatographic technique and characterized on the basis of its spectral analysis.
Keywords : *Baker's Yeast, Furfural, ImBY, NADH, Immobilization*.

Introduction

The non-selective reductions often require additional protection–deprotection steps influencing process economy and leading to waste that increases stoichiometrically with increasing number of steps involved in production. Therefore, the reduction in the number of synthetic steps by highly selective and sustainable reduction processes in organic synthesis is of key importance and has influenced the development of reduction processes, reagents, and tools which can give the desired products without increasing the waste. The variety of reducing agents, from simple molecular hydrogen with chiral or achiral catalysts in catalytic hydrogenations to reducing equivalents from inorganic or organic reagents with the required reducing power for the specific reduction, has enabled a large number of selective reduction reactions. The scope of reducing agents has been greatly expanded from the use of hydrogen gas in catalytic hydrogenation, the preparation of non gaseous

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reducing agents like lithium aluminium hydride and sodium borohydride, to the development of highly selective boranes representing a milestone of organic synthesis and optically active organoboranes and providing versatile synthetic methodologies for asymmetric reductions of parochial ketones, whereby the chiral auxiliary is recovered in an easily recyclable form [1–2]. With the growing importance of safety, health, and environment aspects, the nature of the reducing agents, the transition from stoichiometric to catalytic reductions, and the development of sustainable chemistry have received increased attention [3]. Among the many synthetic methodologies available for reduction reactions, biocatalysis [4–14] has become an attractive choice in organic chemistry due to progress in understanding fundamental structure–function relationships and engineering of enzymes, their applications to organic synthesis, and developing novel enzymes to solve synthetic challenges in organic chemistry.

Biocatalytic transformations using bacteria or fungi as biocatalyst are known for many decades. Especially organisms from the group of the yeasts, e.g., *Saccharomyces cerevisiae* have been applied in biocatalytic processes [15-17]. In comparison to isolated enzymes that have the disadvantage of needing expansive cofactor like NADH or NADPH, whole-cell applications have distinct different characteristics. Enzymes utilized as whole cells are usually more stable due to the surrounding of their natural environment. Furthermore, especially in fermentative processes, the cells have internal cofactor regeneration, so that the addition of cheap glucose is sufficient to drive their action. [8, 18]

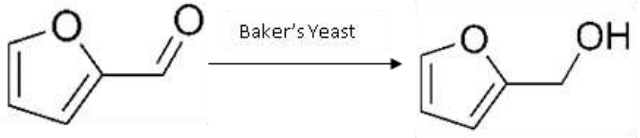
Immobilization of yeast cells has been considered as potential alternative for enhancing ethanol production because immobilizing yeasts reduces the risk of contamination [19,20], make the separation of cell mass from the bulk liquid easy[20], retain stability of cell activities [21] , minimize production costs [22,23,24], enable biocatalyst recycling [20], reduce fermentation time [22,25], and protect the cells from inhibitors [21].

In the present paper, we have used Baker's Yeast (BY) as a whole and its immobilized form (IMBY) for reduction of furfural to furfuryl alcohol. The Baker's Yeast is a most widely used micro-organism that has been used for this purpose since it is more easily available than the purified reductase.The bio catalyst was immobilized into a matrix of acrylamide and bis acrylamide by in situ copolymerization.

Results and Discussion

The effect of immobilization of yeast is the most important amongst reaction conditions. Since polymers on immobilization surround yeast cells tightly, it is reasonable to expect that the immobilizing polymer chemically influences the cell membrane of the yeast, which is in contact with the cell within the resolution of the electron microscope. As a consequence, the concentration and the rate of uptake of substrate through the cell will differ from those of the BY. In addition if the enzyme dehydrogenase involved in the reaction is one of the membrane enzymes and then another important factor would be the effect of glucose concentration on the reduction. The actual reducing agent present in the system is Nicotinamide Adenine Dinucleotide Hydride (NADH). NADH donates H^- (hydride ion) to aldehydes and ketones (and thereby reduces them). The electron lone pair on a nitrogen atom of NADH pushes out H^- which adds to a carbonyl group in another molecule to cause a reduction.

The amount of NADH in the yeast cell is limited to a quite low level. In order to allow the reduction to continue, it is, therefore, necessary to activate another biological pathway to reduce Nicotinamide Adenine Dinucleotide ion (NAD+) into NADH. Yeast contains some saccharides in the cell, which reduce NAD+ to NADH via pentose-phosphate pathway. The addition of glucose to the reaction mixture ensures simultaneous feeding of the yeast cells which ultimately results in enhanced concentration of NADH, which is regenerated from NAD+ via pentose phosphate pathway. Immobilization enhances the operational stability of BY and isolation of the products becomes easier. In addition, reuse of the catalyst is often possible under these conditions and the product formation rates are usually high [26], not only because of the inhibitory influences but also due to high cell population. It also permits easy continuous operation since immobilized cells can be easily removed from the reaction medium simply by centrifugation and can be repeatedly reused although with decreasing activity of the immobilized cells. The baker's yeast mediated reduction of furfural can be represented as:



The results are summarized in following table-

Substrate	Reaction Time (hrs.)	Yield with BY (%)	Yield with ImBY (%)	Mass spectra (m/z)	IR Spectra (cm^{-1})	NMR spectra (δ,ppm)
Furfural	48	74.20	62.46	98,97,31,67	3412,1052,1351	4.25(s),4.53(s) 6.40-7.38(m)

Experimental Methodology

200 mL water was taken in a one-litre round-bottom flask, equipped with a magnetic stirrer (Remi Make). 50 g fresh baker's yeast and 4 g glucose were then added and the suspension was stirred for 30 minutes. The furfural (2 mmol) was separately dissolved in ethanol (50 mL) and the alcoholic solution was poured into Baker's Yeast suspension. The resulting mixture was filled in with water to make a solution of 1 litre. It was then magnetically stirred for a suitable period. The suspension changed its colour from orange to yellow.

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 methylene chloride extract was dried over sodium sulphate and on evaporating it, the product was obtained. The product was purified with the help of semi preparative HPLC (Shimadzu, Japan) and then identification was made with FTIR (Shimadzu, Japan), GC-MS spectrophotometer (Thermo Finnigan Trace-GC) and NMR (JEOL, Japan, 300 MHz) techniques.

Immobilization of Baker's Yeast by polyacrylamide Gel

Micro-organism entrapment has been reported in a gel or a membrane or within microcapsules [19, 27-30]. The Polymerization of unsaturated monomers in the presence of an enzyme often results in its occlusion within the interstitial spaces of the gel.

The polyacrylamide gel is prepared with the help of these solutions.

1.0 mL of solution E

0.5 mL of solution F

0.5 mL of solution G

2.0 mL of solution H

The composition of these solutions is given below –

Solution E = 10 g Acrylamide and 2.5 g N, N′ - Methylene bis acrylamide in 100 mL double distilled water (DDW)

Solution F = 5.98 g Tris*, 0.46 mL TEMED** and 48 mL 1N HCl to 100 mL solution

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

Solution H = 34.2 g SUCROSE in 100 mL DDW

After preparation of above solutions, they are added in the following manner.

E + F + H (B. Yeast) + G

*Tris = Trihydroxy methyl amino methane

**TEMED = N, N, N′, N″ – tetramethylethylenediamine

For 5% gel the above solutions were mixed and deaerate for half an hour. 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 BY.

Conclusions

The bio catalytic methods have potential to replace well-established classical methods which are in general material consuming (expensive reagents, dry solvents) for the synthesis of chiral alcohols using baker's yeast in free form and immobilized form. Immobilization of the BY has made it a powerful biocatalyst and consequently made this method attractive. . BY in immobilized form not only allows carrying out reduction at room temperature but also an easy work-up procedure for the product and good yields with a quite simple instrumentation.

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