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# Biocatalytic Reduction of Selected Cyclohexanones

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**Abstract**: The environmental friendly reduction of 2-tertbutylcyclohexanone, 4-tertbutyl cyclohexanone and 4-Ethylcyclohexanone was carried out by Baker's Yeast (in free as well as in immobilized forms). Glycerol was employed as an alternative green reaction medium in various carbonyl reduction Methodologies. Several catalytic and non-catalytic reactions were successfully performed in glycerol. Products thus obtained were isolated, purified and characterized by combined application of chromatographic and spectroscopic techniques.
**Keywords:** 2-tertbutylcyclohexanone, 4-tertbutylcyclohexanone, 4-Ethylcyclohexanone, Baker's Yeast (BY), Immobilized Baker's Yeast (ImBY).

## Introduction:

Biotransformation and Biocatalysis are the novel highly selective and greener processes, which can provide novel potential chiral synthons for the pharmaceutical and fine chemical industry. The chemo-, regio-, and stereoselective properties of biocatalysts enabled difficult synthesis to be circumvented, especially in the synthesis of single enantiomeric form. Whole cell system acts as a catalyst and laborious isolation of the enzyme and the use of a coenzyme are unnecessary. The problems related to side reactions or low selectivity is then solved as well (1). Biocatalytic methods, as alternative to traditional methods, have gained wide interest among chemists for the production of chiral compounds (2). The asymmetric reduction of ketones is one of the most important, fundamental and practical reactions for producing non-racemic chiral alcohols, which can be transformed into various functionalities, without racemization, to synthesize industrially important chemicals such as pharmaceuticals, agrochemicals and natural products (3). Biocatalytic processes are environmentally friendly in contrast to conventional chemical catalytic processes, especially when these utilize heavy-metal based catalysis. As such, biocatalysis is a highly promising field of research, especially for the development of sustainable technologies for the production of fine chemicals (4).

Baker's Yeast (*Saccharomyces cerevisiae*) mediated enzymatic transformations of organic compounds are well known reactions in organic chemistry (5). Immobilization of the cells can be carried out by entrapment in a gel, aligenate, K-carragenum, polyurethane, poly vinyl alcohol. Immobilization also causes the difference in chemical as well in optical Yield (6-7).

Many compounds show low water solubility and are toxic for yeast cells. These results in low reaction rates and yields in water medium, furthermore the observed enantiomeric excess is often low due to the competing activities of many oxidoreductases in yeast cells. Replacing the more customary aqueous

reaction environment, an organic solvent has been used as a medium for yeast reactions. The main advantage associated with the use of an organic solvent is the simplicity with which pure product can be isolated and has been shown that significantly better yields and enantioselectivities can be achieved. Baker's Yeast cells have been the most popular biocatalyst for asymmetric ketone reductions, acceptance of this organism is very broad, and making use of commercially available whole cells is both experimentally simple and inexpensive (8). Glycerol, which is a non-toxic, biodegradable, and recyclable liquid manufactured from renewable sources, has a high potential to serve as alternative green solvent for organic reactions (9). Glycerol's promising physical and chemical properties make it an ideal reaction medium for various catalytic and non-catalytic organic syntheses. Specifically, it has a high boiling point and negligible vapor pressure (10-11).

Cyclohexanol derivatives, chemically derived from furnagillol ,a hydrolysate of furnagillin ,which has been known as an antibiotic agent and an antiprotozoal agent , have a action of inhibiting angiogenesis and an antitumor action(12). Cyclohexanol derivative like 2-tret-butyl -4-methyl cyclohexanol is a versatile scent which can be used in acidic as well as in alkaline media and has an earthy woody vetiver – like fragrance (13). 2-(2-alkoxy-1-methylethyl)-5-methyl cyclohexanol has the properties like it refrigerates not only the mouth mucosa but also the skin, is practically odorless and is dissolved in various bases (14). Chiral juvenoids (Ethyl N-{2-[4-(2-hydroxy-1-cyclohexylmethyl) - phenoxy] ethyl} carbamate) can be synthesized using 2-substituted cyclohexanols (15).

2-tertbutylcyclohexanol has camphoraceous – piney and minty odor and is used in perfumes where woody, Cedar-like, Pine-like or even Patchouli-like effects are emphasized. However, its major use is as a starting raw material for OTBCHA.OTBCHA (Ortho tertiary butyl cyclohexyl acetate) has a fruity citrus odour with a woody camphoraeous undertone. Due to its good stability, the product is used as a perfume material for soaps, detergents, body care formulations and household products.

4-tertbutylcyclohexanol has a dull woody camphoraceous odor and is used in perfumes to provide these impressions. However, its major use is as a starting raw material for PTBCHA, a very important perfumery chemical. PTBCHA (Para tertiary butyl cyclohexyl acetate) is widely used in perfumeries along with ionones, cedarwood products, floral and non-floral perfume chemicals. It is used as a blender/modifier in countless types of fragrances varying from woody to floral; from pine to rose etc. 4-Ethylcyclohexanol is an important intermediate in the formation of ionic crystals.

The present work therefore describes use of free Baker's yeast as well as Baker's Yeast immobilized in polyacrylamide to bring about biotransformation of selected cyclohexanone to Cyclohexanols.

## Experimental

1) **Reduction using Free Baker's Yeast**: - Biotransformation of chosen compounds were carried out as follows:
In a 500 ml flat bottom flask, equipped with a magnetic stirrer (Remi-2MLH make) fresh BY (10 g), a mixture of water and glycerol (50:50) and isopropanol (25ml) were placed and corresponding suspension was stirred for 30 minutes. The alcoholic solution of compounds (2mM) was poured gradually into BY suspension. The resulting solution was magnetically stirred for suitable period (Table 1). After completion of the reaction, the product was filtered using celite (HIMEDIA grade), the filtrate was saturated with sodium chloride and extracted with diethyl ether (3x25ml), and ether extracts were combined and dried over sodium sulphate. After evaporation, the product was isolated, purified and characterized by combined application of chromatographic techniques and spectroscopy.

2) **Reduction using Immobilized Baker's Yeast**: - The experiment was performed under similar conditions with Immobilized Baker's Yeast, obtained insitu immobilization of Baker's Yeast in polyacrylamide gel. The details of immobilization of Baker's Yeast in polyacrylamide gel are given below:
The gel was prepared using the following solutions.

**Solution A**: - Acrylamide (10 g) and N, N'-methylene bisacrylamide (2.5 g) in DDW (100 ml),

**Solution B**: - Tris (5.98 g), TEMED (0.46 ml) and 1N HCl (48 ml) solution to 100ml,

**Solution C**: -APS (560 mg) in DDW (100 ml),

**Solution D:** - Isopropanol (25 ml), where- TRIS= Trihydroxy Methyl Amino Methane, TEMED= N,

N, N', N" Tetramethyl, Ethylenediamine, APS= Ammonium Persulphate, DDW= doubly distilled water.

Above solutions were mixed in following way- sol. A (10 ml) + sol. B (5 ml) + BY (2g) + sol. C (5 ml). And then solution D was added and then deaerated for 30min.

The resulting final products obtained were characterized by spectral analysis viz. IR, NMR, Mass. The purity of products was checked by single spot obtained by thin layer chromatography (TLC). NMR spectra were recorded in CDCl3 solution on Joel (Japan) 300 MHz spectrophotometer and IR spectra were recorded by using Nicolet (USA) FTIR Spectrophotometer. Samples were sent to CDRI for mass spectral analysis. These results are shown in Table-1.

## Result and Discussion:-

1) **Reduction using BY and ImBY:** - The actual reducing agent which is present in this is NADH (Nicotinamide Adenine Dinucleotide hydride) in limited amount. After reducing the substrate it is itself oxidised to $NAD^+$. Therefore, to continue reduction process it is necessary to reduce $NAD^+$ (Nicotinamide Adenine Dinucleotide ion) into NADH. Yeast contains some saccharides in the cell, which reduce $NAD^+$ to NADH via pentose-phosphate pathway. To activate this pathway isopropanol is added to the reaction mixture, which is oxidized to acetone and regenerates NADH from $NAD^+$. In asymmetric reduction of carbonyl compounds using whole cells of Baker's Yeast as biocatalysts two enzyme systems are involved. One of them is the enzyme catalyzing the asymmetric reduction and other is the cofactor regeneration system, which supplies NADPH or NADH from $NADP^+$ or $NAD^+$ through the oxidation of the energy source such as carbohydrates. Immobilization enhances the stability of FBY and isolation of the product is easier. Immobilized cells can be reused, and yield is also good.

**Table-1 Spectroscopic data for microbial reduction of compounds**

| Product Name | Reaction time(In hours) | Boiling point ($^0$C) | Yield BY | Yield ImBY | IR Data (cm$^{-1}$) | NMR Data (δ- Value) | Mass Spectra (m/z) |
|---|---|---|---|---|---|---|---|
| 2-tertbutyl cyclohexanol | 72 | 207 | 79 | 84 | 3310(OH Str)<br>2930(CHStr)<br>1440&1330(CH ben)<br>1365&1395(Tertbut yl)<br>1110(C-O) | 2.1(OH)<br>3.14(CH)<br>1.47(CH)<br>1.39(CH2)<br>1.43(CH2)<br>1.58(CH2)<br>1.04(CH3) | 156<br>82<br>67<br>57 |
| 4-tertbutyl cyclohexanol | 72 | 213 | 82 | 87 | 3440(OH)<br>2945(CHStr)<br>1435&1320(CHben)<br>1365&1390(Tertbut yl)<br>1130(C-O) | 2.3(OH)<br>1.56(CH2)<br>3.16(CH)<br>1.39(CH)<br>1.41(CH2)<br>1.07(CH3) | 156<br>82<br>57<br>67 |
| 4-Ethyl cyclohexanol | 72 | 184 | 80 | 86 | 3370(OH)<br>2975(CHStr)<br>1475&1385(CHben)<br>1150(C-O) | 2.4(OH)<br>1.28(CH2)<br>1.42(CH)<br>1.37(CH2)<br>3.16(CH)<br>1.56(CH2)<br>0.94(CH3) | 128<br>81<br>57<br>43 |

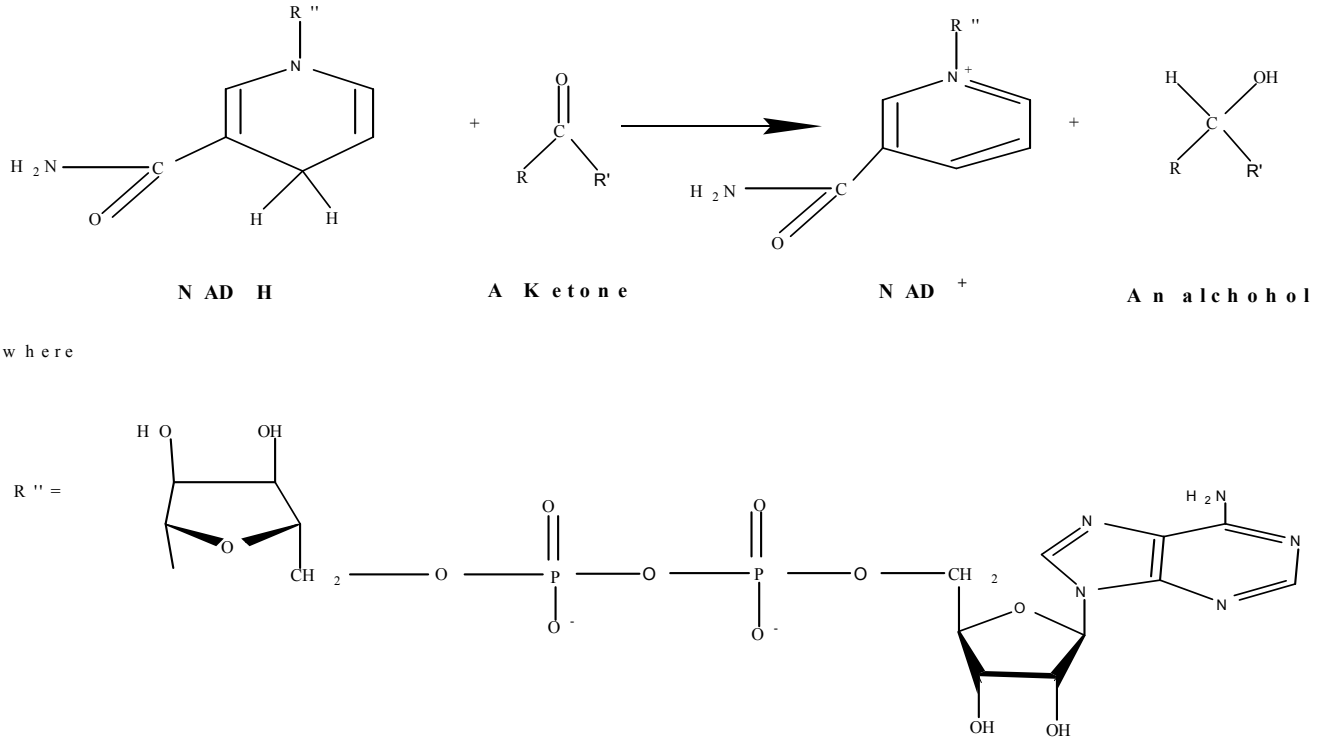


**Fig.1 Mechanism for reduction of carbonyl compound by NADH**

## Conclusion:

Performing the reaction in a mixture of glycerol and water combined the advantages of the two solvents and allowed easier and more efficient product extraction. Glycerol was successfully employed as an environmentally friendly solvent in the reductions reactions. The present work is an attempt to apply alternative synthetic routes using microbial catalyzed reduction of substrates into useful products and has merits like specificity & cost effectiveness. It is expected to reduce the ever-increasing problem of pollution caused by hazardous, corrosive chemicals and harsh reaction conditions.

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