Reductive Biotransformation of Ethyl Acetoacetate: A Comparative Studies using Free and Immobilized Whole Yeast Cells

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Abstract: Bioreduction of ethyl acetoacetate with free and immobilized yeast whole cell was achieved by using water and sucrose combination. After detachment from immobilized beads under basic condition, the corresponding ethyl(S)-(+)-3-hydroxybutanoate was isolated with 98 to 100% yield. Immobilized beads of yeast whole cell were prepared at different temperature which affects the morphology and physiology of the beads for the diffusion of the enzyme, which shown the maximum conversion of the substrate to products as compared to the free yeast whole cell.

Keywords: Biotransformation; Ethyl acetoacetate; Ethyl(S)-(+)-3-hydroxybutanoate; Immobilized yeast whole cell; Free yeast whole cell.

1. Introduction

Biotrasnformation processes for the synthesis of organic compounds have expanded in number to include a rather large group of examples and a diverse selection of microorganisms and enzymes[1]. Advantages of using enzymes in biotransformation include a) their ability to carry out a wide range of organic reactions, often at much higher reactions rates than those observed using classical organic synthesis, and b) their selectivity respective reaction and substrates type and their general regiospecific and stereospecific nature. The products of biotransformation may be present in a highly pure form. In reactions involving formation of an asymmetric carbon, the stereochemical designation at those carbon groups is usually predominately (R)- or (S)- configuration, thereby avoiding difficulties of resolving racemic mixtures of product which often result via classical organic synthesis[1]. Biotransformation operates at relatively mild physical conditions of pH and

temperature, which preserve the functional integrity of the catalysts and advantageous when labile substrates or products are involved[2].

Immobilization of cells or enzymes may extend the life of the biocatalyst, facilitate recovery and re-use, simply broaden the range of reaction conditions. Where cells are used as biocatalytic reagents, the system must allow adequate rates of penetration and diffusion of substrate into, and product from the cells; further, enzyme reactions involving formation of undesirable by-products or degradation of the desired product have to be inhibited or minimized[3].

Bakers yeast gives enantioselective reductions of carbonyl compounds. One of the compounds most widely subjected to bakers yeast reduction is ethyl acetoacetate giving rise to ethyl(S)-(+)-3-hydroxybutanoate. Ethyl acetoacetate is reduced by using commercially available dried yeast, table sugar and tap water. Thereby making this an extremely cheap source of valuable homo chiral synthon[4-6].

The use of immobilized bakers yeast is known to cause differences in chemical and optical yield in comparison to dry bakers yeast, variation also occur between different methods of immobilization. The most common method of immobilization involves entrapment of yeast cells in gel or membrane, usually alginate[7,8] K-carageenan[9] and polyurethane[10]. Also bakers yeast immobilized with calcium alginate in hexane was achieved, much of this work has been carried out by Naoshima[12,13] *et.al*.

The method of preparation of immobilization of bakers yeast also causes differences in chemical and optical yield to some extent[11-19].

The first yeast reduction of a β-keto ester was reported[20] in 1918 and in recent years. There has been an enormous resurgence of interest in the application of this most widely known whole cell biotransformation. The first report of use of immobilized bakers yeast to achieve stereochemical control was by Ohno[21] *et.al*. In 1985, bakers yeast immobilized with polyurethane was used in aqueous System to reduce β-keto ester to ethyl-(S)-4-chloro-3-hydroxybutanoate.Reduction of prochiral carbonyl groups by baker's yeast (*Saccharomyces cerevisiae*) is a well-known process and β- ketoesters are unquestionably the compounds of reference[22]. As a standard compound, ethyl acetoacetate has been reported to undergo reduction by baker's yeast to the corresponding ethyl 3-hydroxybutanoate, with the (*S*) configuration in 97-100% under various reaction conditions aqueous solution[23,24], different temperature conditions and immobilized yeast.

From the past work, it was decided to achieve the maximum chemical yield with less impurity with clear optical chiral compound and easy workout using cheap raw materials. So in this

paper we studied the reductive biotransformation using the free and immobilized whole yeast cell and to compare the results.

2. Experimental Part

General Procedures. The reaction was performed on IKA.420 magnetic stirrer. Chemical yield were determined on VARIAN-CP-3800 gas chromatography using chloroform as solvent.The downstreaming process were performed on HIEDOLPH rotary evaporating machine and vacuum distillation machine.All the chemicals were purchased from SIGMA and DOW company Ltd.

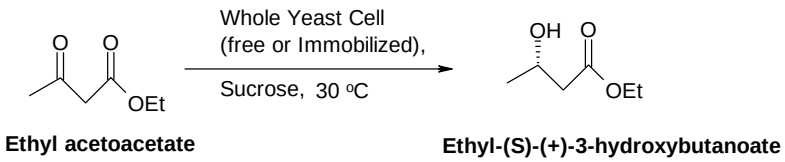
Biotransformation of ethyl acetoacetate with free whole yeast cell[8]

A 2l three necked round bottom flask equipped with thermometer, was charged with tap water (800ml), sucrose (150gm), yeast (20gm) added in this order then mixture was stirred very gently (15 r.p.m.) at 30°C for 1hr (at the end of 1 hr carbon dioxide should be evolved at approximately 1-2 bubbles/ sec.). Ethyl acetoacetate (9.5ml) was added drop wise to the fermenting solution and the mixture stirred at ambient temperature for 24hr. A warm solution (40°C.) of sucrose (100gm) in tap water (500ml) was then added and the mixture stirred for 1 hr then a further aliquot of ethyl acetoacetate (9.5ml) added. The mixture was then stirred for a further 18 hr. when no more starting material is apparent by Gas Chromatography, the reaction may be terminated. Then filter aid was added to the suspension for filtration, the filtrate was saturated with sodium chloride and extracted with ethyl acetoacetate, combine the extract, dry over the magnesium sulphate, filter and remove the solvent under reduced pressure to afford pale viscous oil. The crude product was then distilled to afford the desired alcohol as clear colorless oil.

Biotransformation of ethyl acetoacetate with immobilized whole yeast cell can be performed by the same way as mentioned above (Instead of free whole yeast cell, immobilized beads whole yeast cell were used.)

Immobilization of whole yeast cell[7,8]

Immobilization can be done by using sodium alginate and water in the calcium chloride solution (The temperature of calcium chloride solution can be maintained at 45°C, 37°C, and 15°C for preparation of beads of whole yeast cell, which means 45°C, 37°C,15°C beads)



Scheme 1. Biotransformation of Ethyl acetoacetate in to Ethyl-(S)(+)-3-hydroxybutanoate

3. Results and Discussion

The reduction of ethyl acetoacetate using free whole yeast cell 97.54% product and 1.5% by-products. Immobilized whole yeast cell reduction of ethyl acetoacetate gives the 98.4% product and 0.86% byproducts. Prior to the choice of dry whole yeast cell for the bioreduction, attempts were made to perform the same bioreduction using Immobilized whole yeast cell beads reused 2-3 times gives the conversion with 98.57% and 98.45% yield respectively.

Among the commercially available immobilizing agent, alginate was chosen as support, since its chemico-physical features allow easy isolation of product. The variation can be seen in the chemical yield of the beads prepared at different temperatures.It has been found that by maintaining the low temperature of the beads (15°C.) in calcium chloride solution has proved the best, as it gives the maximum chemical yield. A new concept has been focused on the chemical yield of the chiral compound, by means of temperature maintenance for good quality beads further to give good quality chiral compounds as product. The basic mechanism of immobilization is the diffusion of enzyme through the membrane of beads for the stereospecific control on the substrate.We observed that, reusing these beads at second time gives the satisfactory results. The period of time for the total raw material consumption could be made reduced, while we mainly focused in carrying out maximum chemical yield with cheap and friendly method. Thus the procedure given in the experimental section showed satisfactory results. The Table no.1 shows the details of chemical yield obtained by using free whole yeast cell and immobilized yeast prepared at different temperature parameters.

Table 1. Chemical yield obtained from ethyl acetoacetae by various

Sr. No.	Parameters applied	Retention time (Min)	Yield(%)
1	Free whole yeast cell reduction of ethyl acetoacetate.	1.81	97.54
2	Immobilized whole yeast cell reduction of ethyl acetoacetate.	1.81	98.49
3	The reduction of ethyl acetoacetate by using Immobilized whole yeast cell beads at second time.	1.81	98.57
4	The reduction of ethyl acetoacetate by using Immobilized whole yeast cell beads at third time.	1.81	98.45
5	Effect of 45°C. temperature on Immobilized whole yeast cell beads for reduction of ethyl acetoacetate.	1.81	67.36
6	The reduction of ethyl acetoacetate by using Immobilized whole yeast cell beads prepared at 45°C.	1.81	98.22
7	The reduction of ethyl acetoacetate by using Immobilized whole yeast cell beads prepared at 37°C.	1.81	90.83
8	The reduction of ethyl acetoacetate by using Immobilized whole yeast cell beads prepared at 15°C.	1.81	98.31
9	The reduction of ethyl acetoacetate on second time by using Immobilized whole yeast cell beads prepared at 45°C.	1.81	97.49
10	The reduction of ethyl acetoacetate on second time by using Immobilized whole yeast cell beads prepared at 37°C.	1.81	98.42
11	The reduction of ethyl acetoacetate on second time by using Immobilized whole yeast cell beads prepared at 15°C.	1.81	100
12	The reduction of ethyl acetoacetate on third time by using Immobilized whole yeast cell beads prepared at 37°C.	1.81	97.73
13	The reduction of ethyl acetoacetate on third time by using Immobilized whole yeast cell beads prepared at 15°C.	1.81	99.27

4. Conclusion

Thus the overall performance of bioreduction reactions shows that, the immobilized whole yeast cell beads gives the maximum conversion as compared to the free whole yeast cell, but the reused beads prepared at 15°C found to be the best of all with 100% conversion (See Table 1). Increasing the reaction temperature simply decreases the yield of the product. Even though the beads prepared at various temperatures in calcium chloride solution gives the maximum conversion with less impurities. The maximum conversion was found when beads were reused.

5. Acknowledgement

Authors are thankful to **Ms. Sweety Laddha,** Patron, SLR Foundation, Umarkhed, for encouragement and finanacial assistance, **Prof. S. P. Bhatnagar,** Advisor, Technology Development and Transfer, Birla Institute of Technology, Mesra, Ranchi, and **Dr. R. H.**

Iyer, Ex-Head, Radiochemistry Division, Bhabha Atomic Research Centre, Mumbai, for thier guidance and help.

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