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Green Chemical Synthesis of Optically Pure 1-(4-Aminophenyl)Ethanol

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ABSTRACT

The synthesis of 1-(4-Aminophenyl) ethanol was carried out using the microbial catalysts 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. The enantiomeric excess was calculated with the help of polarImetric method and the product was found to high optical purity (enantiomeric excess, ee %).

1. Introduction

Microbial transformations using bacteria or fungi as catalyst are known since many decades. Especially organisms from the group of the yeasts, e.g., *Saccharomyces cerevisiae* were applied in biocatalytic processes [1, 2]. In comparison to isolated enzymes that have the disadvantage to need 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 [3, 4].

The vast majority of new chemical entities that reach the market these days are chirally pure. As a result, the need for effective, selective reactions to create chiral building blocks to make single isomer drugs is only going to increase. In addition to this, these reactions need to be cost effective, high yielding and devoid of use of dangerous or corrosive reagents. It is hardly surprising therefore, that lots of synthetic efforts are put in by chemical companies, pharmaceutical companies and chemists in our laboratory [5-7] to create new and better processes. Chiral alcohols are important intermediate in the synthesis of precursors used in pharmaceutical activities. Dawpharma has developed a catalytic route to chiral 1-aryl-2-imidazol-1-yl ethanols using asymmetric transfer hydrogenation [8].

In the present paper, we use Baker's Yeast (BY) as whole and their immobilized form (IMBY) for reduction of p-amino acetophenone into optically pure 1-(4-amino phenyl) ethanol. The Baker's Yeast is a common micro-organism that can be used for this purpose since it is more easily available than the purified reductase.

2. Experimental Methods

About 200 mL water was taken in a one-litre round-bottom flask, equipped with magnetic stirrer (Remi Make). Then 50 g fresh baker's yeast and 4 g glucose were added and the suspension was stirred for 30 minutes. The p-amino acetophenone (2 mmol) was separately dissolved into ethanol (50 mL) and ethanolic solution was poured into Baker's Yeast suspension. The resulting mixture was filled in with water to make a solution of one litre and 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 (Thermofinnigan Trace-GC) and NMR (JEOL, Japan, 300 MHz) techniques. The optical rotation was measured by using a polarimeter.

2.1 Immobilization of Baker's Yeast by polyacrylamide Gel

Micro-organism entrapment has been reported in a gel or a membrane or within microcapsules [10, 11]. 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 and 2.0 mL of solution H.

The composition of these solutions are,

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 per sulphate in 100 mL DDW);
Solution H = 34.2 g SUCROSE in 100 mL DDW;

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

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

*Tris = Trihydroxy methyl amino methane
**TEMED = N, N, N′, N′′ – tetra methyl ethylenediamine

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.

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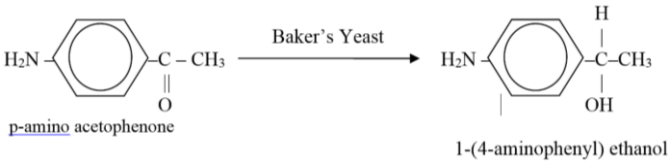
3. Results and Discussion

The most important amongst reaction conditions is the effect of immobilization of yeast. Since polymers on immobilization surround yeast cells tightly, it is reasonable to expect that the immobilizing polymer chemically influence 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 in present 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 continuously, it is therefore necessary to active 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 ensure simultaneous feeding of the yeast cells which ultimately results in enhanced concentration of NADH, which is regenerated from NAD+ via pentose phosphate pathway. This will ultimately ensure increase in the enantiomeric excess (ee) of the product. 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 [9], not only because of the inhibitory influences but also high cell population. It also permits easy continuous operation since immobilized cells can be easily removed from the reaction medium and can be repeatedly reused although with decreasing activity of the immobilized cells. The baker's yeast mediated reduction of p- amino acetophenone can be represented by Scheme 1. The results are summarized in following Table 1.

Table 1 Bio catalytic reduction by Baker's Yeast (BY) and Immobilized Baker's Yeast (ImBY)

Substrate		p-amino acetophenone	
Reaction Time	(hrs.)	48	
Yield with BY	(%)	82.35	
Yield with ImBY	(%)	79.42	
Mass spectra	(m/z)	137,136,	92,45
IR Spectra	(cm^{-1})	3404,3425(s)	3445(b)
		1605-1635,1061	
NMR spectra	(δ,ppm)	7.15-7.20(d,2H)	7.27-7.32(d,2H),
		7.00(S,2H)	4.76(S,1H),
		4.00(q,1H)	1.51(d,3H)
ee		70(BY)	81(ImBY)



Scheme 1 Baker's Yeast mediated reduction of p-amino acetophenone

4. Conclusion

The biocatalytical 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 catalyst made this method more attractive and it becomes a powerful tool. Baker's yeast allow to carry out reduction at room temperature with an easy work-up of product and good yields with a quite simple installation.

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