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RESEARCH ARTICLE

Biocatalytic and Electrochemical Reduction of Ethyl Levulinate

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ABSTRACT

This paper reports biocatalytic and electrochemical reduction of ethyl levulinate to synthesize ethyl 4-hydroxy valerate. Hydroxyacids represent one such class which serves as useful chiral synthetic building blocks for other fine chemicals and pharmaceuticals and it can be prepared by biocatalytic reduction of keto esters using microbial catalyst [Baker's Yeast (Saccharomyces cerevisiae)] in its free (FBY) as well as in immobilized (ImBY) form. Substrates showed the higher conversion rate in immobilized form. The electrochemical behavior of ethyl levulinate was investigated cyclic voltammetrically andelectrochemical reduction was then carried out galvanostatically using economically viable stainless steel (SS-316) electrodes. The reduction products were isolated and purified by chromatographic techniques and characterized by spectral analysis.

***Key words**: ethyl levulinate, Baker's yeast (BY), Biocatalytic reduction, Immobilized Baker's yeast (ImBY), Cyclic Voltammetry, galvanostatically Stainless Steel Electrode (SS-316)*

INTRODUCTION

Modern chemistry plays an important role in the improvement of quality of life around the world. However, these advances created an increase in contamination of the environment by toxic substances. Therefore, the so-called Green Chemical Processes where the "best available technology" not entailing excessive cost and aspiring to "performance without pollution" can be used in industrial processes. Some of the most active areas of Green Chemistry research and development are use of Microbial transformation in conventional organic synthesis (1-3). Biocatalysts are truly "Green Catalysts" with little to no toxicity in the process. Enantioselective reduction of ketones to optically active secondary alcohols is one of the most interesting areas of research. But separation of alcohol racemate is not easy; certain methods are used for catalytic enantioselective hydrogenation of the corresponding ketone. General drawbacks of these reactions are the requirement of often expensive chiral metal-complex catalysts, contamination of end product with catalysts, vigorous reaction conditions such as high pressure, flammable reaction media, or cryogenic conditions and the range of possible products is limited.

Employing biocatalysts for the asymmetric reduction is advantageous since reactions proceed at low temperature and in the absence of high hydrogen pressure. Use of the whole cell such as baker's yeast (Saccharomyces Cerebefovisiae) for chiral reduction is more attractive from economical, environmental and handling points of view. Thus biotransformations are eco-friendly, regio- and stereoselective processes. Present study describes biocatalytic reduction ethyl levulinate using free as well as immobilized whole cell biocatalyst, Baker's Yeast. Aim of present investigation is to explore electrochemical reduction of ethyl levulinzte. Before this at first cyclic voltammogram of ethyl levulinate was recorded at different pH and different scan rate to check the reversibility of the process. On the basis of results obtained from cyclic voltammetry, conditions were determined for electrolysis at stainless steel electrode (SS-316) galvanostatically.

MATERIALS AND METHODS

All the chemicals used were of AR (Analytical Reagent) grade and triply distilled water was used for the making of solution and Baker's Yeast purchased was of food grade.

EXPERIMENTAL

Reduction Using Free Baker's Yeast:

Biotransformation of levulinic acid (A) and ethyl levulinate (B) was carried out as follows: In a one liter round bottom flask, equipped with a magnetic stirrer (Remi- 2MLH make) water (200ml), fresh BY (10 g) and isopropanol (25ml) were placed and corresponding suspension was stirred for 30 minutes. The alcoholic solution of compounds (A/B) (2mM) was poured gradually into BY suspension. The resulting solution was magnetically stirred for suitable period (12 hr). The suspension changed its colour during the course of reaction. 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, 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.

Reduction Using Immobilized Baker's Yeast:

Immobilization of Baker's Yeast (2g) in polyacrylamide gel. The details of immobilization are given below: Immobilization of BY in polyacrylamide gel: Washed Baker Yeast (2g) is suspended in 5 ml ice-cold water and mixed with 10 ml ice-cold freshly prepared monomer solution in 0.2M potassium phosphate buffer (pH=7) containing 2.85g acrylamide, 0.15 g N,N-methylene-bis-acrylamide, 20 mg tetra methylene diamine and 10 mg ammonium persulfate. The reaction mixture is immediately poured onto a glass plate equipped with stainless steel spacers (0.7 mm). Polymerization starts within 1 minute and completes after 1 hr. The gel sheet is then cut into cubic shaped pieces of equal sizes.

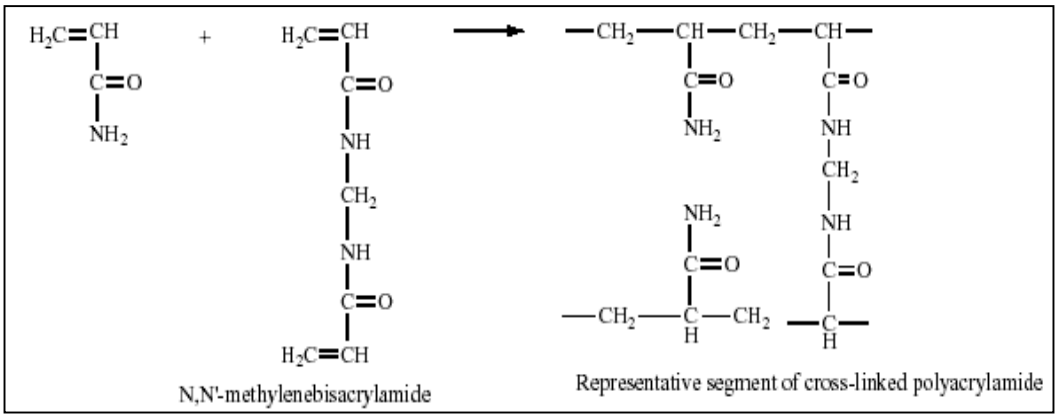

Fig. 1: Formation of polyacrylamide gel

Reduction Using Electroanalytical Technique:

First of all cyclic voltammogram were recorded at different pH and different scan rate using a computer based Basic Electrochemistry sysem ECDA-001, supplied by Con-serv enterprises, Mumbai, using 3 electrode cell assembly with 1mm diameter glassy carbon as working electrode, Ag/AgCl as reference electrode and Pt wire as counter electrode. The voltammographic curves were recorded for compounds in aqueous solution using 1M potassium chloride as supporting electrolyte and BR buffer of different pH (5, 7, and 9) at platinum electrode to determine the optimum conditions for electrochemical reduction. These conditions were subsequently applied for carrying electrochemical reduction at stainless steel electrode (SS-316) galvanostatically. The conventional H-type cell with two limbs separated by G-4 disc was used for electrolysis. The supporting electrolyte (1M) sodium acetate was filled in both the limbs. The reactants (0.001M) were dissolved in water and placed in cathodic chamber

and the pH of cathodic solution was 9. The stainless steel (SS-316) was used as cathode as well as anode. The constant current of 1 amp was passed through the electrolyte for suitable period (4 hr) with the help of a galvanostate (CDPE make, University of Rajasthan, Jaipur). There after the working up of the reaction mixture involved extracting the aqueous solution with diethyl ether (3×25ml). The ether layer was then separated and washed with aqueous saturated NaCl solution. The organic extracted were dried over anhydrous Na_2SO_4 and then characterized.

CHARACTERIZATION OF THE PRODUCT

The products were characterized on the basis of their chromatrographic, IR, NMR & Mass spectral analysis. Optical activity of products was measured by using a polarimeter and enantiomeric excess (ee) was calculated.

RESULT AND DISCUSSION

Reduction Using Baker's Yeast in Free and in Immobilized Form:

1) The actual reducing agent is NADH (Nicotinamide Adenine Dinucleotide Hydride) donates hydride ion (H^-) to aldehydes and ketones and thereby reduces them. The electron lone pair on nitrogen atom of NADH pushes out the hydride ion that is added to a carbonyl group of another molecule to cause its reduction. The process is completed by addition of proton to the carbonyl oxygen.

2) Immobilization of yeast by acrylamide increases yield, purity and catalytic performance of products. It increases control over enzymatic reaction as well as high volumetric productivity with lower residence time. It improves tolerance of cells from substrate and endpoint inhibition. The reuse of same biocatalyst for prolonged period of time due to constant cell regeneration is yet another advantage. Immobilization also improves substrate utilization and reduces risk of microbial contamination.

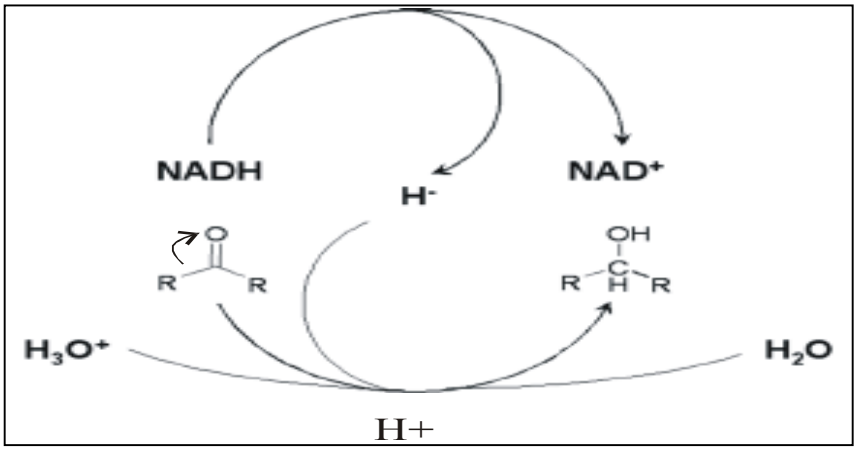

Fig. 2: Mechanism of Reduction

CYCLIC VOLTAMMETRIC STUDY

Effect of PH:

The reduction of carbonyl compounds in aqueous solution depends on the pH of the system. From cyclic voltammograms shown in fig.1) it can be clearly concluded that at lower pH i.e. at pH 5.0, there is no appearance of peak in cyclic voltammogram. As pH increases a cathodic peak begins to appear & with increasing pH its appearance becomes clearer. Accordingly at pH 7.0, slight peak appears and at pH 9.0, the peak shows prominent appearance. From above Cyclic Voltammetric studies it can be concluded that the process of reduction is easier in basic media as compared to acidic and neutral media. In alkaline solutions electrons come from water which decomposes to yield hydrogen and hydroxyl ion

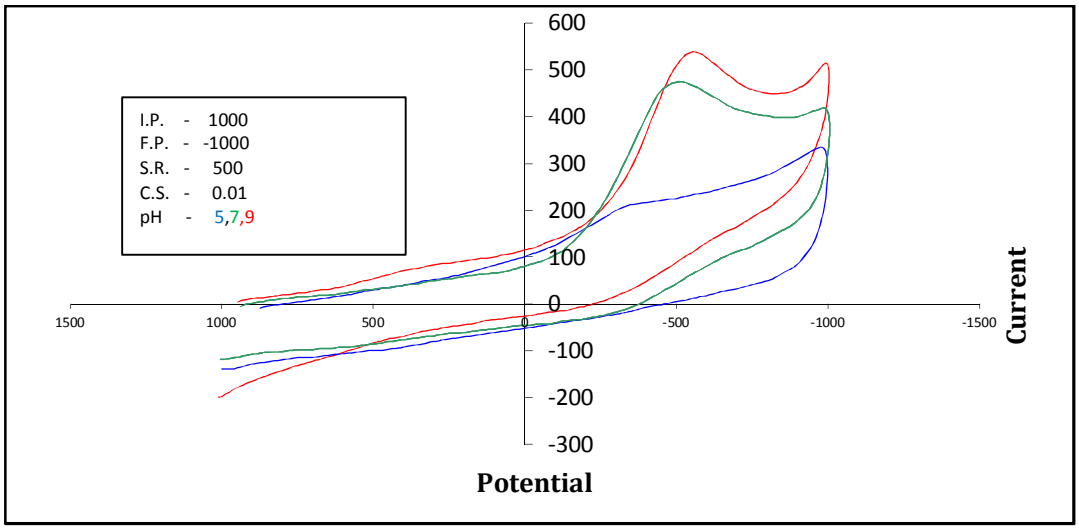

Fig. 3: Effect of pH on reduction of ethyl Levulinate

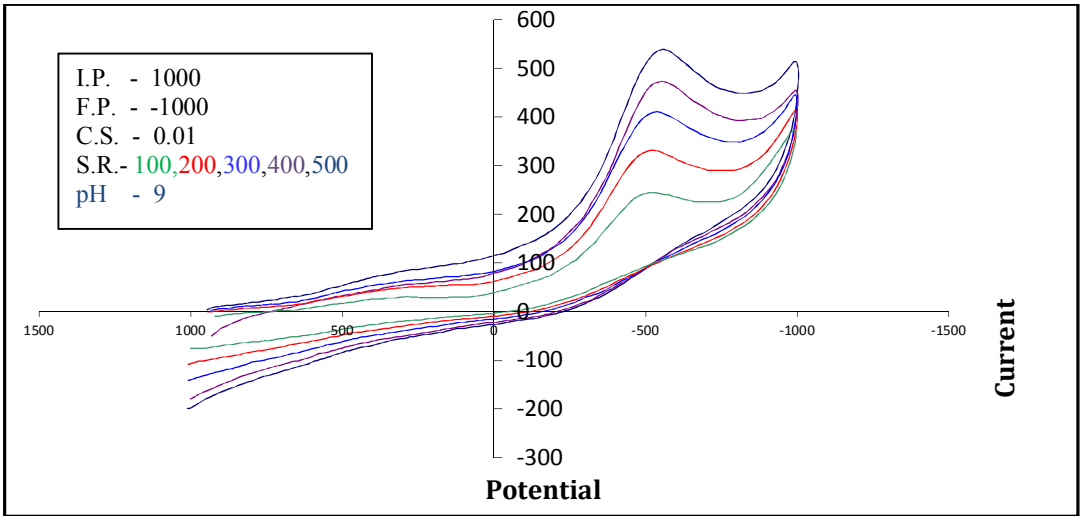

Fig. 4: Effect of Scan rate on reduction of ethyl levulinate

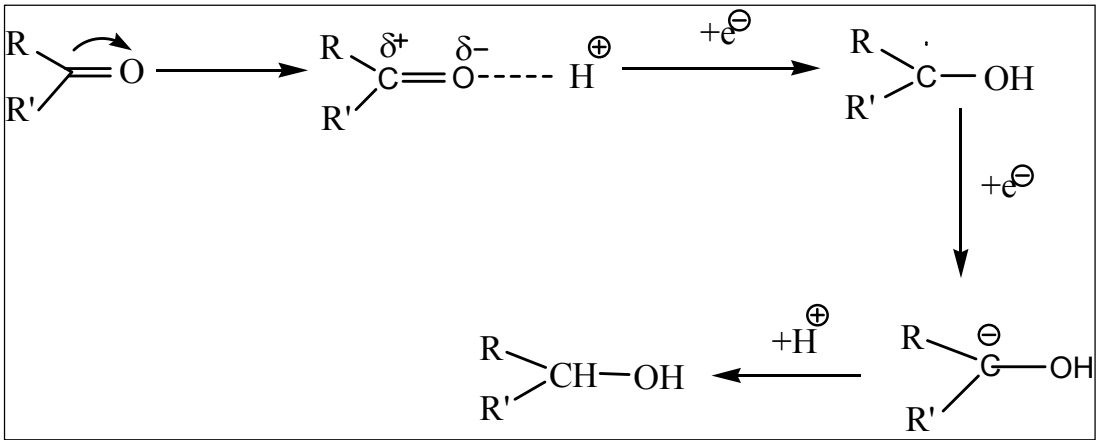

Fig. 5: Mechanism of electrochemical reduction

Effect of Scan Rate:
From cyclic voltammograms (fig.2), it is clear that as the sweep rate was gradually increased to 100,200,300, 400 and 500,800 and 1000 mV/sec, peak potential (Ep) gradually shifted towards higher values. The cathodic peak current (Ip) increases with increasing scan rate.

Mechanism for Electrochemical Reduction:
From cyclic voltammogram it is clear that process of reduction is ir-reversible and it completes with transfer of two electrons.

SPECTROSCOPIC RESULTS OF PRODUCTS

Name of Reactant	Reaction Time (In Hours)	B.P (^{0}C)	Yield (%)	IR Data (cm^{-1})	NMR Data (δ- Value)	Mass Spectra (m/z)	Compound formed
Ethyl levulinate	8 hrs	207	97%	3450 (O-H str) 2970 (C-H str) 1740 (C=O str) 1370 (C-H def) 1240 (C-O str)	4.12 (CH2) 3.39 (CH) 2.25 (CH2) 2.0 (OH) 1.83 (CH2) 1.30 (CH3) 1.21 (CH3)	146 145 129 99 89 83 73 59 55 45 31	Ethyl 3 - hydroxyl valerate

CONCLUSION

The present paper gives excellent methodsfor reduction of ethyl levulinate follows Green Chemical routes. The processes are environmental friendly, specific and cost effective. Such techniques are real solution of increasing problem of pollution caused by harmful chemicals and harsh reaction conditions.

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