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Green Chemical Methods for the Reduction of Keto Esters

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Abstract: This paper reports green chemical reduction of selected keto esters like ethyl 3-oxo hexanoate, ethyl levulinate, ethyl 2-chloro acetoacetate and ethyl 2-methyl acetoacetate involving microbial transformation as well as electrochemical techniques. In case of microbial transformation Baker's Yeast (*Saccharomyces cerevisiae*) was used in free as well as in immobilized form. Immobilized cell showed maximum conversion of the products as compared to the free yeast cells. The electrochemical behavior of substrates was studied with the help of cyclic voltammetry. Information obtained from cyclic voltammetric studies was used for establishing optimum conditions for electrochemical reduction. Electrochemical 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 on the basis of spectral analysis.

Keywords: Ethyl 3-oxo hexanoate, ethyl levulinate, ethyl 2-chloro acetoacetate, ethyl 2-methyl acetoacetate Cyclic Voltammetry, Stainless Steel Electrode (SS-316), Biotransformation, Baker's Yeast (BY) and Immobilized Baker's Yeast (ImBY).

Introduction

Modern chemistry plays a key role in the improvement of quality of life around the world. However, these advances frequently came with an increase in contamination of the environment by toxic substances. Nowadays steps are being taken, mainly due to increasing economic, social, legal, and environmental pressures, to avoid further degradation. 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. The Green Chemistry has emerged since 1990s as away that the skills, knowledge, and talents of chemists can be used through its application to avoid threats to human health and the environment in all types of chemical processes. A central driving force in this increasing awareness is that it accomplishes both economic and environmental goals simultaneously through the use of sound, fundamental scientific principles. Some of the most active areas of Green Chemistry research and development are application of biocatalyst and electro-analytical methodology developments in conventional organic synthesis (1-3).

Demand for enantiopure chiral compounds continues to rise, primarily for use in pharmaceuticals but also in three other sectors: flavor and aroma chemicals, agricultural chemicals and specialty materials. Biotransformation has a number of advantages when compared to the corresponding chemical methods. Biocatalysts are known to posses some interesting and advantageous features i.e. high efficiency, mild environmental friendly operation conditions, versatility and last but not least have high selectivity (chemeo, regio, and stereoselectivity). The selectivity and particularly the stereochemical preference are observed when biocatalyst act on their substrates.

Besides this economically, some biotrans - formation can be cheaper and more direct than their chemical analogues and the conversion normally

proceeds under conditions that are regarded as ecologically acceptable. Because of these reasons the use of enzymes for biotransformation of man-made organic compounds has been used for more than hundred years where in whole cells, organelles or isolated enzymes were employed (4-5).

In the field of electrochemistry, electro-analytical techniques like Polarography, Cyclic Voltammetry etc. and synthetic techniques like Electrolysis at Constant Current and Constant Potential are now known and can be employed for analytical and synthetic purposes. Cyclic Voltammetry, is finding extensively use to provide information regarding potential corresponding to reduction, oxidation and formation of intermediates and also about the reversible nature of electrode transfer processes. Shape of cyclic voltammograms provides valuable information related to the kinetics of the electrode processes and on the rates of the processes. A number of industrial processes have now replaced routine methods of their synthesis by the electrochemical methods. Since, electro–organic synthesis is much more economical, eco-friendly, avoid massive chemical effluents. Such type of reactions is easy to control automatically as well as the reactions conditions are generally mild and effects of potential, pH, buffer, solvent and structural modifications of substrate can also be studied. It is with this background then it is proposed to make use of these electroanalytical techniques for carrying out synthesis of the selected substances (6-9).

The aim of present investigation is to explore a novel ecofriendly method of synthesis of optically pure alcohol using free Baker's Yeast (BY) (*Saccharomyces cerevisiae*) as well as immobilized Baker's Yeast (ImBY). The Baker's Yeast is a common micro-organism that can be used for this purpose since it is economical and more easily available. When whole microbial cells, such as baker s yeast, are used as the catalyst for the asymmetric reduction of carbonyl compounds, two enzyme systems are mainly involved in the production reaction. One is the enzyme catalyzing the asymmetric reduction of prochiral carbonyl compounds to chiral alcohols, i.e. carbonyl reductases. The other is a cofactor regeneration system, which supplies NADH or NADPH through the oxidation of the energy source, such as carbohydrates and alcohols. The use of immobilized Baker's yeast (ImBY) as biocatalyst has several advantages such as removal of the biocatalyst from the reaction mixture is easy and its repeated use is also possible(10-14).

The present work therefore describes use of free Baker's yeast as well as Baker's Yeast immobilized in polyacrylamide gel have been used to bring about biotransformation of keto esters to optically active hydroxy esters. Homochiral hydroxy esters so formed have been used as chiral starting materials for the synthesis of a variety of important chemicals including lactams, insect pheromones, and carotenoids. Then for electrochemical reduction, first cyclic voltammogram of keto esters were 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.

Experimental

1) **Reduction using Free Baker's Yeast**: - Biotransformation of chosen compounds were carried out as follows:
In a one liter round bottom flask, equipped with a magnetic stirrer (Remi-2MLH make) water (200 ml), fresh BY (10 g) 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). 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.

2) **Reduction using Immobilized Baker's Yeast**: - The experiment was performed under similar conditions with Immobilized Baker'sYeast, obtained insitu immobilization of Baker's Yeast (2g) 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= Trihydrixy 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.

3) **Reduction using electroanalytical Technique: -** First of all cyclic voltammogram were recorded at different pH and different scan rate using a computer based Basic Electrochemistry system 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 (table -2) hours 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 Na2SO4 and than characterized.

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 Phosphate 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^+. Immobilization enhances the stability of FBY and isolation of the product is easier. Immobilized cells can be reused, and yield is also good.

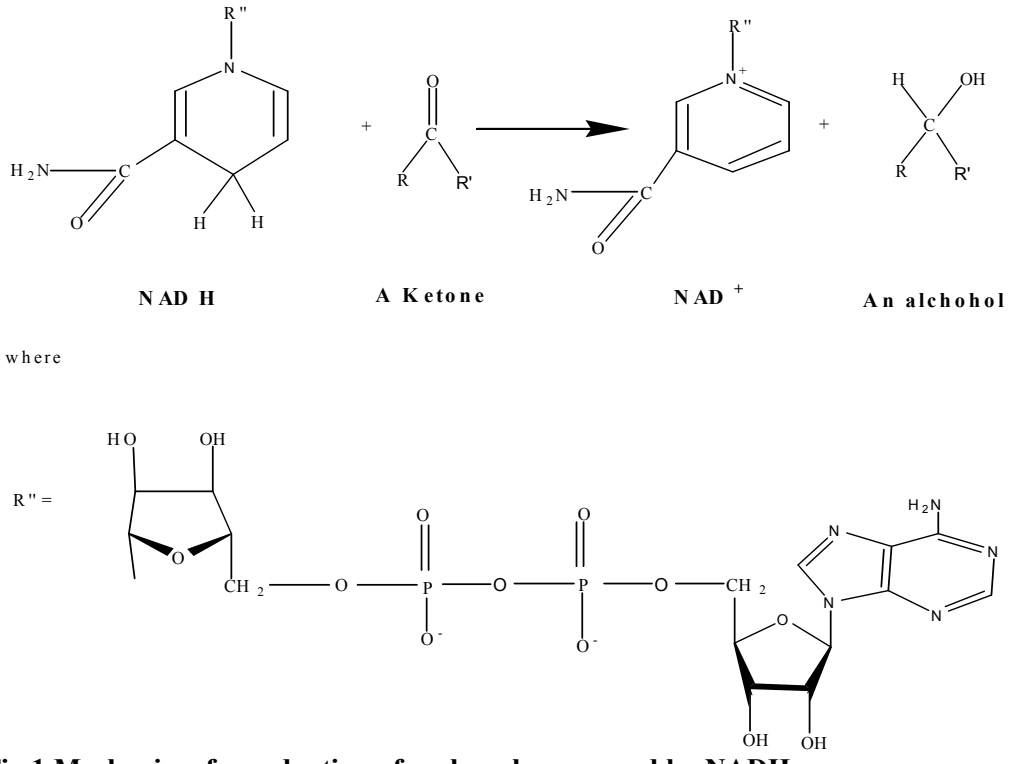


Fig.1 Mechanism for reduction of carbonyl compound by NADH

2) **Reduction using electroanalytical technique: -** The reduction of carbonyl compounds in aqueous solution depends on the pH of the system. From cyclic voltammograms shown in **fig (2-5)** 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.

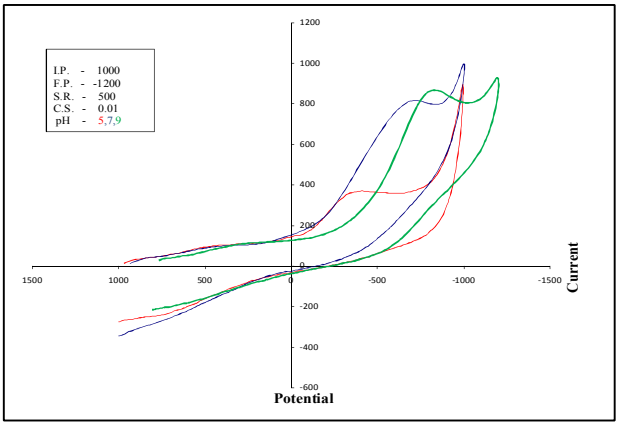


Fig.2 Effect of pH on reduction of ethyl 3-oxo hexanoate

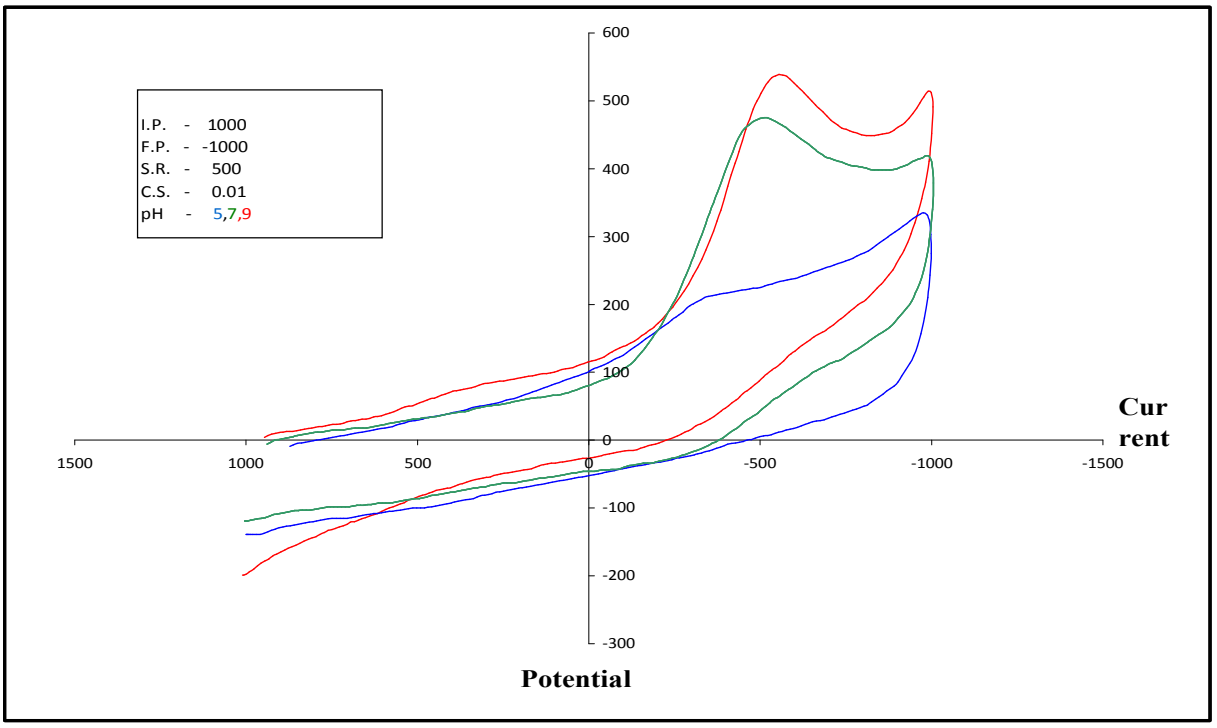


Fig.3 Effect of pH on reduction of ethyl Levulinate

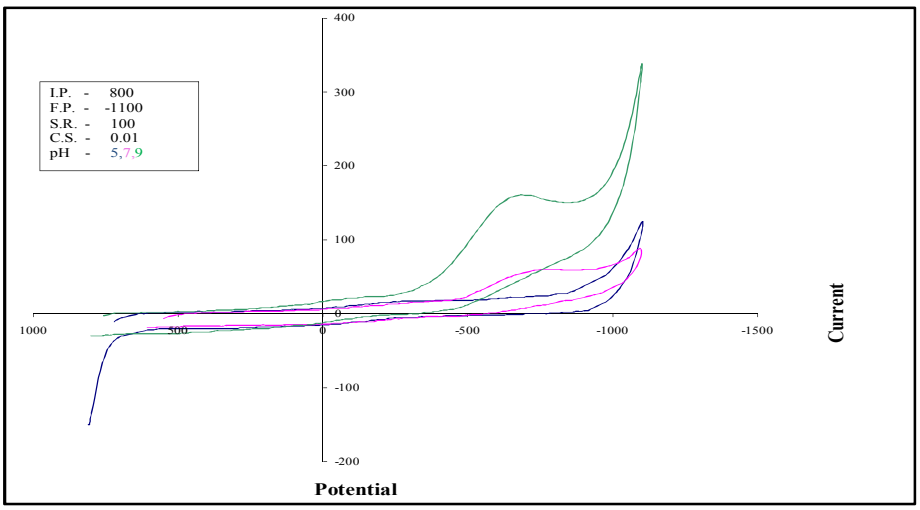


Fig.4 Effect of pH on reduction of ethyl 2-chloro acetoacetate

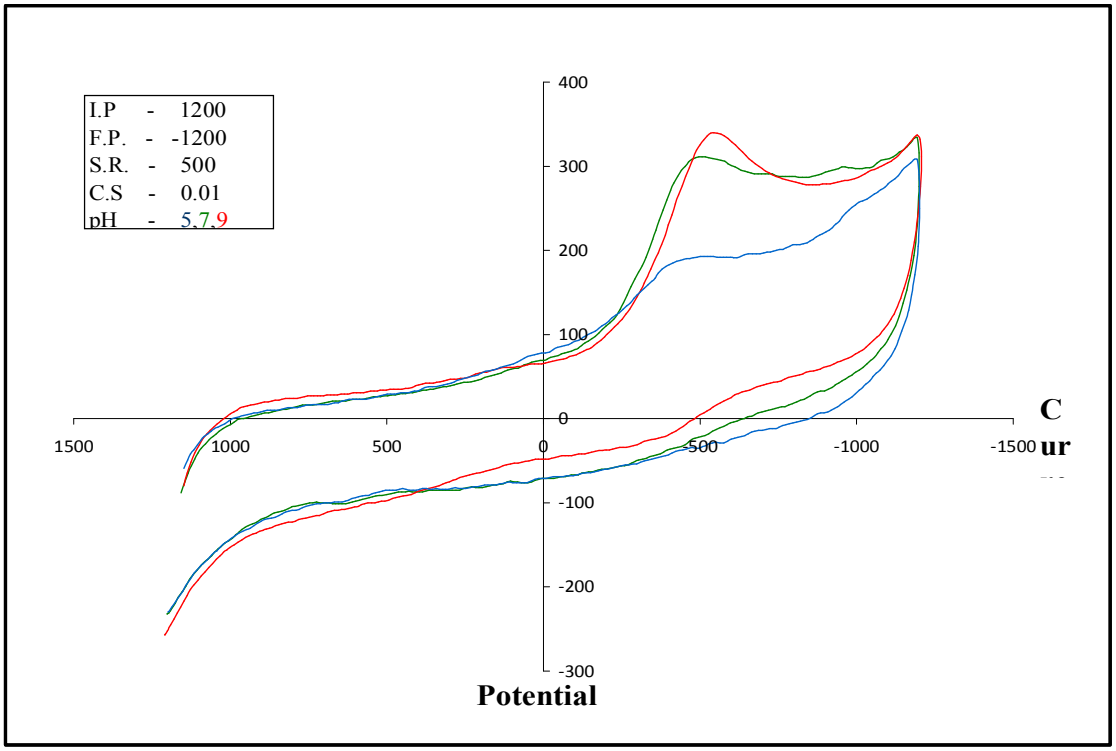


Fig.5 Effect of pH on reduction of ethyl 2-methyl acetoacetate

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.

Effect of scan rate- From cyclic voltammograms (fig6-9), 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. The current function (Ip /√ ν) has been found to be fairly constant with respect to scan rates' indicating that the electrode process is diffusion controlled.

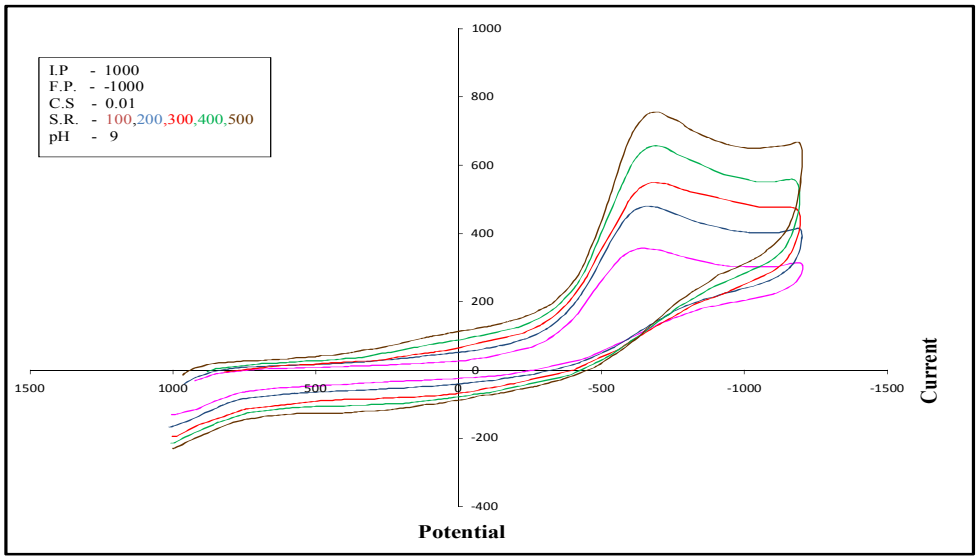


Fig.6 Effect of Scan rate on reduction of ethyl 3-oxo hexanoate

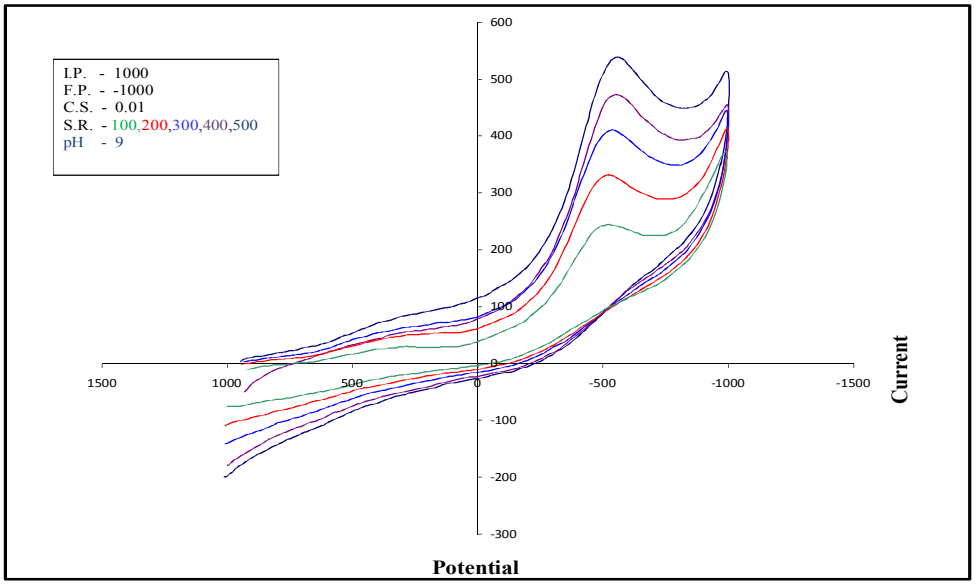


Fig.7 Effect of Scan rate on reduction of ethyl levulinate

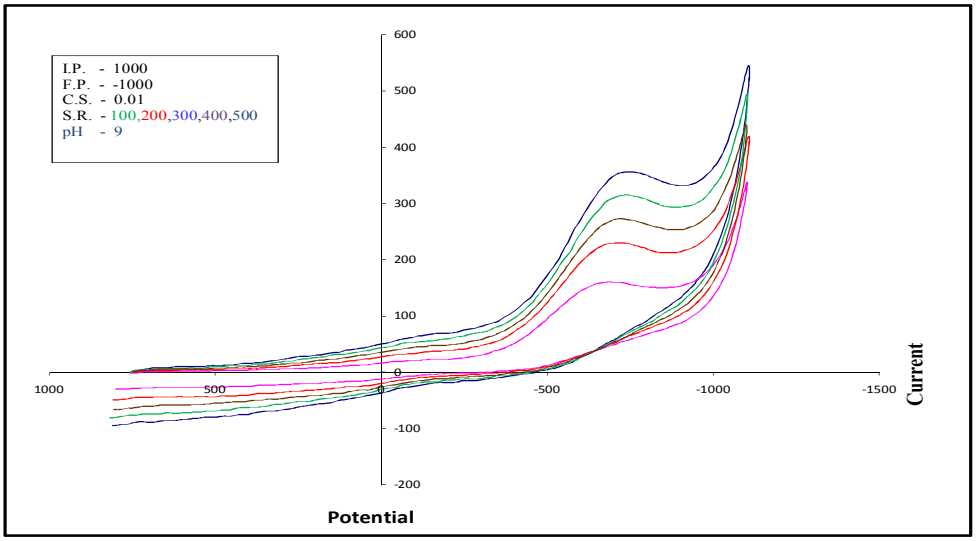


Fig.8 Effect of Scan rate on reduction of ethyl 2-chloro acetoacetate

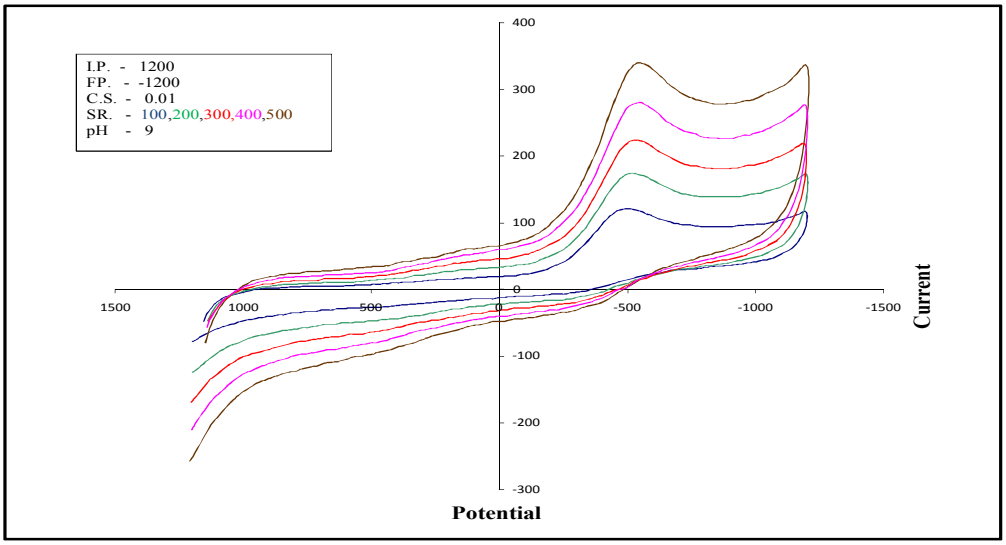
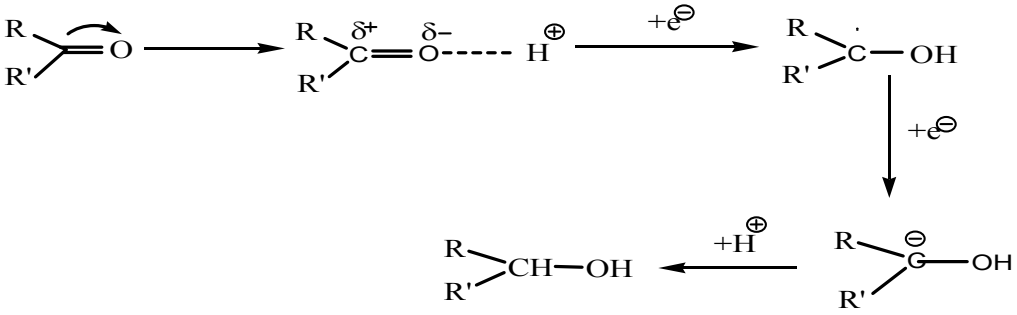


Fig.9 Effect of Scan rate on reduction of ethyl 2-methyl acetoacetate

Proposed Mechanism for Electrochemical Reduction: - Cyclic voltammogram(fig. 6-9) clearly indicates that in all cases the reduction is ir-reversible and involves transfer of two electrons.



Conclusion

The present work is an attempt to apply alternative synthetic routes using electrochemical as well as 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. Both methods can bring about the reduction in good yield but it is only the microbial catalyzed reduction which is selective in nature. Immobilization of the microbial catalyst has further additional advantages like reuse and easy workup besides cost effectiveness.

Table-1 Spectroscopic data for microbial reduction of compounds

S. No	Substrate Name	Reaction Time (In Hours)	B.P (^{0}C)	Yield Free BY (%)	Yield IMB BY (%)	IR Data (cm^{-1})	NMR Data (δ-Value)	Mass Spectra (m/z)	ee (%) Free BY	ee (%) IMB BY	Compound Confirmed
1	Ethyl 3-oxo hexanoate	48	98	76	84	3450 (O-H str) 2870 (C-H str) 1735 (C=O str) 1470 (C-H def) 1140 (C-O str)	4.12(CH2) 3.85(CH) 2.40(CH2)2.0(OH) 1.44(CH2) 1.33(CH2) 1.30(CH3)0.96(CH3)	159 131 117 97 89 71 60 55 45 43 39 29	89.4	92.6	Ethyl 3-hydroxy hexanoate
2	Ethyl levulinate	72	207	78	88	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	91	94.5	Ethyl 3-hydroxy valerate

3.	Ethyl 2-chloro acetoacetate	48	155	84	92	3450 (OH str) 2980 (CH str) 1740 (C=O str) 1350 (C-H def) 1180 (C-O str)	4.38(CH) 4.34(CH) 4.12(CH2) 2.0(OH) 1.30(CH3) 121(CH3)	165 151 137 112 89 55 45 43 39 29	92	93.5	Ethyl 2-chloro 3-hydroxy butanoate
4.	Ethyl 2-methyl acetoacetate	48	170	88	96	3450 (O-H str) 3060 (C-H str) 1765 (C=O str) 1470 (C-H def) 1140 (C-O str)	4.12(CH2) 4.02(CH) 2.57(CH) 2.0(OH) 1.30(CH3) 1.24(CH3) 1.21(CH3)	155 146 117 101 73 55 45 39 29	91.5	94.5	Ethyl 2-methyl 3-hydroxy butanoate

Table-2 Spectroscopic results of products obtained by electrochemical reduction:

S. No	Substrate Name	Reaction Time (In Hours)	B.P (^{0}C)	Yield (%)	IR Data (cm^{-1})	NMR Data (δ-Value)	Mass Spectra (m/z)	Compound Confirmed
1.	Ethyl 3-oxo hexanoate	6 hrs	98	98%	3450 (O-H str) 2870 (C-H str) 1735 (C=O str) 1470 (C-H def) 1140 (C-O str)	4.12(CH2) 3.85(CH) 2.40(CH2) 2.0(OH) 1.44(CH2) 1.33(CH2) 1.30(CH3) 0.96(CH3)	159 131 117 97 89 71 60 55 45 43 39 29	Ethyl 3-hydroxy hexanoate

2.	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
3.	Ethyl 2-chloro acetoacetate	6 hrs	155	96%	3450 (OH str) 2980 (CH str) 1740 (C=O str) 1350 (C-H def) 1180 (C-O str)	4.38(CH) 4.34(CH) 4.12(CH2) 2.0(OH) 1.30(CH3) 121(CH3)	165 151 137 112 89 55 45 43 39 29	Ethyl 2-chloro 3-hydroxy butanoate
4.	Ethyl 2-methyl acetoacetate	4 hrs	170	98%	3450 (O-H str) 3060 (C-H str) 1765 (C=O str) 1470 (C-H def) 1140 (C-O str)	4.12(CH2) 4.02(CH) 2.57(CH) 2.0(OH) 1.30(CH3) 1.24(CH3) 1.21(CH3)	155 146 117 101 73 55 45 39 29	Ethyl 2-methyl 3-hydroxy butanoate

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