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BIOCATALYTIC AND ELECTROCHEMICAL REDUCTION OF SELECTED PHENYL ALKANONES WITH ANTIBACTERIAL ACTIVITY OF THE CORRESPONDING PHENYL ALKANOLS

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ABSTRACT: This paper reports the production of some phenyl alkanols by the biocatalytic and electrochemical reduction of selected Phenyl alkanones viz. Propiophenone, Butyrophenone, and Valerophenone. Baker's yeast was used in its free as well as immobilized form for biocatalytic reduction. Substrates showed the higher conversion rate when the cells were used in immobilized form. The electrochemical behavior of substrates was investigated cyclic voltammetrically to explore electrochemical reduction as alternative synthetic route for the preparation of alcohols. Results obtained from cyclic voltammetric studies were used for establishing optimum conditions for electrochemical reduction which 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. The products thus obtained also exhibited significant antibacterial activity against four strains of bacteria viz. *Staphylococcus aureus, Enterococcus faecaulis, Escherichia coli, Pseudomonas aeruginosa.*

Key words: Propiophenone, Butyrophenone, Valerophenone, Baker's yeast (BY), Biocatalytic reduction, Immobilized Baker's yeast (ImBY), Antibacterial Activity, Cyclic Voltammetry, Stainless Steel Electrode (SS-316)

INTRODUCTION

Enantioselective ketone reduction is the most popular method for producing the chiral compounds which are used as the building blocks in organic synthesis (1). Although a large range of selective organic reactions available for most synthetic needs, but most of them are not stereospecific (2).Chemists have now become interested in reactions which are mediated by biocatalysts such as microorganisms or isolated enzymes. One of the major reactions which can be performed by Alcohol dehydrogenase, present in baker yeast is to enantioselective reduction of prochiral ketone to corresponding chiral alcohol (3). Baker's Yeast is an economical catalyst neither toxic nor pathogenic and yet has features like high efficiency, mild operational conditions, versatility and last but not the least high selectivites (chemo, regio, and stereroselectivity) (4).

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 reducates. The other is a cofactor regeneration system, which supplies NADH or NADPH through the oxidation of the energy source, such as carbohydrates and alcohols (5-8).
The work up procedure, efficiency & cost of the system can be enhanced by immobilization of the biocatalyst. 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. Immobilization of yeast was carried out by polyacrylamide gel (9-14).

Electro-analytical techniques like Polarography, Cyclic Voltammetry etc. and synthetic techniques like Electrolysis at Constant Current and Constant Potential can also be employed for analytical and synthetic purposes (15).This apporch avoids undesirable by products and simplifies the otherwise cumbersome workup procedure and also reduces pollution problem. Cyclic voltammetry (CV) (16) provides valuable information related to the kinetics of the electrode processes and on the rates of the processes which help in deciding optimum condition for reduction at constant current or constant potential (17-18).

Antimicrobial compositions are provided wherein there is obtained an enhancement of activity by combining the antimicrobial agent with an effective amount of a potentiator. Phenyl alkanol can be used as a potentintor.The compositions (antimicrobial agent + potentiating agent) are useful as surgical scrub solutions and in dressing topical wounds where the presence of blood and wound exudate would otherwise inhibit the action of the antimicrobial agent if it were to be used alone (19). Methyl phenyl pentanol derivatives are also used in augmenting or enhancing the aroma or taste of consumable materials including perfume compositions, colognes, perfumed articles, foodstuffs, chewing gums, medicinal products and chewing tobaccos.

A series of n-alkanols and phenyl-substituted n-alkanols were characterized for their ability to block action potentials (APs) in frog sciatic nerves. Phenyl group addition to an alkanol markedly increases the molecule's potency (20). Derivative of phenyl alkanol *i.e.1RS, 2SR*)-(±)-2-Amino-1-phenyl-1-propanol is a good vasoconstricting agent and clinically used as a nasal decongestant (21). Phenyl alkanols and esters of phenyl alkanols are used in augmenting, enhancing or modifying the aroma of perfume compositions and colognes (22-23). 1-Phenyl 1-propanol used in industry as a heat transfer medium, in the manufacture of perfumes, and as a choleretic in medicine. 1-Phenyl 1-pentanol is a synthetic derivative of an ingredient of Curcuma longa that is used as a condiment and dye. 1-Phenyl-1-pentanol also plays an important role in release of secretin, gastrin, and pancreatic secretion of bicarbonate and protein in both dogs and humans (24). It is a useful agent for release of secretin in subjects with achlorhydria, severe hyposecretory state, or total gastrectomy. Phenyl alcohols can be used as a cosurfactant in microimulsions (25).The aim of present work is to explore a novel ecofriendly method of synthesis of optically pure alcohol using free Baker's Yeast as well as immobilized Baker's Yeast and Electrochemical methods also. Inhibitory effect of the products on the biological activities of some bacteria was also studied.

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 Propiophenone, Butyrophenone, and Valerophenone was carried out as follows:

In a 500 liter round bottom flask, equipped with a magnetic stirrer (Remi-2MLH make) water (100 ml), fresh BY (10 g) and isopropanol (25ml) were placed and corresponding suspension was stirred for 30 minutes. The substrate (2 m mol) was separately dissolved in to ethanol (50 ml) and ethanolic solution was poured into Baker's Yeast suspension. The resulting mixture was made up with water: alcohol solution (50:50 v/v) to about 500 ml and it was magnetically stirred for a suitable period. 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) and the alcohol in the reaction mixture was separated by the distillation, after separation of alcohol, the resulting solution was saturated with sodium chloride and extracted with diethyl ether, and the 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: - The experiment was performed with Immobilized Baker's Yeast obtained insitu immobilization of Baker's Yeast (2g) in polyacrylamide gel under condition similar to those used for free Baker's Yeast. Details of immobilization of Baker's Yeast in Polyacrylamide gel are as follows. The solution used for preparation of immobilized BY in polyacrylamide gel was the following.
Solution A: - Acryl amide (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.

The above solutions were then mixed in following sequence-

Sol. A (10 ml) + sol.B (5 ml) + BY (2g) + sol.C (5 ml)

Solution D was added to the above solution & the whole solution was then deareated for 30 minutes.

Reduction using electroanalytical Techniques: - Cyclic voltammogram were recorded Using Pt electrodes at different pH and different scan rate with the help of a computer based Basic Electrochemistry system ECDA-001, supplied by Con-serv enterprises, Mumbai, using three electrode cell assembly with 1mm diameter glassy carbon as working electrode, Ag/AgCl as reference electrode and Pt wire as counter electrode. Blank voltammogram were also recorded after deareation of the solution. The voltammographic curves were recorded for compounds using potassium chloride (1M) as supporting electrolyte, methanol as a solvent in BR buffer at different pH (5, 7, and 9). On the basis of the results optimum condition for electrochemical reduction was determined. 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 sodium acetate (1M) was filled in both the limbs. The reactants (0.01M) were dissolved in alcohol and placed in cathodic chamber and the pH of cathodic solution was 9.Methanol was also added in cathodic chamber. 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 was worked up by extracting 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.

Characterization of the Product: -The characterization of the products was done on the basis of their IR, NMR& Mass spectral data which were recorded after checking their purity by thin layer chromatography. NMR spectra were recorded in $CDCl_3$ 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. Optical activity of products was measured by using a polarimeter and enantiomeric excess (ee) was calculated. These results are shown in Table-1

Antibacterial activity: **-** The synthesized compounds were screened for their antibacterial constituents against four strains of bacteria i.e. *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (MTCC 439), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) by Well diffusion method. Nutrient agar was used as culture medium. All compounds were dissolved in Ethanol. Streptomycin (2.5mg) was used as reference antibiotic and distilled water as control. The zones of inhibition formed were measured in mm. Results are shown in Table-3.

Table-2 Spectroscopic results of products obtained by electrochemical reduction

Compound	Reaction Time (In Hours)	B.P(^{0}C)	Yield (%)	IR Data (cm^{-1})	NMR Data (δ- Value)	Mass Spectra (m/z)
1-Phenyl-1-propanol	6	218	78	3445 (OH) 1605,1500&1470(C=C) 3005(ArCHStr) 2865(C-H) 1100&1285(C-OStr Sec.alcohol)	7.18(CH) 4.48(CH) 2.0(OH) 1.80(CH2) 0.97(CH3)	136 107 79 77 51
1-Phenyl-1-butanol	6	168	81	3360(OH) 1610,1515&1460(C=C) 3015(ArCHStr) 2870(C-H) 1100&1290(C-OStr Sec.alcohol)	7.17(CH) 4.51(CH) 2.1(OH) 1.75(CH2) 1.32(CH2) 0.96(CH3)	150 107 79 77 51
1-Phenyl-1-pentanol	7	135	83	3300(OH) 1610,1530&1465(C=C) 3010(ArCHStr) 2900(C-H) 1100&1295(C-OStr Sec.alcohol)	7.16(CH) 4.47(CH) 2.1(OH) 1.74(CH2) 1.29(CH2) 1.32(CH2) 0.94(CH3)	164 107 79 77 51

Table-1 Spectroscopic data for microbial reduction of compounds

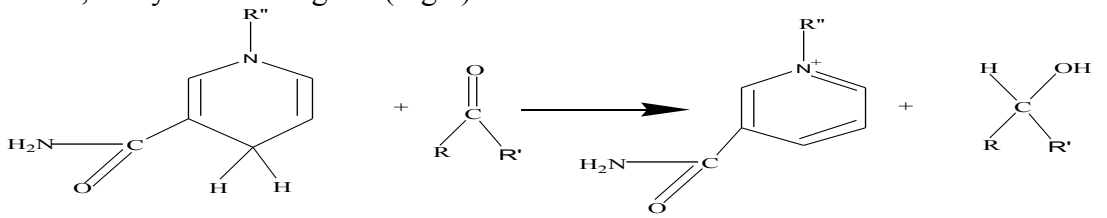
Compound	Reaction time(In Hours)	B.P (^{0}C)	Yield Free BY (%)	Yield ImBY (%)	IR Data (cm^{-1})	NMR Data (δ- Value)	Mass Spectra (m/z)	ee(%) Free BY	ee(%) ImBY
1-Phenyl-1-propanol	72	218	82	88	3400 (OH) 1605,1510&1470 (C=C) 3005(ArCHStr) 2850(C-H) 1100&1285(C-OStr Sec.alcohol)	7.18(CH) 4.48(CH) 2.1(OH) 1.82(CH2) 0.97(CH3)	136 107 79 77 51	89.5	92
1-Phenyl-1-butanol	96	167	87	96	3350(OH) 1600,1515&1460 (C=C) 3015(ArCHStr) 2865(C-H) 1100&1290(C-OStr Sec.alcohol)	7.17(CH) 4.51(CH) 2.0(OH) 1.74(CH2) 1.32(CH2) 0.95(CH3)	150 107 79 77 51	92	94.3
1-Phenyl-1-pentanol	72	136	78	89	3300(OH) 1610,1530&1465 (C=C) 3010(ArCHStr) 2905(C-H) 1100&1295(C-OStr Sec.alcohol)	7.16(CH) 4.47(CH) 2.1(OH) 1.76(CH2) 1.28(CH2) 1.31(CH2) 0.95(CH3)	164 107 79 77 51	91.5	94.6

Table-3 Effect of products on the growth of tested Bacteria

Sample Bacteria	Streptomycin (Reference)	1-Phenyl-1-propanol	1-Phenyl-1-butanol	1-Phenyl-1-pentanol
1.Staphylococus aureus (Gram positive)	42 nm	22 nm	30 nm	26nm
2.Enterococcus faecaulis (Gram positive)	40 nm	25 nm	29 nm	22 nm
3.Pseudomonas aeruginosa (Gram negative)	43 nm	20 nm	25 nm	31 nm
4.Escherichia coli (Gram negative)	35 nm	28 nm	30 nm	24 nm

RESULTS AND DISCUSSION

1) **Reduction using BY and ImBY:** - The actual reducing agent which is present in BY and ImBY is NADH (Nicotinamide Adenine Dinucleotide hydride) in limited amount. After reducing the substrate it is itself oxidised to NAD^+. Therefore, in order make reduction process continuous it is necessary to regenerate NADH from NAD^+ (Nicotinamide Adenine Dinucleotide Phosphate ion) .To achieve this isopropanol is added to the reaction mixture, which regenerates NADH from NAD^+ & is itself oxidized to acetone . 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).



where

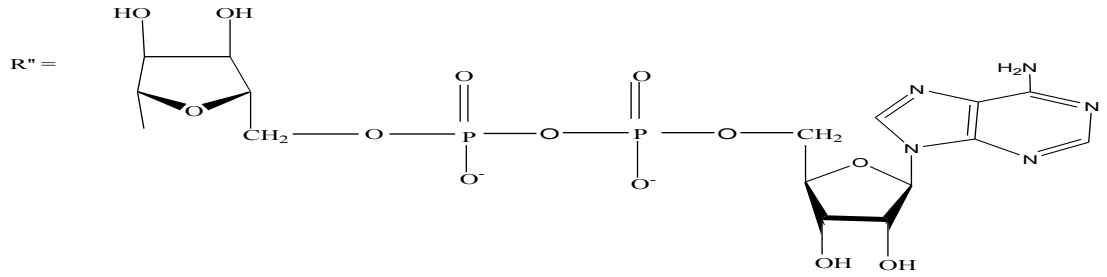


Fig.1 Mechanism for reduction of carbonyl compound by NADH

The classical methods generally involve use of either corrosive reagent or yield product which is burden to the ecosystem. The use of baker's yeast however offers an alternative to carry out reduction at room temperature using simple installation with an easy work-up. The process is essentially green and the yields are very good.

2) **Reduction using electroanalytical technique: -** The reduction of carbonyl compounds in aqueous solution depends on the pH of the system. Cyclic voltammograms were recorded in acidic, neutral and basic conditions. In acidic medium stainless steel electrode cannot be used hence no attempt were made to synthesize the product. The present method therefore offered single product phenyl alkanol in alkaline conditions. Parameters evaluated from cyclic voltammograms are shown in Table 4.

Table 4: Voltammeteric data evaluated from cyclicvoltamogram at pH 9.0

Compound	Scan rate(υ) (mV/s)	Epc(mV)	Ipc(µA)	Ip/√ υ
Propiophenone	100	-671	144	14.4
	200	-707	202	14.28
	300	-717	236	13.62
	400	-732	275	13.35
	500	-755	295	13.19
Butyrophenone	100	-737	154	15.4
	200	-750	212	14.99
	300	-767	256	14.78
	400	-776	293	14.65
	500	-788	343	15.33
Valerophenone	100	-654	153	15.3
	200	-671	215	15.20
	300	-681	268	15.47
	400	-699	310	15.5
	500	-705	365	16.32

3) **Effect of scan rate-** From cyclic voltammograms (fig2-4), it is clear that as the sweep rate was gradually increased to 100,200,300, 400 and 500mV/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.

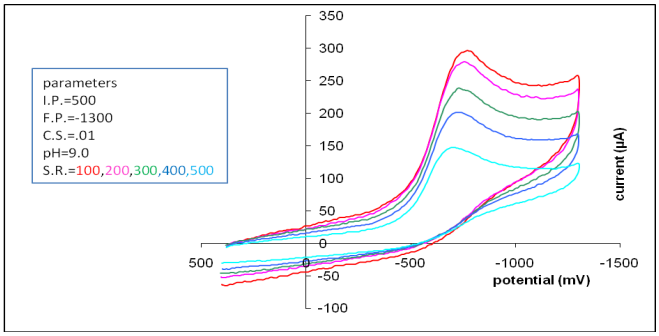


Fig 2: Effect of Scan rate on reduction of Propiophenone

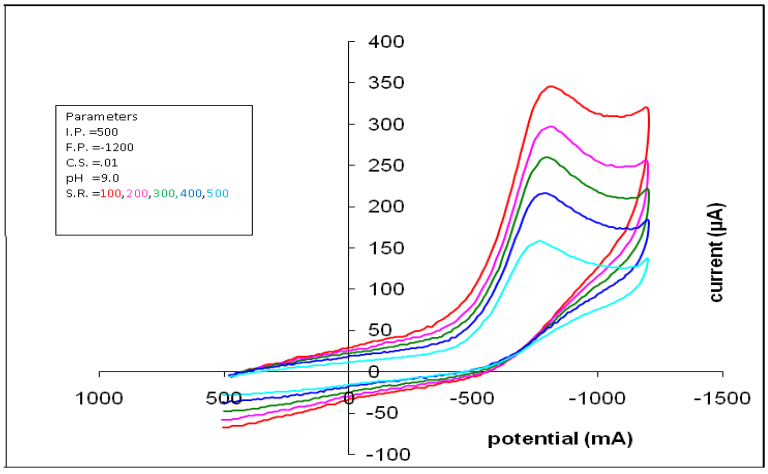


Fig3: Effect of scan rate on reduction of Butyrophenone

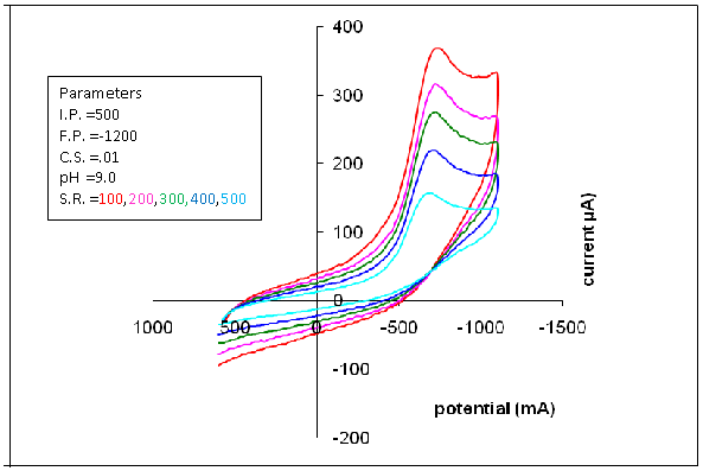


Fig 4: Effect of scan rate on reduction of Valerophenone

The dependence of the cathodic peak current on the squre root of sacn rate is linear with correlation coefficients close to unity at all the pH(Graph 1).

4) Effect of pH: Typical voltammogram of Propiophenone, Butyrophenone and Valerophenone has been shown in Fig. 5-7 from which it is clear that the reduction of compound can be carried out in acidic, neutral and basic medium. As it is clear from cyclic voltammogram peak height is high in case of basic medium so the reduction is expected to be feasible and will provide good yield. It was concluded that reduction can be best carried out in basic media due to these reasons: -

1. A sharp peak obtained in basic media.

2. With increase in pH potential have negative shift that shows reduction is easier in basic media.

3. Stainless steel (SS-316) electrode is best work in basic medium.

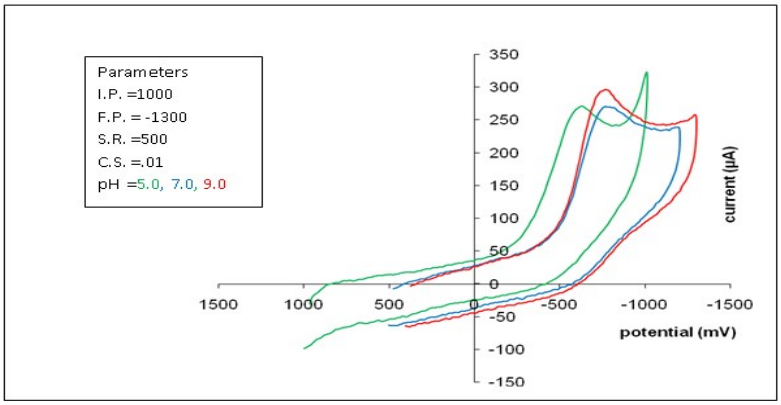


Fig 5: Effect of pH on reduction of Propiophenone

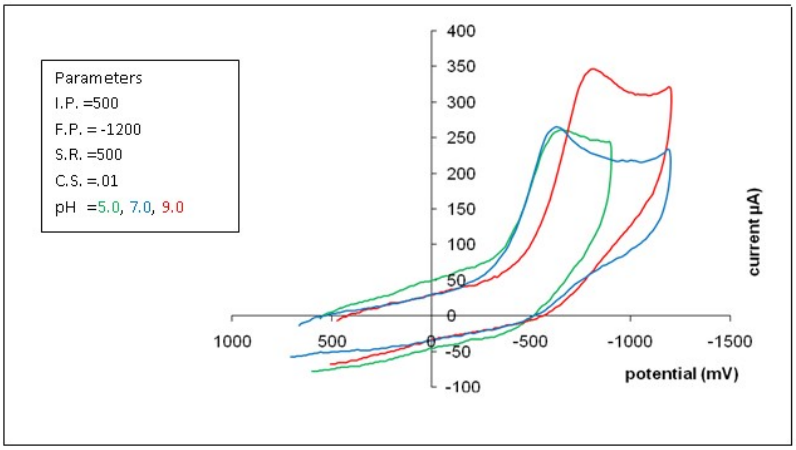


Fig 6: Effect of pH on reduction of Butyrophenone

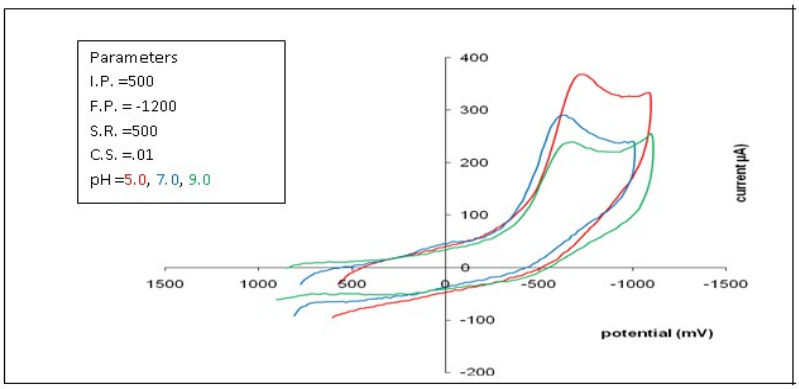
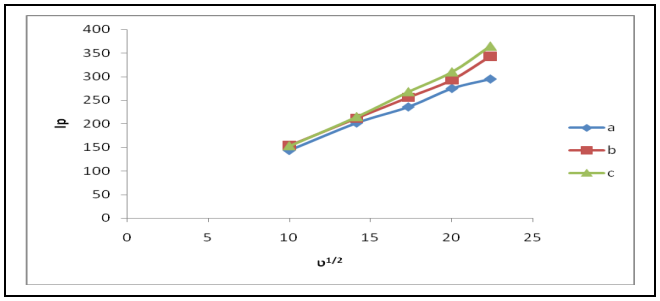


Fig 7: Effect of pH on reduction of Valerophenone

5) Antibacterial Activity: Phenyl alkanols synthesized via baker yeast mediated reduction were examined against four bacterial species i.e. *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (MTCC 439), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa (*ATCC 27853). Thus the results clearly demonstrate that compounds have good bactericidal activity.

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. The products thus obtained also exhibited significant antibacterial activity against four strains of bacteria viz. *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa.*



Graph 1: Variation of the cathodic peak current (Ip) with $\upsilon^{1/2}$ for a (Propiophenone), b (Butyrophenone), c (Valerophenone) at pH 9.0

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