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Research article

NOVEL ECO-FRIENDLY APPROACHES FOR THE REDUCTION OF SELECTED CARBONYL COMPOUNDS

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ABSTRACT: This paper reports reduction of (-)-verbenone, 1,3-indanedione, 3-indolyl methyl ketone and 2-furyl methyl ketoneinvolving two green and environment friendly methods viz. biotransformation using Baker's yeast as microbial catalyst in free as well as immobilized form and electrochemical method. Microbial transformation was carried out in water – isopropanol mixture (4:1) for selected substrates. The electrochemical reduction of these substrates was also carried out at constant current by using Stainless Steel Electrode (SS-316). The reduction products were isolated and purified by chromatographic techniques and characterized on the basisof spectral analysis.

Key words: Eco-friendly, Carbonyl compounds, Reduction

INTRODUCTION

The asymmetric reduction of ketones is one of the most important and fundamental techniques for producing non-racemic chiral alcohols which find practical application in obtaining industrially importantchemicals such as pharmaceuticals, agrochemicals andnatural products. Biotransformation using micro-organisms and electrochemical techniques using electrons as reducing agent has been employed as a novel and eco-friendly route to furnish chiral building blocks for producing fine chemicals (Mahmoodi N.O. and Navrood M. N , 2007). Biocatalysts due to their specific synthetic utility can prove to be boon for organic chemists to prepare chiral synthons. They can be constituted by whole cells of animals, plants and microorganisms (Mizrahi A, 1986, Arathoon W R, Birch J R, 1986). Baker's yeast (*Saccharomyces cerevisiae*) has been the most popular whole-cell biocatalyst, particularly for asymmetric reduction of carbonyl compounds which are building blocks for the formation of useful products for mankind (Yadav S R et al 2005, Mahmoodi N et al, 2003).

Verbenol, a bicyclic secondary allylic alcohol, with pronounced camphor and mint flavour,is mainly used as food flavouring in soft drinks, soups, meats, sausages and ice cream. It is also used to control harmful insects and hence has potential for use in agriculture and is an intermediate in the synthesis of valuable perfume and medicinal substances.1-(2-furyl)-ethanol, a chiral compound with a reactive furan moiety, is useful for cyclo-additionreactions (Rao S C V et al, 2003). 3-hydroxy-1-indanone is used as a chiral synthons in the preparation of wide range of compounds. 1-(3-indolyl)-ethanol and its derivatives are used in the preparation of anti-inflammatory drugs.

In this paper we are reporting the enantioselective reduction of the selected compounds to the corresponding alcohols by using Baker's yeast in free as well as immobilized form. The electrochemical reduction of these compounds using stainless Steel Electrode (SS - 316) at constant current is also being reported simultaneously. Prior to electrolysis,electrochemical behaviour of the substrates was investigated by cyclic voltammetry to establish optimum conditions for electrochemical reduction such as pH, solvent media, reduction potential range, supporting electrolyte and also the irreversible nature of the reaction (Vijay M et al, 2007).

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MATERIALS AND METHODS

All the chemicals were used of AR grade, triply distilled water was used for the making solutions and Baker's yeast used was of food grade and purchased from a local grocery shop.

Experimental

Reduction using Free Baker's Yeast

Biotransformation of (-)-verbenone, 1,3-indanedione, 3-indolyl methyl ketone and 2-furyl methyl ketone was carried out as follows:

In a one litre flat bottom flask, equipped with a magnetic stirrer (Remi – 2MLH make), 100 ml of water, 10 gm fresh Baker's yeast and 25 ml of isopropanol were placed and the suspension was stirred for 30 minutes and thereafter reactant (2mm) was dissolved in minimum quantity of absolute alcohol. The alcoholic solution was poured gradually into the Baker's yeast suspension. The resulting mixture was magnetically stirred for appropriate time (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 (3 times) and ether extracts were combined dried over sodium sulphate. After evaporation of ether, the product was isolated, purified and characterized by spectral techniques such as IR, NMR and Mass spectroscopy.

Reduction using immobilised Baker's Yeast.

For preparation of immobilized baker's yeast (ImBY) using 2 gm BY in 5% polyacrylamide gel, mixing the following solution in the given proportion:

Solution A: Acrylamide (10g) and N,N'-methylene bisacrylamide (2.5g) in 100 ml DDW.

Solution B: Trihydroxy methyl Amino Methane, TRIS (5.98g), N,N,N',N",-tetramethylEthylenediamine, TEMED (0.46g) and 1NHCl solution (48 ml) to 100 ml.

Solution C: Ammonium per sulphate (560 mg/100ml) in DDW.

Solution D: 25 ml of isopropanol.

All these solutions were mixed in following proportion and then dearated for 30 minutes: 10.0 ml of solution A + 5.0 ml of solution B + 2 gm of Baker's Yeast + 5.0 ml of solution C + 10.0 ml of solution D. The optimum pH and temperature were determined and found to be 5.0 – 7.0 and 27 °C respectively. Time required for maximum product formation was determined by analysing the product after every 30 minutes interval. An agitation speed of 120 rpm and biotransformation time were employed (Table 1). Optimum solvent/emulsifier type and concentration to be employed was standardized by addition of these to Baker yeast cells. During our investigations, it was observed that BY is an effective reducing agent in all the media and yield varied from 95-70%. Immobilized baker yeast cell could be re-employed twice after the first use. Gel pieces were activated in nutrient rich media (sucrose solution) at pH 7 and 27°C for 12 hrs, before the first use. The product concentrations during the first and second reuse were 65% and 50%.

Reduction using Electro – Analytical Technique.

The cyclic voltammograms of selected compound were recorded at different pH and at different scan rate by using a Computer based ECDA-001 supplied by Con – Serv Enterprises, Mumbai (INDIA) and three electrode cell assemblies with 1 mm diameter glassy carbon as working electrode, Ag/AgCl as reference electrode and Pt wire as counter electrode. This experiment was carried out using 1 ml of KCl (1M) as electrolyte, 5 ml of BR buffer(0.04N) of desired pH (5, 7 or 9), 1 ml of reactant (0.01M) and methanol or DMF as a solvent for make up the final solution to 10 ml. Thereafter the mixture was purged with nitrogen gas for 10 minutes to remove the dissolved oxygen and the voltammographic curves were recorded at different pH viz. 5, 7 & 9 and scan rates of 50, 100, 200, 300, 400 & 500. The voltammogramwith blank was taken prior to this. On the basis of these results the optimum conditions w.r.t. nature of solvent and pH for electrochemical reaction are determined which were subsequently applied for the galvanostatic electrochemical reduction at stainless Steel Electrode (SS-316). The conventional H-type cell with two limb separated by G-4 disc was used for electrolysis. The sodium acetate solution (1M) was filled in both limbs as electrolyte and pH was maintained at 5.0. The reactant was dissolved in minimum quantity of alcohol and placed in cathodic limb of cell slowly. The stainless steel electrodes (SS-316) were used as cathode as well as anode. The constant current electrolysis was done for 6-8 hrs by using galvanostate designed and developed by CDPE (Centre for Development for Physical Education), University of Rajasthan, Jaipur (INDIA). The reaction mixture was extracted with diethyl ether (3x25 ml). The ether layer was separated and washed with aqueous saturated NaCl solution. The organic extract was dried over anhydrous Na_2SO_4 and finally the product was characterized by spectroscopically.

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RESULTS AND DISCUSSION

Biotransformation:

Baker's yeast (BY) is a common microorganism and it can be easily used in free and immobilized form (ImBY) for synthesis of optically pure chiral alcohol.Two enzyme systems are used in whole cell catalyst biotransformation, one is carbonyl reductases which converts prochiral carbonyl compound to chiral alcohol and other is a cofactor regeneration system which provides NADH or NADPH through the oxidation of energy source, such as carbohydrates and alcohols (Ojha P et al, 2011). The actual reducing agent in present system is NADPH (Nicotinamide Adanine Dinucleotide Phosphate Hydride) which donates hydride ion ($:H^-$) to aldehydes and ketones. It reduces them and is itself oxidised to $NADP^+$. The lone pair of electrons on a nitrogen atom of NADPH pushes out $:H^-$ which adds to a carbonyl group of the substrate to cause a reduction. The amount of NADPH in the yeast cell is limited, after reducing the substrate it is itself oxidised to $NADP^+$. Therefore, to make the reduction process continuous, it is necessary to reduce $NADP^+$ (Nicotinamide Adenine Dinucleotide Phosphate ion) in to NADPH. Regeneration of NADPH from $NADP^+$ can also be done chemically by adding isopropanol which is oxidized to acetone.Addition of sucrose activates the pentose-phosphate pathway and thus by converts $NADP^+$to NADPH. Immobilization enhances the operational stability of FBY and isolation of the product is easier. In addition, reuse of the catalyst is often possible under these conditions and yield is also good. Under these conditions, the product formation rates are usually high.

Table 1: Reduction using FBY &ImBY technique.

S. No.	Product name	Reaction time (hrs.)	Yield %		BP (°C)	IR Values (cm^{-1})	NMR Values (δ)
			With FBY	With ImBY			
1.	(-)-verbenol	72	78	80	215	3350, 3030, 2930, 1750, 1460	1.0-1.9, 2.1-2.4, 4.4, 5.3
2.	3-hydroxy-1-indanone	72	82	86	343	3350, 1450, 1720, 3030	2.6, 3.0, 5.4, 7.3-7.9
3.	1-(3-indolyl)ethanol	72	80	84	174	3370, 1460, 2940, 3040	1.2, 3.4, 3.6, 3.9, 6.7-6.9
4.	1-(2-furyl)ethanol	72	82	85	167	3360, 1470, 2930, 3030	1.2, 4.6, 6.1, 6.2, 7.3

Table 2: Reduction using electrochemical technique

S. No.	Name	Reaction time (hrs.)	Yield %	BP (°C)	IR Values (cm-1)	NMR Values (δ)
1.	(-)-verbenol	8	80	215	3350, 3030, 2930, 1750, 1460	1.0-1.9, 2.1-2.4, 4.4, 5.3
2.	3-hydroxy-1-indone	8	82	343	3350, 1450, 1720, 3030	2.6, 3.0, 5.4, 7.3-7.9
3.	1-(3-indolyl)ethanol	8	84	174	3370, 1460, 2940, 3040	1.2, 3.4, 3.6, 3.9, 6.7-6.9
4.	1-(2-furyl)ethanol	8	82	167	3360, 1470, 2930, 3030	1.2, 4.6, 6.1, 6.2, 7.3

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Table 3: Current potential measurements by cyclic voltammetry of (-)-verbenone

Condition applied:
Initial potential E_i: 1000 mV
Switching potential E_s: -1500 mV
Current Sensitivity: - 0.01mA

Electrodes applied:
Working Electrode: Platinum
Reference Electrode: Ag/AgCl
Auxilliary Electrode: Platinum

S.N.	Medium	Scan Rate	Anodic Wave		Cathodic Wave		Effect of scan rate	Remark (Cathodic wave)
	(pH)	(mV/s)	E_{pa}(mV)	I_{pa}(µA)	E_{pc}(mV)	I_{pc}(mA)	(Cathodic wave)	
1	7	100	no peak		-472	123	with increase in peak scan rate peak potential shifts to negative direction	Irreversible
2	7	200	no peak		-497	167		Irreversible
3	7	300	no peak		-516	182		Irreversible
4	7	400	no peak		-554	253		Irreversible
5	7	500	no peak		-646	290		Irreversible

Table 4: Kinetic Parameters for the reduction of (-)-verbenone

scan rate	pH	Ep	Ip	BV	a	a/2	IP/2= BV+a/2	√v	IP/√v	Ep/2	α n a
200	5.0	-536	176	145	31	15.5	160.5	14.14214	12.44508	-712	0.002716
300	5.0	-528	192	156	36	18	174	17.32051	11.08513	-754	0.002115
400	5.0	-546	233	182	51	25.5	207.5	20	11.65	-769	0.002143
500	5.0	-573	264	217	47	23.5	240.5	22.36068	11.80644	-828	0.001875
100	7.0	-472	123	96	27	13.5	109.5	10	12.3	-702	0.002078
200	7.0	-497	167	128	39	19.5	147.5	14.14214	11.80868	-748	0.001904
300	7.0	-516	182	171	11	5.5	176.5	17.32051	10.50777	-799	0.001689
400	7.0	-554	253	233	20	10	243	20	12.65	-824	0.00177
500	7.0	-646	290	232	58	29	261	22.36068	12.96919	-901	0.001875
100	9.0	-446	137	108	29	14.5	122.5	10	13.7	-899	0.001055
200	9.0	-496	209	155	54	27	182	14.14214	14.77853	-898	0.001189
300	9.0	-513	236	168	68	34	202	17.32051	13.62547	-897	0.001245
400	9.0	-522	257	216	41	20.5	236.5	20	12.85	-896	0.001278
500	9.0	-566	333	245	88	44	289	22.36068	14.89221	-895	0.001453

Table 5: Current potential measurements by cyclic voltammetry of 1,3-indanedione

Condition applied:
Initial potential E_i: 1000 mV
Switching potential E_s: -1500 mV
Current Sensitivity: - 0.01mA

Electrodes applied:
Working Electrode: Platinum
Reference Electrode: Ag/AgCl
Auxilliary Electrode: Platinum

S.N.	Medium	Scan Rate	Anodic Wave		Cathodic Wave		Effect of scan rate	Remark (Cathodic wave)
	(pH)	(mV/s)	E_{pa}(mV)	I_{pa}(µA)	E_{pc}(mV)	I_{pc}(mA)	(Cathodic wave)	
1	7	100	no peak		-550	110	with increase in peak scan rate peak potential shifts to negative direction	Irreversible
2	7	200	no peak		-572	156		Irreversible
3	7	300	no peak		-602	194		Irreversible
4	7	400	no peak		-634	225		Irreversible
5	7	500	no peak		-662	242		Irreversible

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Table 6: Kinetic Parameters for the reduction of 1,3-indanedione

scan rate	pH	Ep	Ip	BV	a	a/2	IP/2= BV+a/2	√v	IP/√v	Ep/2	α n a
100	7.0	-550	110	76	34	17	93	10	11	-694	0.003319
200	7.0	-572	156	97	59	29.5	126.5	14.14213562	11.03087	-712	0.003414
300	7.0	-602	194	127	67	33.5	160.5	17.32050808	11.2006	-754	0.003145
400	7.0	-634	225	152	73	36.5	188.5	20	11.25	-769	0.003541
500	7.0	-662	242	164	78	39	203	22.36067977	10.82257	-828	0.00288
100	9.0	-505	122	87	35	17.5	104.5	10	12.2	-702	0.002426
200	9.0	-560	162	115	47	23.5	138.5	14.14213562	11.45513	-748	0.002543
300	9.0	-590	191	130	61	30.5	160.5	17.32050808	11.02739	-799	0.002287
400	9.0	-593	224	147	77	38.5	185.5	20	11.2	-824	0.002069
500	9.0	-617	270	197	73	36.5	233.5	22.36067977	12.07477	-901	0.001683

Table 7: Current potential measurements by cyclic voltammetry of 3-indolyl methyl ketone

Condition applied:

Initial potential E_i: 1000 mV

Switching potential E_s: -1300 mV

Current Sensitivity: - 0.01mA

Electrodes applied:

Working Electrode: Platinum

Reference Electrode: Ag/AgCl

Auxilliary Electrode: Platinum

S.N.	Medium	Scan Rate	Anodic Wave		Cathodic Wave		Effect of scan rate	Remark (Cathodic wave)
	(pH)	(mV/s)	E_{pa}(mV)	I_{pa}(µA)	E_{pc}(mV)	I_{pc}(mA)	(Cathodic wave)	
1	9	100	no peak		-2006	1064	with increase in peak scan rate peak potential shifts to negative direction	Irreversible
2	9	200	no peak		-2009	1340		Irreversible
3	9	300	no peak		-2016	1640		Irreversible
4	9	400	no peak		-2018	1825		Irreversible
5	9	500	no peak		-2018	2200		Irreversible

Table 8: Kinetic Parameters for the reduction of 3-indolyl methyl ketone

scan rate	pH	Ep	Ip	BV	a	a/2	IP/2= BV+a/2	√v	IP/√v	Ep/2	α n a
100	7.0	-1918	1076	870	206	103	973	10	107.6	-959	-0.0005
200	7.0	-1926	1300	1033	267	133.5	1166.5	14.14214	91.92388	-963	-0.0005
300	7.0	-1932	1567	1391	176	88	1479	17.32051	90.47079	-966	-0.00049
400	7.0	-1934	1823	1709	114	57	1766	20	91.15	-967	-0.00049
500	7.0	-1938	2445	2266	179	89.5	2355.5	22.36068	109.3437	-969	-0.00049
100	9.0	-2002	1064	928	136	68	996	10	106.4	-1001	-0.00048
200	9.0	-2009	1340	1146	194	97	1243	14.14214	94.75231	-1004.5	-0.00048
300	9.0	-2016	1640	1539	101	50.5	1589.5	17.32051	94.68544	-1008	-0.00047
400	9.0	-2010	1825	1679	146	73	1752	20	91.25	-1005	-0.00048
500	9.0	-2018	2200	2018	182	91	2109	22.36068	98.38699	-1009	-0.00047

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Table 9: Current potential measurements by cyclic voltammetry of 2-furyl methyl ketone

Condition applied:
Initial potential E_i: 1000 mV
Switching potential E_s: -1300 mV
Current Sensitivity: - 0.01mA

Electrodes applied:
Working Electrode: Platinum
Reference Electrode: Ag/AgCl
Auxilliary Electrode: Platinum

S.N.	Medium	Scan Rate	Anodic Wave		Cathodic Wave		Effect of scan rate	Remark (Cathodic wave)
	(pH)	(mV/s)	E_{pa}(mV)	I_{pa}(µA)	E_{pc}(mV)	I_{pc}(mA)	(Cathodic wave)	
1	9	100	no peak		-424	162	with increase in peak scan rate peak potential shifts to negative direction	Irreversible
2	9	200	no peak		-462	226		Irreversible
3	9	300	no peak		-516	278		Irreversible
4	9	400	no peak		-530	332		Irreversible
5	9	500	no peak		-580	370		Irreversible

Table 10: Kinetic Parameters for the reduction of 2-furyl methyl ketone

scan rate	pH	Ep	Ip	BV	a	a/2	IP/2= BV+a/2	√v	IP/√v	Ep/2	α n a
100	7.0	-500	131	100	31	15.5	115.5	10	13.1	-694	0.002464
200	7.0	-515	169	154	15	7.5	161.5	14.14213562	11.9501	-712	0.002426
300	7.0	-548	210	179	31	15.5	194.5	17.32050808	12.12436	-754	0.00232
400	7.0	-567	239	200	39	19.5	219.5	20	11.95	-769	0.002366
500	7.0	-596	261	206	55	27.5	233.5	22.36067977	11.67227	-828	0.00206
100	9.0	-424	162	151	11	5.5	156.5	10	16.2	-702	0.001719
200	9.0	-462	226	196	30	15	211	14.14213562	15.98061	-748	0.001671
300	9.0	-516	278	233	45	22.5	255.5	17.32050808	16.05034	-799	0.001689
400	9.0	-530	332	292	40	20	312	20	16.6	-824	0.001626
500	9.0	-580	370	345	25	12.5	357.5	22.36067977	16.5469	-901	0.001489

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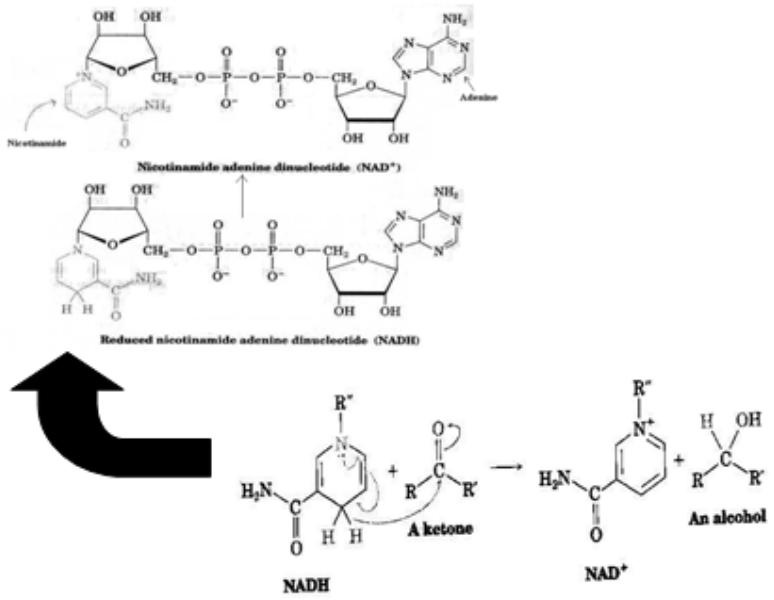

Fig. 1: Diagrammatic representation of reduction

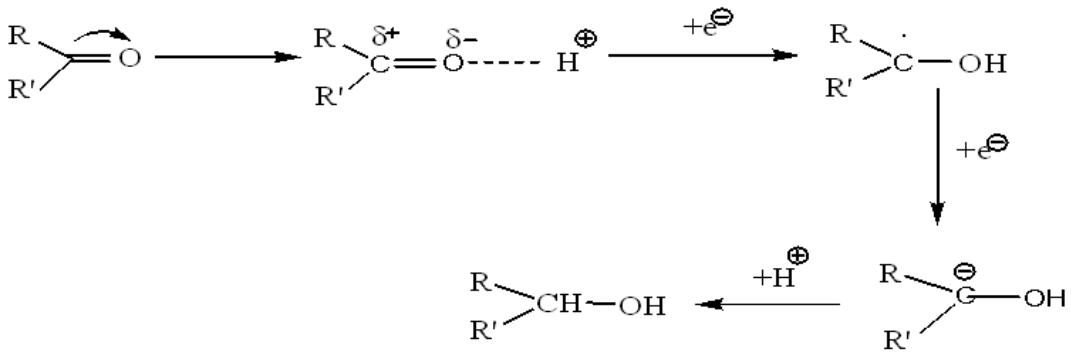

Fig. 2: Proposed Mechanism showing electrochemical reduction

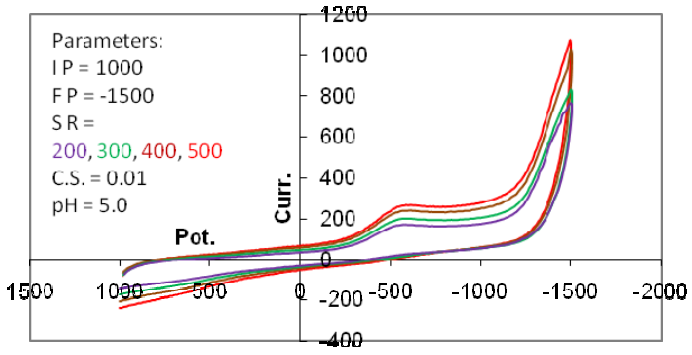

Fig-3: Cyclic voltammogram of (-)-verbenone at pH 5

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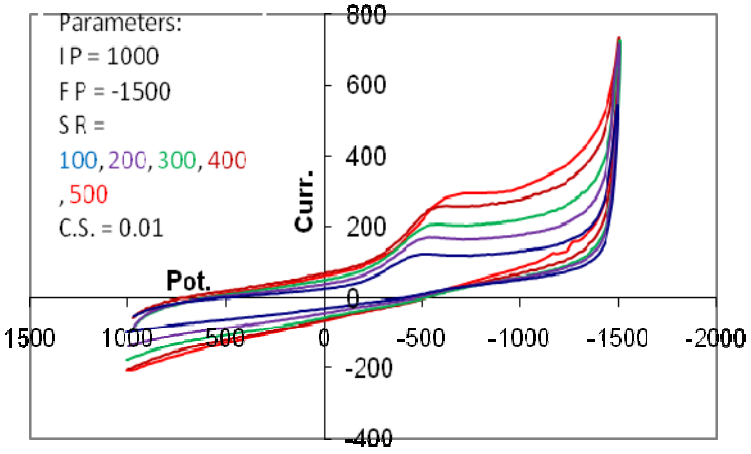

Fig-4: Cyclic voltammograms of (-)-verbenone at pH 7

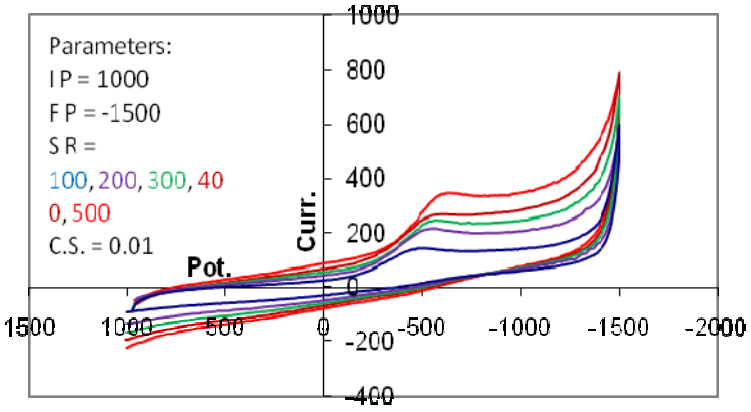

Fig-5: Cyclic voltammograms of (-)-verbenone at pH 9

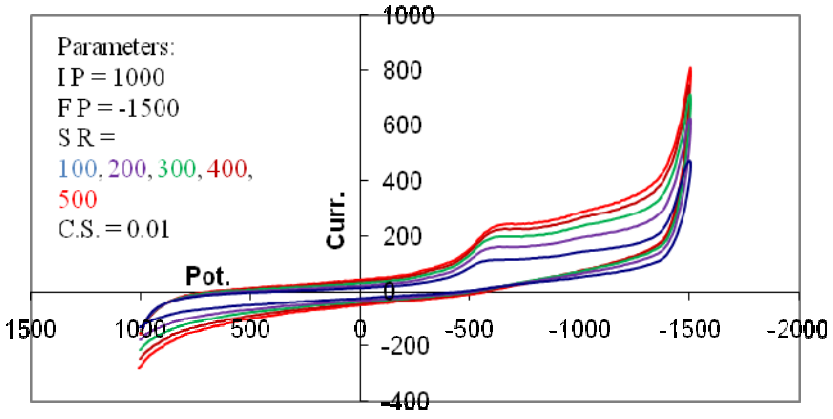

Fig-6: Cyclic voltammograms of 1,3-indandione at pH 7

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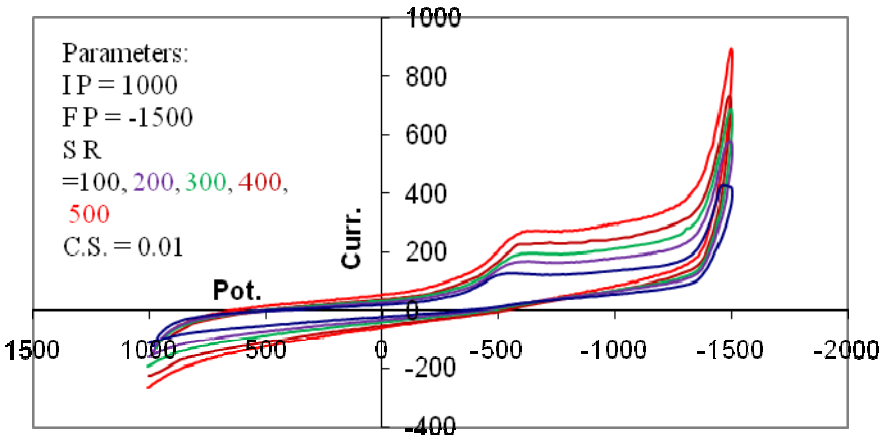

Fig-7: Cyclic voltammograms of 1,3-indandione at pH 9

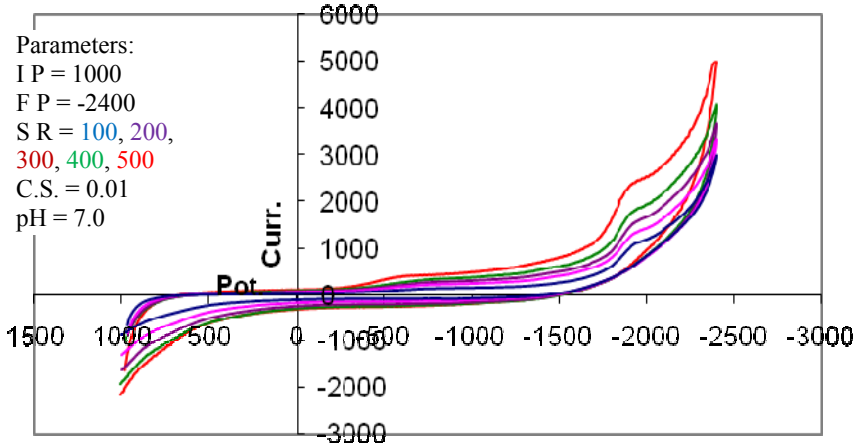

Fig-8: Cyclic voltammograms of 3-indolyl methyl ketone at pH 7

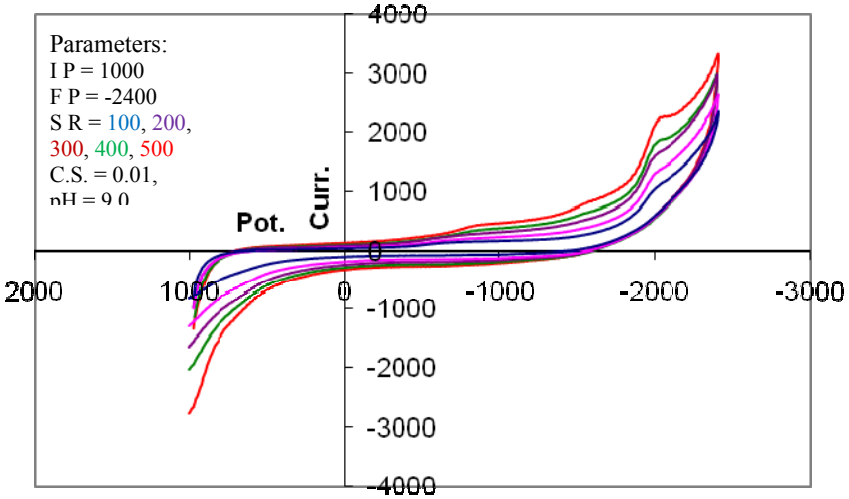

Fig-9: Cyclic voltammograms of 3-indolyl methyl ketone at pH 9

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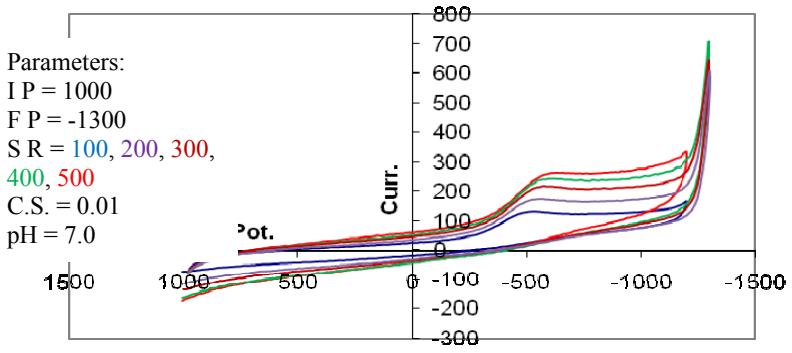

Fig-10: Cyclic voltammograms of 2-furyl methyl ketone at pH 7

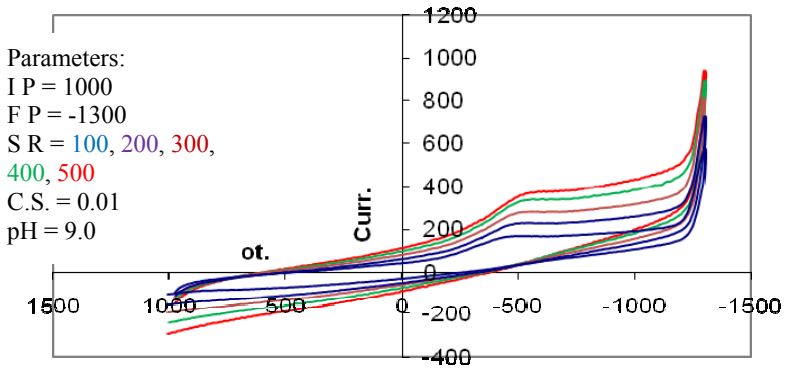

Fig-11: Cyclic voltammograms of 2-furyl methyl ketone at pH 9

Electrochemical Transformation:

Most cyclic voltammograms wererecorded with a potential range of 1000 mV (Ei) to -1500 mV (Es) at different scan rates (viz. 50, 100, 200, 300, 400, and 500mV/sec.) and different pH (pH 5, 7 and 9). In all cases single irreversible peak was observed. Electrochemical reduction of (-)-verbenone, 1,3-indanedione, 3-indolyl methyl ketone and 2-furyl methyl ketone has been carried out using constant current electrolysis.The products were obtained in reasonably good yields. The purity of compound was checked by single spot TLC and product was characterized by spectroscopically.

Effect of scan Rate and pH: The effect of scan rate on peak potential, a peak current was analysed at different pH using BR buffer. In all cases, the peak potential (Ep) shifts towards more negative side as scan rate (√) increases, which indicate irreversible nature of electrochemical process. The cathodic peak current (Ip) increases with increasing scan rate. The current function ($ip/\sqrt{v}$) has been found to constant with respect scan rate that mean the process is diffusion controlled. The reduction of carbonyl compounds in aqueous solution is pH dependent. In all cases, only one reduction peak appears in all pH.

CONCLUSION

Baker's yeast in free as well as in immobilized form successfully employed in the asymmetric reduction of (-)-verbenone, 1,3-indanedione, 3-indolyl methyl ketone and 2-furyl methyl ketone to the corresponding alcohols. The electrochemical reduction at constant current provides an alternative synthetic route for the synthesis of these products. Both processes in contrast with conventional chemical method involve clean and green methodology and have merits like specificity, cost effectiveness and therefore they are expected to reduce the ever increasing problem of pollution caused by hazardous, corrosive and harsh reaction conditions.

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