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Synthesis of 3-Hydroxy cyclohexanone by Electrochemical and Microbial techniques

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Abstract: 3-hydroxy cyclohexanone was synthesized by two-ecofriendly procedures viz. electro-organic synthesis using Stainless Steel (SS-316) electrodes in alkaline medium under Galvanostatic (constant current) conditions and biotransformation using Baker's Yeast in polyacrylamide gel. Biotransformation offered optically pure hydroxy ketone (ee=93.3%).
Keywords : Stainless Steel (SS-316), Controlled Current Synthesis, Biotransformation, Baker's Yeast (BY) and Immobilized Baker's Yeast(ImBY).

1. Introduction

The present research work describes the two-ecofriendly procedures viz. electro-organic synthesis using Stainless Steel (SS-316) electrodes and biotransformation involving use of free as well as immobilized baker's Yeast.

Electro-organic synthesis is now a well-established technique (1-2) to synthesize the desired compounds by oxidation or reduction appropriate substrates. Such reactions follow a green pathway, since the electrons, obtained during electrochemical reaction act as a reagent. 1,3-diketones represent an interesting class of compounds to study their reduction reactions. Some diketones have been reduced at platinized platinum electrode in acid medium to hydrocarbon (3-4) where others at dropping mercury electrode in a protic medium are reduced to keto alcohol or pinacol and formation of which depends upon the potential of the electrode (5-6). The reduction of diketones depends upon the pH of the solution and the electrode material. Intramolecular interaction between the two C=O functions has been detected during electrochemical reaction (7-8).

Biotransformations (9) utilize biological systems to carry out chemical transformations. Isolated enzymes or whole cells are used to bring about the desired in biotransformations. Such processes are now playing an increasingly important role in research laboratory as well as in industry because they occur under extremely mild as well as environmentally friendly conditions and have selectivity, which often greater that the one achieved encountered with syntheses using traditional laboratory reagents. Thus biotransformation (10) present viable alternatives to purely chemical synthetic routes or they be used in a complimentary manner in a

multi-step organic synthesis. Recently a concise introduction to this burgeoning field including biotrans formation of drugs in humans has been reported (11).

A vast number of biotransformation (12-13) of organic compounds has been carried out using free Baker's Yeast (BY); and its coenzyme NADH (Nicotinamide Adenine Dinucleotide Hydride) (Enzyme dehydrogenase of BY are involved in bringing about the reduction and the reduction is stereospecific) The use of immobilized Baker's yeast (ImBY) (14-20) as biocatalyst has several advantages such as removal of the biocatalyst from the reaction mixture is easy and it's repeated use is also possible. Inspite of these attractive features of immobilized microorganism the use of ImBY has surprisingly been not so frequent. 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 1, 3-cyclohexanedione to 3-hydroxy cyclohexanone.

2 Experimental

2.1 Electro-organic Synthesis of 3-hydroxy cyclohexanone

Preparative electro-organic synthesis, utilizing the optimum conditions derived from cyclic voltammetric studies, was then carried out at pH= 9.0 in a conventional H-type cell having two limbs separated by G-4. The supporting electrolyte sodium acetate (250 ml, 1M) was filled equally in both the limbs. 1, 3-cyclohexanedione (4.4850 gm) was dissolved in the alcohol and placed in the cathodic chamber. The Stainless Steel (SS- 316) electrodes having an area 2 x 3 cm^2 were used as cathode as well as anode. The constant current (1 amp.) was passed through the electrotype for 8 hours with the help of a Galvanostat (Designed and made by the Center for Development of Physics Education (CDPE), University of Rajasthan, Jaipur).

2.2 Biotransformation of 1, 3-cyclohexanedione to 3-hydroxy cyclohexanone

In a one litre round bottom flask, equipped with magnetic stirrer 200 ml water, 5 gm fresh Baker's Yeast (BY) and 4 gm glucose were placed and the suspension was stirred for 30 minutes. The reactant (2 m mol) was separately dissolved into absolute alcohol (minimum quantity) and ethanolic solution was poured into the BY suspension. The resulting solution was magnetically stirred for suitable period.

The Biotransformations was performed with Immobilized Baker's Yeast (ImBY) obtained from 10gm Baker's Yeast in polyacrylamide gel in place of free BY and rest part of reaction procedure was the same in both cases. Immobilization of the BY in polyacrylamide gel was carried out by the procedure reported earlier our group (21).

2.3 Workup and characterization of the product

After the completion of the reaction in the above two experiments (electrochemical synthesis and biotransformation) the resulting mixture was filtered. The solution was concentrated by removing water from the solution by distillation. The residue was then extracted repeatedly with diethyl ether. The ether layer was allowed to evaporate. After evaporation product was isolated, purified and characterized by combined application chromatographic techniques and spectroscopy viz. IR (Model FTIR-8400 S Shimadzu), NMR (Model JEOL-AL-300) (H^1, C^{13}), Mass data given in Scheme 1 and Table 2 & 3.

Optical rotation (22) of the products was measured using a polarimeter (Model 343 S Polarimeter 60 Hz, Sigma).

2.4 Calculation of Enantiomeric excess (ee) or optically purity

$$\text{Enantiomeric excess (ee \%)} = \frac{[\alpha]_{mix}}{[\alpha]_{pure}} \times 100$$

2.4a For Free Baker's Yeast

$$\text{Enantiomeric excess (ee \%)} = \frac{16.7}{18} \times 100$$

$$= 92.77\ \%$$

2.4b For Immobilized Baker's Yeast

$$\text{Enantiomeric excess (ee \%)} = \frac{16.8}{18} \times 100$$

$$= 93.3\ \%$$

2.4c For electrochemical reaction there is racemic product is obtained.

3 Results and Discussion

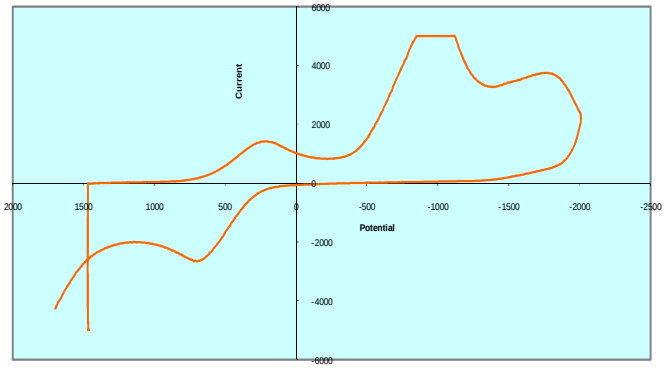
Cyclic voltammograms were recorded by using Basic electrochemistry system (ECDA-001 model; Con-serve Enterprises Mumbai India).

The voltammographic curves of 1, 3-cyclohexanedione 0.1M in aqueous medium, potassium chloride 1M as supporting electrolyte and BR buffer (pH= 4.0, 7.0 and 9.0) at glassy carbon electrode using Ag/AgCl as reference electrode are recorded.

3.1 Effect of Scan rate

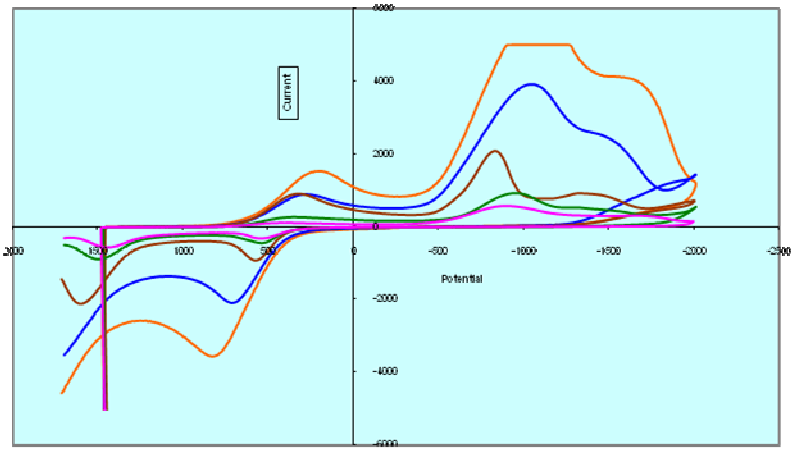
The cyclic voltammograms were recorded with an initial potential Ei 1700mv and final (switching) potential E_s of –2000mv at different scan rates and different pH which shows irreversibility nature of the system that is given in Fig. 1, 2 & 3 and data in Table 1. Double cycle voltammograms were recorded to monitor the fate of products generated in first cycle.

Fig.1 Cyclic Voltammograms of 1,3-Cyclohexanedione at different scan rates



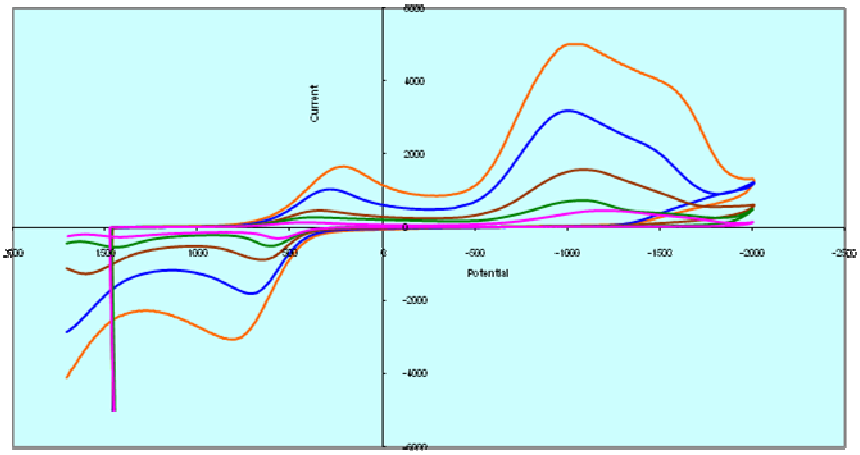
Parameters
IP=1700 mV
FP=-2000 mV
SR=50, 100, 200, 500,1000
CS=0.1 µA
PH=4.0

Fig.2 Cyclic Voltammograms of 1,3-Cyclohexanedione at different scan rates



Parameters
IP=1700 mV
FP=-2000 mV
SR=50, 100, 200, 500,1000
CS=0.1 µA
PH=7.0

Fig.3 Cyclic Voltammograms of 1,3-Cyclohexanedione at different scan rates



Parameters
IP=1700 mV
FP=-2000 mV
SR=50, 100, 200, 500,1000
CS=0.1 µA
PH=9.0

Table 1: Current potential measurements by cyclic voltammetry for 1, 3-cyclohexanedione

Condition applied:
Initial potential E_i : 1700 mV
2000mV

Electrodes applied:
Working Electrode: Glassy Carbon Switching potential E_s: -
Reference Electrode: Ag/AgCl
Auxillary Electrode: Platinum

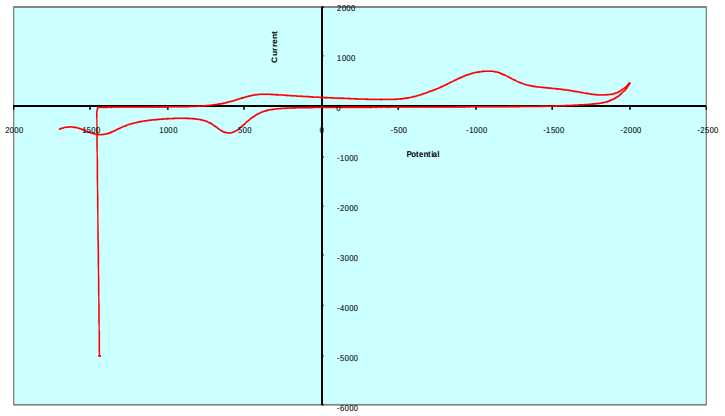
S.N	Medium	Scan rate	Cathodic Wave		Effect of scan rate	Remark (Cathodic wave)
	pH	(mV/s)	E_{pc}(mV)	I_{pc}(µA)		
1	4.0	50	-734	513	With increase scan rate peak potential shifts towards negative side of potential	irreversible
2	4.0	100	-762	1262		Irreversible
3	4.0	200	-810	2097		irreversible
4	4.0	500	-880	3008		irreversible
5	4.0	1000	-992	4198		irreversible
6	7.0	50	-946	565		irreversible
7	7.0	100	-978	931	Peak potential show considerable cathodic shift of potential with increasing scan rates.	irreversible
8	7.0	200	-1005	861		irreversible
9	7.0	500	-1074	3907		irreversible
10	7.0	1000	-1101	4998		irreversible
11	9.0	50	-1108	434		irreversible
12	9.0	100	-1119	717		irreversible
13	9.0	200	-1128	1569	Peak potential shifts towards negative side of potential with increasing scan rates.	irreversible
14	9.0	500	-1193	2810		irreversible
15	9.0	1000	-1224	4633		irreversible

3.2 Effect of pH

Optimum conditions for electrochemical synthesis were ascertained with the help of cyclic voltammetry (Fig.4) and 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.4 Cyclic Voltammogram of 1,3-Cyclohexanedione (Effect of pH)



Parameters
IP=1700 mV
FP=-2000 mV
SR=100
CS=0.1 μA
PH= 4.0, 7.0, 9.0

3.3 Effect of Biotransformation

The actual reducing agent in this system is NADH. NADH transfers the hydride (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 in completed by addition of proton to the carbonyl oxygen. The scheme of the reduction is depicted in the following figure 5.

The amount of NADH in the yeast cell is limited to a very low level. In order to allow the reduction continuously, it is therefore, necessary to activate another biological pathway to reduce NAD^+ in to NADH Yeast contains some saccharides in the cell, which reduces, NAD^+ to NADH via pentose phosphate pathway. The addition of glucose to reaction mixture also activates the pentose-phosphate pathway, with simultaneously feeding of yeast cells, which results in an increased concentration of NADH & this ultimately ensure an increase in the enantiomeric excess of the product.

Immobilization enhances the operational stability of FBY and isolation of the products becomes easier. Under these conditions, the product formation rates are usually high(23). It also permits easy continuous operation since the immobilized cells can be easily removed from the reaction medium and can be reused repeatedly although with decreasing activity of the immobilized cells. In contrast to enzyme immobilization, a required coenzyme is supplied and regenerated with in the intact cell.

The reduced product 3-hydroxy cyclohexanone was obtained in reasonably good yield. Single spot TLC (R_f =0.433) checked the purity of compound. The product has M.P. = 95^0 C which is in good agreement to the reported B.P. of 3-hydroxy cyclohexanone.

The identity of 3-hydroxy cyclohexanone was further confirmed by spectroscopic analysis (Table 2, 3).

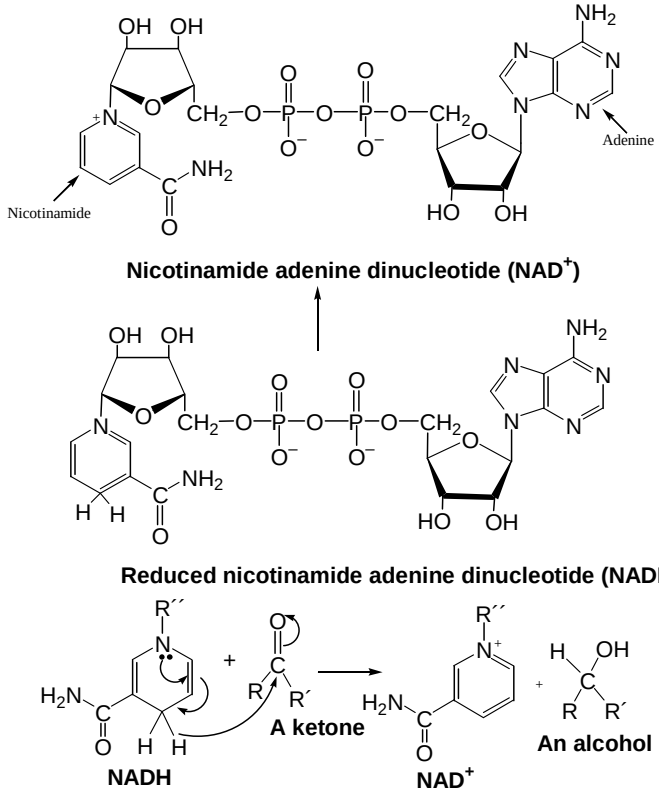
Table 2: Spectroscopic data of 3-hydroxy cyclohexanone which will obtained by electrochemically reduction of 1, 3 cyclohexanedione substrate

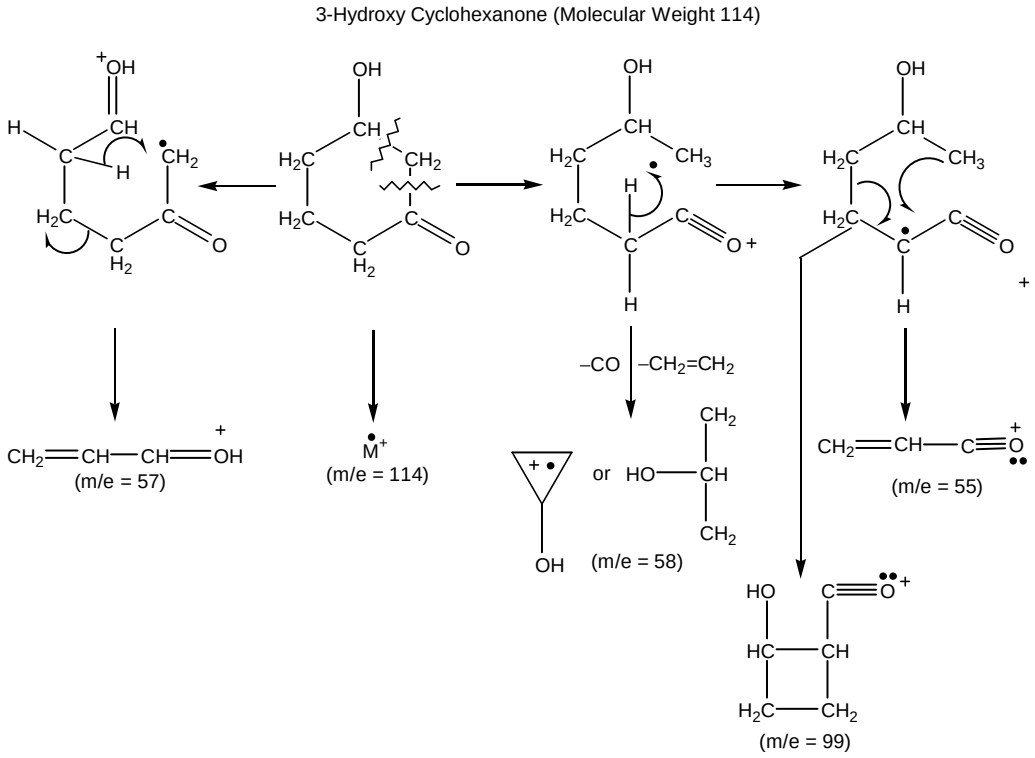
Name of Substrate	**Reaction Time (In Hour)**	**M. P. (^{0}C)**	**IR Data (cm^{-1})**	**NMR Data (δ Value)**	**Mass Spectra (m/z)**	**^{13}C NMR (δ Value)**	**Yield (%)**
1,3-Cyclohexanedione	8	96	3300-3450 2931 1655 1384-1260 1196	1.8 (2H) 2.0 (2H) 2.4(2H) 2.7(2H) 4.1(1H) 2.5(OH)	55 57 58 99 114	25.3 33.1 35.5 41.8 69.5 77 209	85.90

Table 3: Spectroscopic data of 3-hydroxy cyclohexanone which will obtained by Free and Immobilized Baker's Yeast reduction of 1, 3 cyclohexanedione substrate

Name of Substrate	**Reaction Time (In Hour)**	**M.P. (^{0}C)**	**IR Data (cm^{-1})**	**NMR Data (δ Value)**	**Mass Spectra (m/z)**	**^{13}C NMR (δ Value)**	**ee (%) Free BY**	**ee (%) IMBY**
1,3-Cyclohexanedione	48	95	3381-3450 2959 1667 1400-1350 1043	1.8 (2H) 2.0 (2H) 2.4(2H) 2.7(2H) 4.1(1H) 2.5(OH)	55 57 58 99 114	25.3 33.1 35.5 41.8 69.5 77 209	92.77	93.3

Fig.5 : Depicting biological pathway for reduction of carbonyl group by NADH





(Scheme 1)

4. Conclusions

The present work is an attempt to apply an alternative synthetic routes involving electrochemical as well as microbial assisted biotransformation of prochiral substrates into useful chiral 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

high yield but it is only the microbial assisted biotransformation which can offer high enantioseletivtiy. Immobilization of the microbial catalyst has further additional advantages like reuse and easy work up besides cost effectiveness.

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