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Studies on Synthesis of Xanthydrol Using Immobilized Biocatalyst and Electro Analytical Technique

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Abstract: The reduction of Xanthone was done using electrochemical as well as microbial techniques. Xanthydrol formed purified and characterized using different spectral techniques. The optical purity of the product is being stabilized. Also electrochemical reduction of Xanthone was carried out.
Keywords: Xanthone, Xanthydrol, Electrochemical, Microbial and Spectral Techniques.

1. Introduction

Biotransformation is a green chemical technique i.e. synthesis mediated by microbial catalyst such as Baker's yeast. The need for effective, selective reactions to create chiral building blocks to make single isomer drugs is increasing. In addition to this the need for these reactions to be cost effective, high yielding and devoid of use of dangerous or corrosive reagents. It is surprising therefore, that lots of synthetic efforts are put in chemical companies, pharma companies and chemists in universities to create new and better processes.

Organic chemists recognized the ability of Baker's yeast to transform unconventional substrates stereo selectivity since a long time ago, as one judge from the considerable amount of work in the area reported in the early literature.[1] However, there has been a recent revival of interest for the use of baker's yeast in organic synthesis, in the more general context of a preparative approach to enantiomerically pure forms of biologically active natural products and drugs based on the use, as starting materials, of readily available natural products.[2]

Chiral reagents and chiral building blocks play a role in the drug discovery process and in the manufacture of modern pharmaceutical drugs. The role of chirality is gaining importance in the drug discovery process due

to the improved and increased application of modern target-based biorational techniques. Biocatalysts, being chemo-, region- and stereo-selective, offer a great potentially as an alternative tool.[3] Among the various chiral compounds, chiral alcohols represent a highly versatile and attractive group of chiral building blocks for the synthesis of various drugs and drug intermediates. Microbial reductions of ketones to prepare chiral alcohols have provided chemists with the opportunity for the development of a novel chemical synthesis.

Electrochemistry has been widely used in industry in effluent treatment, corrosion prevention and electroplating as well as in electro-chemical synthesis. Electro-organic synthesis is now a well-established technique [4–5] for synthesis of the desired compound by oxidation or reduction of substrate. Here electron obtained during electrochemical reaction play on important role by acting as a reagent.

The aim of present investigation is to explore a novel ecofriendly method of synthesis of optically pure alcohol using free Baker's Yeast (BY) as well as immobilized Baker's Yeast (ImBY). In the present work the electrochemical and Baker's Yeast mediated reduction of Xanthone is described. The reduction potential of the reactant was recorded by polarographic techniques. Cyclic voltammetry was used to decide the reversibility of the process. Different natures of cyclic voltammograms were obtained in different media (acidic, basic and neutral). This indicates that in different media, different electrolysis products were obtained.

On constant current electrolysis [6-12] at stainless steel (SS-316) electrode, Xanthone gave same products in different media. But the present investigation is specific to only basic medium because the SS 316 electrode, which is economically viable and ecofriendly, can successfully be used in basic media.

2. Experimental Biotransformation

In a one-liter round-bottom flask, equipped with magnetic stirrer (Remi Make) 200 ml water, 10 gm fresh Baker's yeast and 25 ml isopropanol were placed and the 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 magnetically stirred for a suitable period. The suspension changed its colour from orange to yellow, which indicate the completion of the reaction, therefore the product was separated from the mixture by filtering the solution. The filtrate was extracted with diethyl ether. The extract was dried

over sodium sulphate and on evaporating it, the product was obtained. The product of the reaction was isolated, purified and characterized by combined application of chromatographic technique and spectroscopy.

3. Electrochemical

All the used reagents NaOH, CH_3COONa, KCl, Xanthone etc., were of AR grade. The solutions were prepared in double distilled water. Cyclic voltammograms were obtained on fully computerized controlled Basic Electrochemistry system ECDA 001, using 3 electrode cell assembly with 1mm diameter glassy carbon as working electrode, Ag/AgCl as reference electrode and Pt wire as counter electrode. In aqueous medium, 1.0 mM concentration of reactant, 1.0 M KCl, which is used as supporting electrolyte to maintain the ionic strength of the solution and BR buffer used to maintain the desired pH viz 5, 7 and 9 were taken in 10 ml cell. Galvanostat designed & made by CDPE (Centre of Development of Physics Eduction, University of Rajasthan, Jaipur) was used for carrying out controlled current electrolysis. For constant current electrolysis the conventional H-Cell has been used, stainless steel electrodes were used both as anode & cathode. All electrolysis process was carried out in buffer (1.0 M CH_3COONa + NaOH) and the pH of the solution was maintained constant at 9.0. After electrolysis the water was removed from the solution by distillation. The residue was then extracted with alcohol. The alcohol layer was allowed to evaporate. After evaporation product was isolated, purified and characterized by combined application of chromatographic techniques and spectroscopic methods.

4. Result and Discussion

Biotransformation:

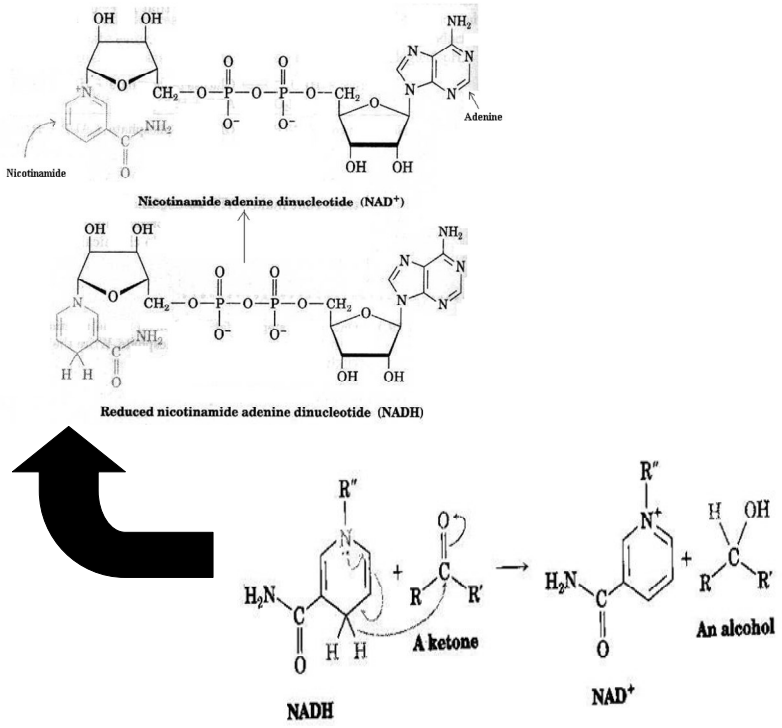
The actual reducing agent in present system is (Nicotinamide Adenine Dinucleotide Hydride) NADH.

NADH donates H^- (hydride ion) to aldehydes and ketones (and thereby reduces them). The electron lone pair on a nitrogen atom of NADH pushes out H^- which adds to a carbonyl group in another molecule to cause a reduction.

The amount of NADH in the yeast cell is limited to a quite low level. In order to allow the reduction continuously, it is therefore necessary to active another biological pathway to reduce (Nicotinamide Adenine Dinucleotide ion) NAD+ in to NADH. Yeast contains some saccharides in the cell, which reduce NAD+ to NADH via pentose-phosphate pathway. The addition of

glucose to the reaction mixture ensure simultaneous feeding of the yeast cells which ultimately results in enhanced concentration of NADH, which is regenerated from NAD+ via pentose phosphate pathway . This will ultimately ensure increase in the enantiomeric excess (ee) of the product. Immobilization enhances the operational stability of FBY and isolation of the products becomes easier. In addition, reuse of the catalyst is often possible under these conditions the product formation rates are usually high (5), not only because of the inhibitory influences but also high cell population. It is also permits easy continuous operation since immobilized cells can be easily removed from the reaction medium and can be repeatedly reused although with decreasing activity of the immobilized cells.

This study will be quite useful in developing green alternative methodology for biotransformation of organic compounds which have merits like specificity, cost effectiveness and they are expected to reduce the ever increasing problem of pollution caused by hazardous, corrossive, chemicals and harsh reaction conditions.



The spectroscopic results of product summarized in table-1, which suggest that the product is alcohol.

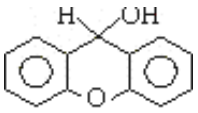
5. Electrochemical

Most cyclic voltammograms were recorded with an initial potential (Ei of 1200 mV) and switching potential Es of (-1000 mV) at different scan rates (vlz. 50,100,200,300,400 and 500 mV/sec) (Fig. 1, 2, 3).

As the sweep rate was gradually increased to (200,300, 400 and 500 mV/sec), peak gradually shifted towards higher values as is expected for an irreversible electron transfer processes.

Electrolytically reduced product Xanthydrol was obtained in reasonably good yields(91.2%). Single spot TLC checked the purity of compounds. The identity of product was further confirmed on the basis of IR and NMR data given in table 1.

Table- 1

Name of Substrate	IR Data (cm^{-1})	NMR Data (& value)	Compound Confirmed	Yield (0%)
Xanthone	3250-3500 cm^{-1} (Broad) due to O-H stretching. 3050-3070 cm^{-1} due to Ar-H Stretching. 2850-2900 cm^{-1} due to C-H Stretching. 1500, 1545, 1595 cm^{-1} due to C= Caromatic. 1200- 1250 cm^{-1} due to C-O stretching. 700-750 cm^{-1} due to (O substitute)	6.5- 7.8 (8 H) 3.98- 4.2 3.98-4.2) (1 H) 2.1 (1 H)	Xanthyrol 	91.2

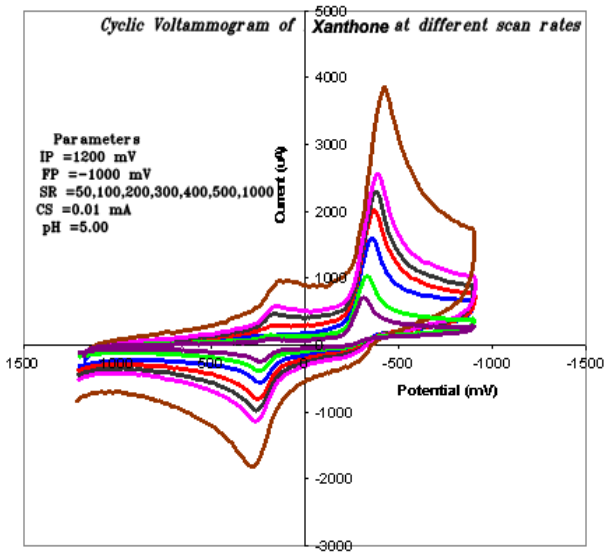


Fig.1.Cyclic Voltammogram of *Xanthone* at different scan rates at pH 5

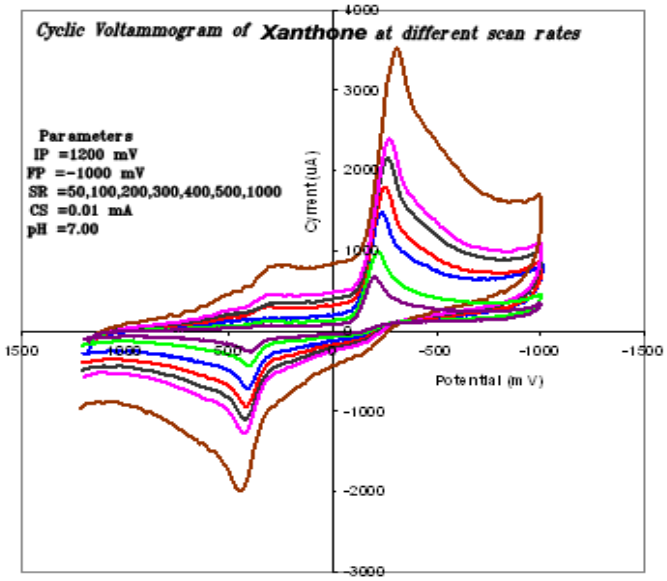


Fig.2.Cyclic Voltammogram of *Xanthone* at different scan rates at pH 7

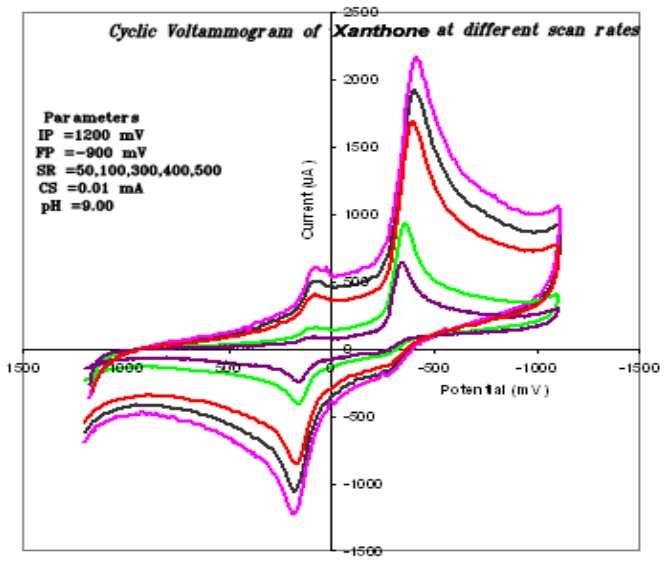
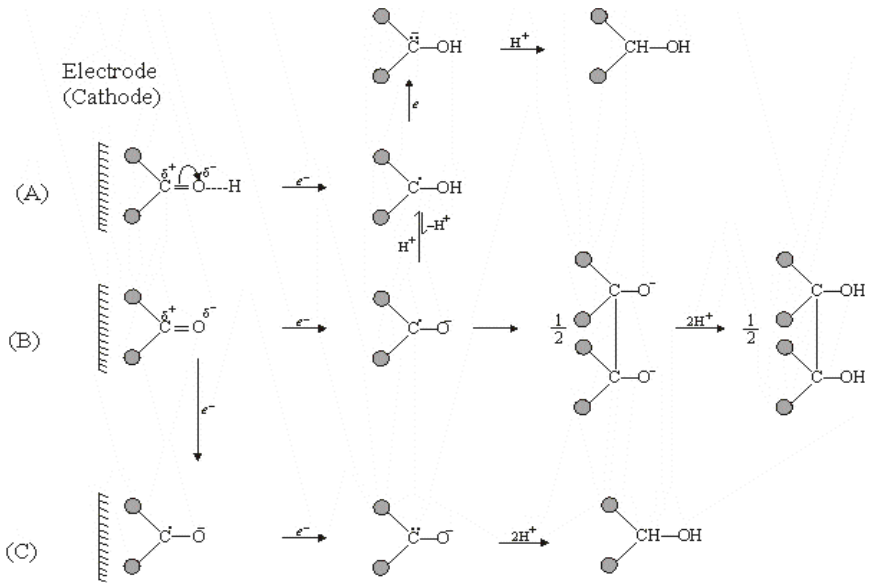


Fig.3.Cyclic Voltammogram of *Xanthone* at different scan rates at pH 9

On the basis of cyclic voltammetric studies proposed mechanism for the reduction of *Xanthone* as follow:-



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