RESEARCH ARTICLE

Study of Reduction Properties of Enzyme Alcohol Dehydrogenase from *Saccharomyces cerevisiae* Meyen *ex.* Hansen on Some Selected Compounds

Saif Yusuf Naaz Khan*
Student, Department of Botany, St Xavier's College (Autonomous), Mumbai, India

Address for Correspondence: Mr. Saif Yusuf Naaz Khan, Student, Department of Botany, St. Xavier's College, Autonomous, Mumbai- 400001, Maharashtra, India

Received: 15 March 2017/Revised: 28 May 2017/Accepted: 21 June 2017

ABSTRACT- *Saccharomyces cerevisiae* (Family: Saccharomycetaceae) is a Basidiomycetes fungus that is used in day to day life for human welfare ranging from food to medicines depending upon the type of strains and uses. Alcohol Dehydrogenase (ADH) is an important enzyme produced by the *Saccharomyces* fungus that catalyzes the many oxidation-reduction reaction in nature. The present study focuses on the reduction properties of the enzyme ADH on compounds like Nicotinamide Adenine Dinucleotide (NAD^+), Dicholorophenol Indophenol (DCPIP), and Acetophenone using Spectrophotometric assays. The aim and hypothesis of present study was to extract the enzyme in Crude and Immobilized form and to check the best reduction of compounds in either form. The activity of enzyme in Crude and Immobilized was mathematically calculated and was expressed in Units of enzyme activity per ml of ADH enzyme on respective compounds used for study. Successfully the ADH was extracted in both forms but reduction of compounds at best was observed in Immobilized Enzyme form.

Key-words- *Saccharomyces cerevisiae*, Alcohol Dehydrogenase, Nicotinamide Adenine Dinucleotide, Dicholorophenol Indophenol, Acetophenone

INTRODUCTION

Alcohol Dehydrogenase (ADH), part of the oxidoreductase family, catalyzes the oxidation of alcohols, using NAD^+ or $NADP^+$ as an electron acceptor. It is constitutive enzyme that reduces the acetaldehyde to ethanol during the fermentation of glucose. The reaction is reversible and substrates can be variety of primary or secondary alcohols and hemiacetals. Alcohol Dehydrogenase is present in most organisms, with that of yeast being the most active form of the enzyme [1]. The alcohol dehydrogenases comprise a group of several isozymes that catalyze the oxidation of primary and secondary alcohols to aldehydes and ketones respectively and also can catalyze the reverse reaction [2]. Generally Baker's Yeast (*Sacchromyces cerevisiae*) is good source of enzyme [1-2].

Access this article online

Quick Response Code

Website: www.ijlssr.com

DOI: 10.21276/ijlssr.2017.3.4.19

Alcohol dehydrogenase is a dimer with 80kDa. The 1990's brought better understanding of the role of zinc metal and the discovery of additional inhibitors. Alcohol Dehydrogenase is a tetramer with each subunit containing one zinc atom [3]. Per sub unit, there are two distinct active site sulfhydryl groups which can be distinguished on the basis of differential activity with iodoacetate and butyl isocyanate. A histidine residue has an essential role. It was found that yeast enzyme is twice the size of mammalian ADH, and approximately 100 times more active [4-5]. Immobilization technique is one of the efficient methods to improve enzyme stability .The immobilization of ADH on various supports has been investigated by several researchers. The advantages of immobilization are prevention of losses due to flushing away of enzyme, more stable enzyme, the possibility to produce enzymes with altered properties Alginate, commonly called Sodium Alginate, is a linear polysaccharide. The process of gelatin, which is simply the exchange of calcium ions, is carried out relatively under mild conditions. [6-8]. It is attracted attention because of its potential applications in the production of various starting materials and intermediates in the chemical industry, the synthesis of chiral compounds, the regeneration of coenzymes NAD(P) and NAD(P)H and

Copyright © 2015-2017| IJLSSR by Society for Scientific Research is under a CC BY-NC 4.0 International License

biosensors. Main applications of ADH are enzymatic determination of primary alcohols, and aldehydes; Synthesis of chiral compounds, Enzymatic catalysis in organic solvents, Studies of the compounds like NAD^+, NADH, NADP+ and NADPH. Alcohol Dehydrogenase is widely used in biochemical, forensic science for estimating the concentration of primary alcohols, NAD^+, ethylene glycol numerous aldehydes and enzymatic catalysis of organic solvents and also in biosensors [9].

MATERIALS AND METHODS

The research was carried out at St. Xavier's College (Autonomous), Mumbai, Department of Botany between November 2016 to April 2017.

Collection of samples

A packet of about 50 gms of Baker's Yeast (*Saccharomyces cerevisiae* Meyen ex. E.C Hansen) was purchased from the General Stores of Kalyanji and Co-operative Society, Opp. to Santacruz Station, Santacruz East, Mumbai-400055, India. The *Saccharomyces* yeast was dry, granular and active.

Extraction of Enzyme Alcohol Dehydrogenase

Yeast obtained in granules was finely crushed using a mortar pestle. It was crush in a very fine powder form. Total 30 gm of finely crushed powder was extracted with 200 ml of 0.066 M disodium phosphate for 2 hours at 37^0C with continuous stirring, followed by extraction stage at room temperature for additional 3 hours. The yeast residue was then centrifuged (Eppendorf AG) at 10000 rpm for 15 minutes at 4^0C. The supernatant solution was brought to 55^0C and maintained at this temperature on water bath for 15 minutes, after cooling, the mixture was again centrifuged at 10000 rpm for 15 minutes. At this stage clear supernatant fluid can be stored at 0^0C overnight [10-11] (Fig. 1).

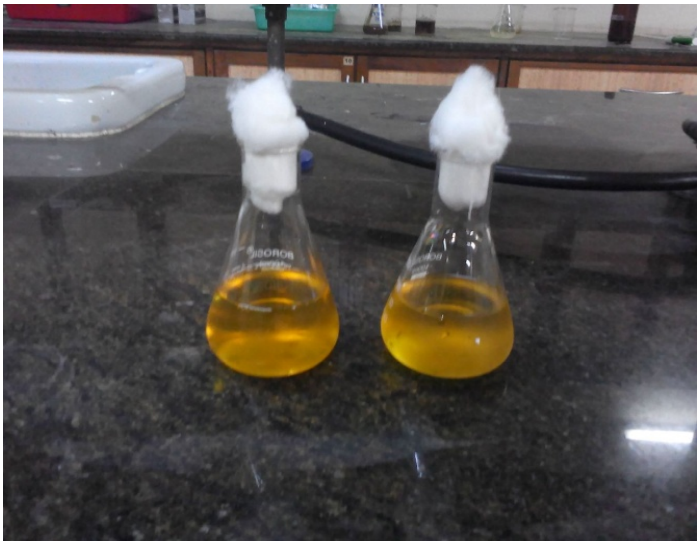

Fig 1: Shown the clear extracted form of ADH Crude enzyme

Immobilization of Crude Enzyme

3 gm of sodium alginate was dissolved in 100 ml of D/W to make a 3% solution. Approximately 0.015 gm of enzyme crude enzyme was separately mixed with 10 ml of 3% sodium alginate. The beads were formed by dripping the polymer solution from a height of approximately 20 cm into an excess Calcium chloride solution using a syringe and needle at room temperature. Pressure and needle gauge can control the bead size. A typical hypodermic needle produces a bead of size 0.5-2 mm in diameter. Beads were left in solution to cure for 0.5-3 hours. The beads were stored under refrigerator up to months till research was carried out (Fig. 2). [12]

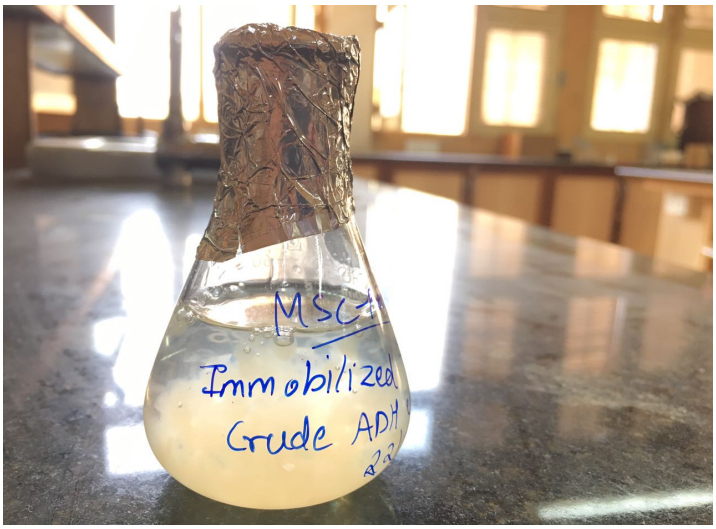

Fig 2: Shown the Immobilized form of Extracted Crude Enzyme

Enzyme assays

Assay for reduction of NAD+

Two tubes were prepared one blank and 2 test tubes, Blank tube consisted of 1.3 ml of 50 mM sodium pyrophosphate buffer of pH , 0.1 ml of 95% ethanol, 1.5 ml of 15mM NAD^+ and 0.1 ml of Enzyme diluents which was prepared by mixing 25 ml of 0.03% BSA and 0.05 ml of Crude enzyme. One test tube consisted of all the chemicals listed above in blank except Enzyme diluents the proper pure extracted 0.1 ml of Crude enzyme was added and to other tube Immobilized form of extracted enzyme *i.e.* 1 bead of sodium alginate was added.

Assay for reduction of DCPIP

For DCPIP reduction assay only two tubes were prepared one blank and other test, Blank tube consisted of 1.3 ml of 50 mM sodium pyrophosphate buffer of pH , 0.1 ml of 95% ethanol, 1.5 ml of 0.2% DCPIP, and 0.1 ml of Enzyme diluents which was prepared by mixing 0.05 ml of 0.03% BSA and 0.05 ml of Crude enzyme. The only test tube consisted of all the chemicals listed above in blank except Enzyme diluents the proper pure extracted 0.1 ml of Crude enzyme only.

Copyright © 2015-2017| IJLSSR by Society for Scientific Research is under a CC BY-NC 4.0 International License

Assay for reduction of Acetophenone

For Acetophenone reduction assay only two tubes were prepared one blank and other test, Blank tube consisted of 1.3 ml of 50 mM sodium pyrophosphate buffer of pH , 0.1 ml of 95% ethanol, 1.5 ml of Pure Acetophenone , and 0.1 ml of Enzyme diluents which was prepared by mixing 0.05 ml of 0.03% BSA and 0.05 ml of Crude enzyme. The only test tube consisted of all the chemicals listed above in blank except Enzyme diluents the proper pure extracted 0.1 ml of Crude enzyme only.

RESULTS AND DISCUSSION

Reduction assay for NAD+

The assay was analyzed using the UV-Visible Spectrophotometer (Shimadzu Co-operation, India) at 340 nm and the considerable reduction of NAD^+ to NADH was observed from the increase in Optical Density (O.D) values (Table 1) .There has been inverse proportionality observed between the reduction of NAD^+ to NADH and increase of O.D. The O.D values were noted for 5 minutes. It was observed that the Immobilized enzyme reduces the NAD^+ in less time than the Crude enzyme (Fig. 2).

Table 1: Indicates the O.D value for each for Crude and Immobilized ADH enzyme at 340 nm

Time (in minutes)	Crude Enzyme	Immobilized enzyme
0	0.168	0.418
1	0.172	0.644
2	0.179	0.856
3	0.188	1.081
4	0.201	1.235
5	0.206	1.378

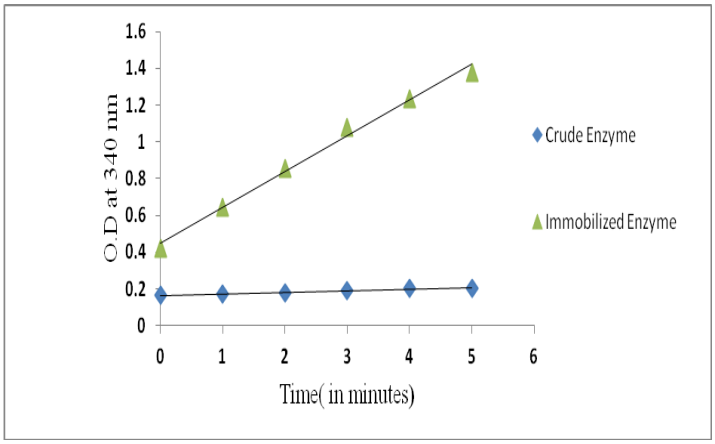

Fig 3: Shows the graphical representation of Crude and Immobilized activity on NAD^+

The enzymatic activity of alcohol dehydrogenase was determined (Table 2) using limiting amounts of enzyme and substrate (ethanol). ADH activity (U/ml) in the crude extract and immobilized enzyme was determined using the formula given below. [12-14]

$$\text{Activity of ADH (U/ml)} = \frac{(A340/\text{min})\ (3)\ (D.F)}{(6.22)\ (0.1)}$$

In the above equation, 3 is the total volume (ml) of assay, A340/min = [Final OD5min – Initial OD1Min / Reaction time (min)], DF is the dilution factor = 500 (0.05 ml of ADH in 25 ml of Enzyme Diluent), 6.22 the millimolar extinction coefficient of NADH at 340 nm and 0.1 is the volume (ml) of enzyme.

Table 2: Shows the activity of ADH

Enzyme type	Activity of ADH (in U/ml)
Crude	16.39
Immobilized	354.01

One unit of ADH activity is defined as the amount of enzyme required for catalyzing the formation of 1.0 mol of acetaldehyde from ethanol per minute at pH 8.8 at R.T. From the (Table 2) and (Fig. 3), observed that the rate of NAD+ reduction increased with time. The reduction of NAD+ was rapidly completed, in the presence of alcohol dehydrogenase and substrate ethanol. Also it indicates that an alkaline pH (8.8) and in presence of ethanol, NAD+ was fully reduced to NADH. For each molecule of NAD+ reduced, 1 molecule of alcohol was oxidized to acetaldehyde. The aldehyde concentration, therefore, was equal to the concentration of NAD+ reduced. Hence it is clear that there was 20 fold increases in activity of ADH of Immobilized enzyme than the Crude form. Immobilized enzymes showed enhanced stability due to formation of aggregates and were separated from reaction mixture, hence greater activity when compared with extracted enzyme of the yeast.

Reduction assay for DCPIP

The assay was spectrophotometrically determined at 620 nm. The (Table 3) shows the O.D values of the assay (Fig. 4). The enzyme ADH has been made to react with DCPIP (2, 6-Dichlorophenolindophenol), DCPIP was reduced by ADH (Fig. 4). Blue DCPIP when made to react with enzyme ADH enzyme was converted to colorless i.e. (Fig. 5) DCPIP was reduced to DCPIP-H_2 (Reduced form).

Copyright © 2015-2017| IJLSSR by Society for Scientific Research is under a CC BY-NC 4.0 International License

Table 3: Shows the reduction of Blue Colour of DCPIP which is indicated by decrease in O.D

Time (in minutes)	O.D at 620 nm
0	0.029
1	0.026
2	0.010
3	0.007
4	0.004
5	0.001

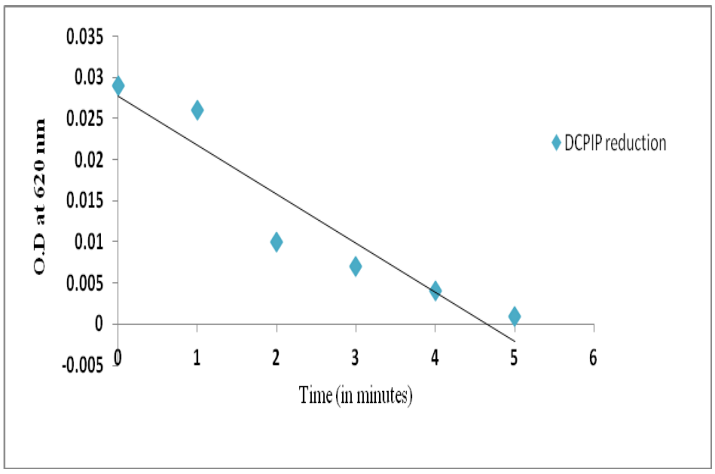

Fig 4: Graph shows reduction of DCPIP

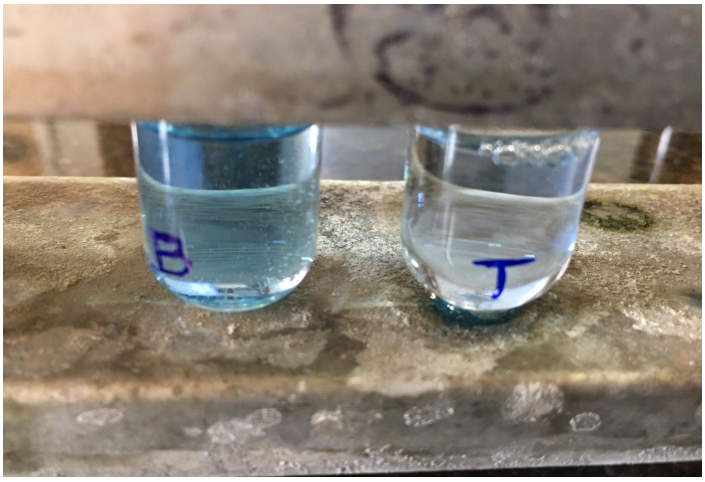

Fig 5: Reduction of Blue color of DCPIP (where B=Blank and T=Test)

Reduction assay for Acetophenone

The assay exhibited the reduction of Acetophenone which was indicated by decrease in O.D at 278 nm, where shown the O.D values and (Fig. 6) depicts the graph in which the Acetophenone reduction is clearly observed.

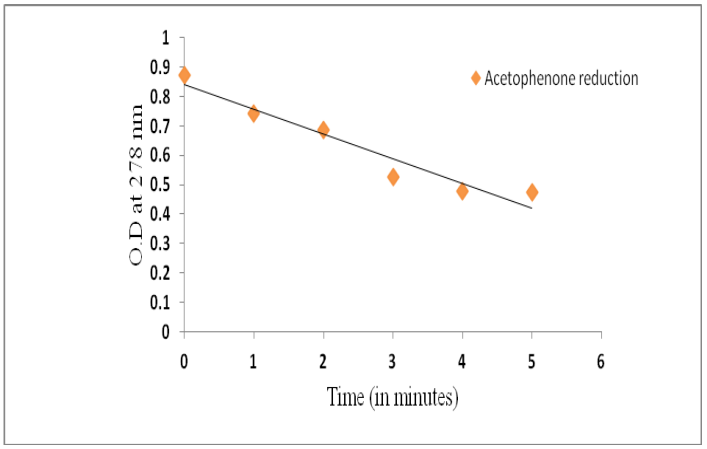

Fig 6: Shows successful reduction of Acetophenone

CONCLUSIONS

Overall it can be seen that the enzyme Alcohol Dehydrogenase (ADH) has effectively reduced the compounds such as Nicotinamide Adenine Dinucleotide, Dichlorophenol Indophenol and Acetophenone. It is seen that during the reduction process ADH enzyme removes the Hydrogen from the substrate used in the process i.e Ethanol and adds these removed Hydrogen to the above substances, which were used as Co-enzymes, so that it gets reduced and the substrate Ethanol gets converted in to an Aldehyde product, which was indicated by change in the O.D on Spectrophotometer. Further as more and more substrate is converted to product the compounds gets more reduced, which is indicated by the colour change specially in Dichlorophenol Indophenol and Acetophenone.

FUTURE PROSPECTS

The research opens up the various future plans such as Enzyme ADH can also be extracted from plant sources and various method of protein precipitation or purification may be tried to get best result. The extracted crude and immobilized enzyme can be lyophilized and brought to powder so that commercially the powder can be sold to research institutes for practical purposes. The enzyme on technological point of view can be made to biosensors that can be used in the forensic sciences to detect the presence of alcohol in severe cases. Several other compounds resembling to the structure of DCPIP and Acetophenone can be tried to reduce with enzyme ADH.

ACKNOWLEDGMENT

I would like to Thank Dr. Rajendra Shine, Vice Principal and Associate Professor in Botany and Prof. Kevin D'cruz, Head of Department and Associate Professor of Botany, St. Xavier's College, Mumbai for their valueable guidance and moral support.

REFERENCES

[1] White and White. Immobilizing enzymes: How to create more suitable biocatalysts. Angewandte Chemie International Edition, 1997; 42:3336-3337.

Copyright © 2015-2017| IJLSSR by Society for Scientific Research is under a CC BY-NC 4.0 International License

[2] Sofer W, Martin F. The genetics of Alochol Metabolism: Role of Alcohol Dehydrogenase and Aldehyde Dehydrogenase Variants. Alcohol Res Health. 2007; 30(1):5-13.
[3] Vallee and Hoch. Metabolically engineered methyl trophic yeast cells and enzymes as sensor recognition elements. FEMS Yeast Res, 1955; 2:307-314.
[4] H.John, R.Eckfeldt, Todd Light. Kinetic ethylene glycol assay with the help of yeast alcohol dehydrogenase. Clin. Chem. 1980; 26(9) 1278-1280.
[5] Hayes and Valeck. Immobilization of yeast alcohol dehydrogenase on magnetic nanoparticles for improving it's stability. Biotechnology letters, 1953; 23:1723-1727.
[6] Madhusudan M.C., Raghavrao K.S.M.S., Nene Sanjay Integrated process for extraction and purification of alcohol dehydrogenase from Baker's yeast involing precipitation and aqueous two phase extraction. Biochemical engineering Journal, 2008; 38:414-420.
[7] Negoro M. and Wakabayashi I. New Simple Method for Purification of Class 1 Alcohol Dehydrogenase. Alcohol Alcohol, 2004; 39:178-182.
[8] Hayes and Waleck. Analysis of alcohol dehydrogenase gene expression in Drosophila. Annual Review of Genetics. 1987; 21:203-205.
[9] Renan Furian. Reduction of Acetophenone to R (+) – Phenylethanol by new alcohol dehydrogenase from *Lactobacillus kefir*, 2014; 53(36):5791-5803.
[10] Ohloson S, Larsson P, and Moshback K. Steroid transformation by living cells immobilized in calcium alginate. European J. Appl. Microbiol. Biotechnol, 1979; 7(2):103-110.
[11] Singh Abhilasha. Enzymatic assay of Alcohol Dehydrogenase. NewLand Publications, 2007; 56-83.
[12] Sadashivam S., Manickam A.Enzyme Immobilization and Entrapment in Alginate Gel, New Age International Publishers, 2010; 143-144.
[13] Rizal Govinda, Karki Shanta. Alcohol Dehydrogenase (ADH) activity in soybean (*Glycine max*) under flooding stress, Electronic Journal of plant breeding, 2011; 2(1): 50-57.
[14] Racker E. Crystalline Alcohol Dehydrogenase from baker's yeast. J. Biol. Chem, 1950; 184:313-320.

International Journal of Life-Sciences Scientific Research (IJLSSR) Open Access Policy
Authors/Contributors are responsible for originality, contents, correct references, and ethical issues.
IJLSSR publishes all articles under Creative Commons Attribution- Non-Commercial 4.0 International License (CC BY-NC).
https://creativecommons.org/licenses/by-nc/4.0/legalcode

How to cite this article:
Naaz Khan SY: Study of Reduction Properties of Enzyme Alcohol Dehydrogenase from *Saccharomyces cerevisiae* Meyen *ex*. Hansen on Some Selected Compounds. *Int. J. Life. Sci. Scienti. Res.,* 2017; 3(4): 1218-1222. DOI:10.21276/ijlssr.2017.3.4.19
Source of Financial Support: Nil, **Conflict of interest:** Nil