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Extraction and purification of glucose oxidase enzyme from Penicillium chrysogenum

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***Abstract*---**The study included the extraction and purification of glucose oxidase enzyme from *Penicillium chrysogenum*, pure of the enzyme by precipitation with ammonium sulfate at a saturation rate of 70% The specific activity in this step reached (10.802) units/mg and fold 1.276, and this was followed by the dialysis step, and the specific activity was 13,593 units/mg and fold 48.184. Finally, ion exchange chromatography using the DEAE-Cellulose column, the specific activity reached 19.355 units/mg, and fold 36.59, after which the enzyme was migrated by the electrophoresis device and one protein bundle appeared.

***Keywords*---**extraction, purification, glucose oxidase enzyme, Penicillium chrysogenum.

Introduction

Glucose oxidase (B-D-glucose:oxygen-1-oxidoreducatase,EC1.1.3.4) symbolizes him GOX or GOD (Bentley, 1963) and It is one of the types of oxidation and reduction enzymes and belongs to the family Oxidoreductase which includes Oxidase , Oxygenase , Peroxidase and Dehydrogenase Which is one of the enzymes that acts as an activator that transports electrons (Trisolini et al ., 2019) The fungus *A.niger* is the most common source of enzyme extraction, followed by the fungus *Penicillium*, which are considered the most industrially producing enzymes (Bhatti and Saleem, 2009) .

Often classified with antioxidants, preservatives and proven properties, there are many food products in which GOX can be used as it can be used to reduce the alcohol content of wine (Röcker et al.,2016). GOX owns many medical and pharmaceutical products. In recent years, the GOX enzyme was used to estimate the level of sugar in the blood and thus detect diabetes (Pullano et al.,2022).

Materials and Methods

Penicillium chrysogenum has been obtained after diagnosing it in Vitek compact , 3,5.dinitrocilcilicacid , Coomasie brilliant blueG250 and KH_2PO_4 from Sigma Chemical USA.

Inoculum preparation (Bhatti et al., 2007)

Pure culture of newly isolated *Penicillium chrysogenum* was maintained on potato dextrose agar (PDA) slants at 4 °C, the growth (3.0 g $NaNO_3$, 1.0 g KH_2PO_4 , 0.5g $MgSO_4.7H_2O$, 0.5g KCl , 0.01g $FeSO_4.7H_2O$, 2.0 g Yeast extract and 80.0g glucose) was autoclaved for 15 min. after cooling the flasks, inoculum was prepared by 10 ml of sterilized distilled water was added to a sporulated 7 days old PDA slant culture, inoculum (5 ml) was added to each flask containing 150 ml of growth medium and flasks was incubated at 28°C on a rotary shaker at 200 rpm for 5 days.

Isolation of glucose oxidase (El-Enshasy,1998)

At the end of fermentation, the mycelium was separated from the culture broth by filtering through a filter paper (Whatman No.1), the filtrate was centrifuged at 8000 rpm for 15 min the supernatant was carefully collected and filtrate take To measure enzymatic activity and protein concentration .

Assay of Glucose oxidase (Gera *et al.*, 2008)

By taking 0.2 ml of the enzyme filtrate and 0.2 ml of glucose solution at a concentration of 1%, adding 0.6 ml of distilled water and 1 ml of phosphate buffer solution of pH 7, the tubes were incubated at 30 ° C for 30 min to allow the enzymatic reaction to take place and then moved to a water bath 100°C for 5 min, the tubes were removed and 2 ml of reagent dye solution was added 3-5-dinitrosalyclic acid (DNSD) which is record according to (Whitaker and Bernhard,1972) Then the tubes were returned to the water bath again for 5 min , as the color of the samples changed due to the bonding of the reagent with monosaccharide. Then the tubes were left to cool and the volume was completed to 24 ml of distlled water. The absorbance of the samples was measured using a spectrophotometer at 540 nm by zeroing the device on the blank solution, which prepare the same method as before replacing the enzymatic solution with distilled water. Enzymatic activity was defined as the amount of enzyme that converts 1 µg of glucose sugar into gluconic acid and H_2O_2 within 30 min.

Glucose standard curve

was measured according to (Imran et al.,2012).

Protein determination

Protein concentration was measured according to (Bradford ,1976).

Enzyme purification

Precipitation of Ammonium sulfate (AWORH and Nakai,1986)

The ammonium sulfate was added in different saturation ratio (20,40,60 ,70 and 80%) to reach the optimum ratio of ammonium sulfate by adding gradually the amount of salt to each 15 ml of the crude enzyme in ice bath and magnetic stirrer, centrifuge the solution for 10 min at 10000 rpm. The filtrate was dropped and take the precipitate and dissolved it in a 5 ml phosphate buffer solution of pH 7 and its volume was measured. and both enzyme activity and protein content were determined for each separate fraction.

Dialysis against phosphate buffer pH 7(AWORH and Nakai,1986)

The precipitate obtained from the precipitation process was collected with ammonium sulfate and conducted on it the membrane osmosis process in dialysis bag against phosphate buffer with pH 7 at a temperature of 4 ° C with the buffer solution changed every four hours. Then the enzymatic extract was collected and its volume was measured and the enzymatic activity and concentration of protein in it.

Ion exchange chromatography technique

Using the DEAE-Cellulose exchanger and following the method described by (Whitaker, 1972) .With a phosphate buffer solution of 0.05, pH 6.5, at a flow rate of 30 ml / hour, at a rate of 2 ml for each fractions.

Electrophoresis

The method of electrophoresis in polyacrylamide gel electrophoresis was used in the absence of teratogenic substances, Polyacrylamide gel electrophoresis according to the method of (Laemmli,1970) and described by (Garfin,1990) to determine the purity of the enzyme using electrophoresis device.

Results and Discussion

Isolation of glucose oxidase

Table (1) shows the steps for extracting and purifying the GOX enzyme, as the enzymatic activity of the crude extract was (5.078) units/ml, the specific activity was (8.463) units/mg , and the protein concentration was (0.6) mg/ml. GOX enzyme was extracted from *P.notatum* using liquid fermentation method, and the specific activity was 5.53 units/mg (Zia et al., 2013).

Table(1) The purification steps of Glucose oxidase from *Penicillium chrysogenum*

Purification steps	Volume (ml)	Activity (U/ml)	Protein (mg/ml)	Specific activity (U/mg)	Total activity (U)	Fold	Yield %
Crude enzyme	25	5.078	0.6	8.463	126.95	1	100
Ammonium sulfate precipitation 70 %	15	4.321	0.4	10.802	64.81	1.276	51.05
Dialysis	15	4.078	0.3	13.593	61.17	1.606	48.184
Ion exchange	12	3.871	0.2	19.355	46.45	2.28	36.59

Glucose oxidase purification

precipitation with ammonium sulfate

The ammonium sulfate used in different saturation ratios (20 ,40 ,60 , and 80)% , then the 70% ratio was selected as best ratio for precipitate the crude extract of enzyme As shown in Figure (1), when the enzyme activity reached to (4.321)units/ml with a purification fold (1.276) and Yield (51.05)% as shown in table (1) while the other saturation ratios (20 ,40 ,60,80) gave low titer of Enzyme activity (2.897,3.861,4.089,2.567) respectively as shown in figure (1) The ammonium sulfate was used in enzyme precipitation because it high soluble and cheap compared with the other salts. The other results of (Hassan et al.,2018) purified GOX from *Penicillium notatum* used ammonium sulfate to precipitate GOX at 60% of saturation ratio, the enzyme activity was (4.235) units/ml with purification fold 1.219.

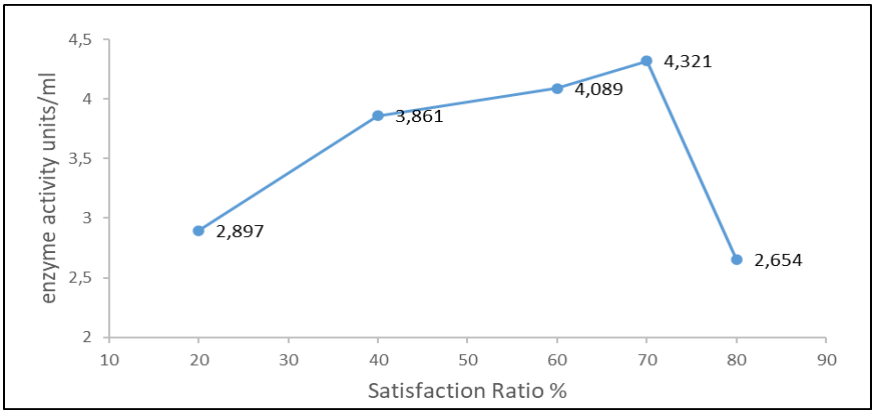


Figure (1) The enzymatic activity of GOX extracted from *Penicillium chrysogenum* with saturation rates (20-80%) of the ammonium sulfate salts

Dialysis against phosphate buffer pH 7

The enzymatic precipitate resulting from the centrifugation process was collected and dissolved with a small amount of phosphate buffer at pH 7 to perform the membrane osmosis process (dialysis) against the phosphate buffer. After the expiration of the time, the enzymatic activity was estimated as (4.078) units/ml and the protein concentration as (0.3) ml/mg, and the specific activity reached (13.593) units/mg, with the number of purification times (1.606) times and with an enzymatic yield of (48.184) % as shown in Table (1).

Ion exchange chromatography

The enzyme solution produced from dialysis was passed through Ion exchange chromatography using DEAE-Cellulose column with dimensions (3 x 15 cm) and equilibrium was carried out using a phosphate buffer with a concentration of (0.05M) and PH = 6.5. The associated fractions were recovered using the same buffer solution containing graduated concentrations of sodium chloride solution at a flow rate of 30 ml per hour and at a rate of 2 ml for each fraction. the fractions were collected from column and measured at 280 nm absorbency. It is noted from the results in figure (2) the appearance of a protein peak in the washing phase of enzymatic activity, and this indicates the release of proteins that were not bound to the ion exchanger and the appearance of a protein peak in the elution phase.

The enzymatic activity was (3.817) units/ml, the protein concentration was (0.2) mg/ml, the specific activity was (19.355) units/mg, the number of purification times was (2.28) and the enzymatic yield was (36.59)% as shown in Table (1). Other studies (Simpson, 2005) When using the DEAE-Cellulose ion exchanger to purify the GOX enzyme from *P.canecense* Tt42, the specific activity was 268.1 units/mg, the protein concentration was 4.1 mg, fold 9.6, and the enzyme yield was 25.4%.

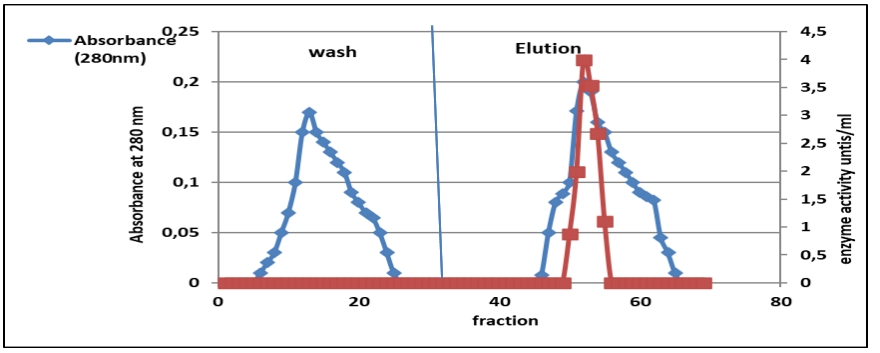
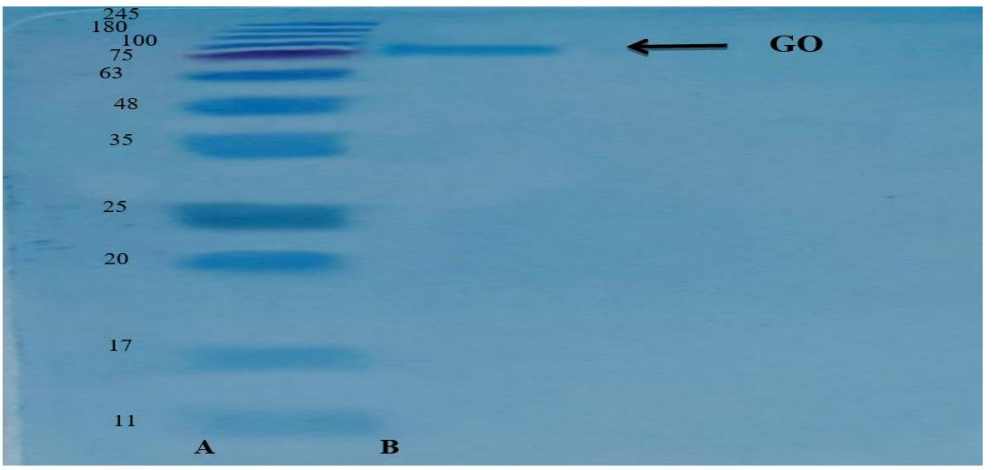


Figure (2) Ion exchange chromatography for purification of GOX enzyme from *Penicillium chrysogenum* using a DEAE-cellulose column with dimensions (3x15cm) and equilibrated using a phosphate buffer (PH= 6.5, 0.05M) and it was recovered using the same buffer solution containing graduated concentrations of Sodium chloride solution with a flow rate of 30 ml/hour and at a rate of 2 ml/fraction

Electrophoresis

Figure (3) shows the result of the electrophoresis of the purified GOX enzyme from *Penicillium chrysogenum* using the precipitation process with ammonium sulfate at a saturation rate of 70% and the process of membrane osmosis, and finally it was passed in an Ion exchange chromatography column using DEAE-Cellulose column. The results show the appearance of one protein bundle and this indicates that the purification steps for the current study were good enough to obtain one protein bundle with a molecular weight of approximately 75 kDa.



Figure(3) Electrophoresis of GOX enzyme in a polyacrylamide gel in the absence of teratogens. A represents the standard marker, known molecular weights, and B is A purified GOX enzyme.

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