Archives of Razi Institute, Vol. 76, No. 4 (2021) 769-779


DOI: 10.22092/ari.2021.355928.1738

***Original Article***

# Extraction and Purification of Extracellular L-Glutamate Oxidase from *Streptomyces*

**Hazim Abdul Hameed, D[1]*, Hussein Ali, E[1]**

*1. Department of Biotechnology, Faculty of Applied Sciences, University of Technology, Iraq*

Received 13 August 2021; Accepted 18 September 2021
Corresponding Author: dinahazim0@gmail.com

**Abstract**

The bacterial isolates *Streptomyces* were obtained from the soil and cultivated in a wheat bran medium, which was used to produce the L–glutamate oxidase enzyme. The extracellular enzyme was then extracted using a cooling centrifugation process to obtain the filtrate that represents the crude enzyme. Afterward, the enzyme purification processes were carried out which included precipitation with ammonium sulfate as a preliminary purification step followed by dialysis to remove the salts. Next, ion-exchange chromatography and gel filtration were used to finish the purification process, and the enzyme activity was determined for each purification step. The results of purification of L-glutamate oxidase enzyme from *streptomyces* using ammonium sulfate showed that the specific activity was 8.25 units/mg protein with a saturation ratio of 60%. Moreover, the results of purification using a dialysis tube indicated that the specific activity was 9.5 units/mg protein. In addition, the result of purification using diethylaminoethyl cellulose ion column revealed that the specific activity was 25 unit/mg protein and the results of purification using gel filtration showed that the specific activity was 56 units/mg protein which was the best step in the purification process due to high specific activity of the enzyme. The optimum temperature and pH for the activity and stability of the enzyme were tested. Based on the findings, the optimum temperature for the activity of the enzyme was 37 °C. In addition, it was found that the optimum temperature range for the stability of the enzyme was 30-50 °C. Besides, the optimum pH for the activity was 7.0 and the optimum pH range for the enzyme stability was 5.0-7.0.

**Keywords:** L-glutamate oxidase, L-glutamate, *Streptomyces*, Specific activity

## 1. Introduction

L-glutamate is an essential amino acid that is commonly utilized as a food additive due to its ability to enhance flavor. In neurochemistry, it is the main excitatory neurotransmitter of the central nervous system and the enteric nervous system (1). Based on this data, it is critical to determine appropriate analytical procedures for the detection of this amino acid generally in simple and dependable methods (2). An enzymatic approach can be used to evaluate L-glutamate. Both glutamate dehydrogenase (GDH) and glutamate decarboxylase (GDC) were used to determine the levels of L-glutamate (3). The GDC and GDH have some problems due to their poor substrate specificity and the need for a costly coenzyme, like NAD+. Instead, L-glutamate oxidase (GLOD) is utilized since it has a higher substrate specificity, compared to GDH and GDC, and requires no further coenzyme (4).

Extracellular L-glutamate oxidase (E.C 1.4.3.11), in the presence of water and oxygen, this enzyme stimulates the oxidative deamination of L-glutamate which leads to the formation of α-ketoglutarate, ammonia, and hydrogen peroxide.


Biosensors for the detection of L-glutamate, L-glutamine, ammonia, and creatinine can be made using the analytic reagent of L-glutamate oxidase. These biosensors can be used in analytical chemistry for qualitative and quantitative enzymatic process tests and food products to determine the quality of the food. They can also be employed in clinical biochemistry for the early detection of heart and liver disorders by evaluating glutamate-pyruvate transaminase and glutamate-oxaloacetate transaminase in biological fluids (5). The present study aimed to isolate, purify, and characterize L-glutamate oxidase from *Streptomyces* culture filtrate.

## 2. Material and Methods

### 2.1. Preparation of Production Medium

*Streptomyces* were grown in a medium of 2 g of wheat bran, 0.5 g of Monosodium Glutamate as a substrate for the enzyme, and 0.5g of NaCl in 100 mL of distilled water as a catalytic medium to the bacteria for enzyme production (6).

### 2.2. Extraction of Enzyme

Bacterial cultures were placed in suitable Falcon tubes.Afterward,the cells were precipitated in a cooling centrifuge at 8000 rpm for 20 min to extract the enzyme. Sincethe enzyme is extracellular, the filtrate is separated from the sediment for the purpose of the purification process.

### 2.3. Estimation of the Activity of L-Glutamate Oxidase

Theperoxidase-catalyzed chromogenic technique was used to determine the enzyme activity. The reaction mixture was made up of 1 ml of 4-aminoantipyrine (2 mM), 2 ml of phenol (3 mM), 0.1 ml of horseradish peroxidase (60 U/ml ), and 0.1 ml ofthe enzyme. The reaction started by adding 0.1 ml (0.01 mM)ofmonosodium glutamate to the reaction mixture after a 2-min pre-incubation at 37 °C to the reaction mixture. After 30 min of incubation at 37 °C with gentle shaking, the absorbance was measured at 500 nm. Under the assay conditions, one unit of enzyme activity is described as the amount of enzyme required to generate 1mol of $H_2O_2$ per min (7, 8).

Enzyme activity (U/ml)=(A×V)/(t×E×v)

A: Absorbance at the corresponding wavelength

V: Total volume of the reaction mixture (ml)

v:enzyme volume (ml)

t:Incubation time (min.)

E: Corresponding extinction coefficient, Equal to 6 X $10^3$ $M^{-1}$ $cm^{-1}$

### 2.4. Estimation of Protein Concentration in the Sample

The protein concentration has been calculated according to the Classics Lowry, Rosebrough (9) method. Thetotal protein content ofthe L-glutamate oxidase enzyme wasdetermined by a spectrophotometer at 600 nm. Bovine serum albumin was usedas the standard protein (9).

### 2.5. Determination of Optimum Temperature for L-Glutamate Oxidase Production

To calculate the optimum temperature for enzyme production, the prepared culture medium (wheat bran), which contained 1 ml of48-h*Streptomyces* culture,wasincubated at different temperatures (20, 25, 30, 37, 40°C) in the shaking incubator for 60 h.Afterward, the activity of the enzyme produced by bacteria was evaluated after extraction to find the optimum temperature for production (10).

### 2.6. Determination of Optimum pH for L-Glutamate Oxidase Production

To determine the optimum pH for enzyme production, the pH of the prepared culture medium (wheat bran) was changed to 3,5,7,8, and 9, which contained1 ml of48-h*Streptomyces* culture,was incubated in the shaking incubator for 60 h. After that, the activity of the enzyme produced by bacteria was measured after extraction to determine the optimum pH for production (11).

### 2.7. L-Glutamate Oxidase Purification Process

**2.7.1. Ammonium Sulfate Precipitation**

The crude enzyme was precipitated with a saturation ratio of 60% of ammonium sulfate $(NH_4)_2SO_4$ by gradually adding salt to the crude enzyme with continuous stirring for 1hbya magnetic stirrer from (GallenKamp company – England). Next, the components were separated by a cooling centrifuge at 10,000 cycles/min for 15 min, the filtrate was neglected,and the precipitate was dissolved in 15 ml of phosphate buffer (pH7.4,0.2M). Afterward, the absorbance was measuredat a wavelength of 500 nm to measure the enzyme activity and specific activity (12).

**2.7.2. Purification Bydialysis Tube**

The dialysis process was carried out for the enzyme resulting from the precipitation step with ammonium sulfate. In total, 15 ml of the enzyme were placed in the dialysis tube, which allows the passage of materials less than 120 kDa. The tube was placed in a container containing a phosphate buffer (pH 7.4, 0.2 M) for 24 h which led to the separation of the saline solution from the enzyme;hence, the enzyme remainedinside the tube.The enzymatic and specific activitiesof the resulting enzyme weremeasured after the dialysis process was completed (13).

**2.7.3. Enzymeseparation from the Ion-Exchange Column by Diethylaminoethyl Cellulose**

The method ofpreparation of ionexchange (diethylaminoethyl cellulose [DEAE-C]) used by Whitaker and Bernhard (14) was applied. In this regard, 20 g of resin was dissolved in 1 L of distilled water and left to settle. Next,the supernatant was removed, and this process was repeated multiple times until the supernatant became pure. The DEAE-Cwas activated for 30 min with 0.25 M HCl, and then filtered through a Buchner funnel with Whatman No.1 filter paper and washed twice with distilled water. The DEAE-Cwas then activated with 0.25 M NaOH followed by two rounds of filtration and washing. The activated DEAE-Cwas equilibrated with phosphate buffer (pH 7.4, 0.2 M) and packed in a column with dimensions of3×13 cm, then 10 ml of the enzyme wasslowly placed on the walls of the ion exchanger using a dropper. Next, the separated portion was collected in appropriate tubes of 5 ml for each portion, anda washing step was performed using a phosphate buffer solution (pH 7.4, 0.2 M). Afterward, the elution step was conducted by applying various sodium chloride concentrations (0.15-1 M NaCl);next,the absorbance of each eluted fraction was measured at 280 nm wavelength for each of the washing and elution steps. Finally, the enzymatic activity was calculated in the collected fractions of the exchanger to determine the fractions containing enzymatic activity (15).

**2.7.4. Purification by Gel Filtration Column Using Sephadex G200**

The gel filtration material was prepared according to the instructions of the suppliercompany by placing 6 g in 200 ml of distilled water to remove the preservatives. Next, it was washed with a phosphate buffer solution (pH 7.4, 0.2 M), heated at a temperature of 90 °C for 3 h,and the air and bubbles were removed by avacuum pump. Subsequently,the material was placed in a column with dimensions of 21×1 cm, and the material was left to precipitate. Afterward,the column was titrated using a phosphate buffer solution, and3 ml of the purified enzyme was taken in the ion exchange step and slowly placed on the walls of the gel filtration column.The enzyme was recovered using the solution used to wash the material. Next,the absorbance of the protein part was determined at a wavelength of 280 nm. Moreover,first, the enzymatic activity of the protein peaks with high absorbency was measured, and thenthe enzymatic activity of all the protein peaks was measured (16).

**2.8. Characterization of L–Glutamate Oxidase**

**2.8.1. Determination of Optimal pH for the Effectiveness of Enzyme**

To determine the optimum pH for enzyme activity, sodium acetate buffer $CH_3NaO_2$was prepared at a concentration of 0.1 M withpH values of 3, 4, and 6, and phosphate buffer was prepared at a concentration of 0.1 M withpH values of 7 and8). Equal volumes of

these solutions were mixed with the substrate solution in a 1:1ratio, and0.1 ml of this mixture was added to 0.1 ml of the enzyme. The enzymatic activity was estimated by measuring the absorbanceat 500 nm. Next,the relationship between the enzymatic activity and the pH was determinedto calculate the optimum pH for theenzymatic activity (17).

**2.8.2. Determination of Maximum pH for Enzyme Stability**

To find thebest pH for enzyme stability, equal volumes of the pure enzyme were mixed with buffer solutions. For this purpose, 0.1 ml of the enzyme was mixed with 0.1 ml of sodium acetate buffer solution at a concentration of 0.1 M with pH values of 3, 4, and 6. Moreover, 0.1 ml of the enzyme was mixed with 0.1 ml of phosphate buffer solutionat a concentration of 0.1 M withpH values of 7 and 8.The solutions were incubated in the incubator at 37 °C for 30 min. The activity of the enzyme was calculatedby a spectrophotometer at 500 nm; moreover, the enzymatic activity was calculated as well. Furthermore, the relationship between the percentage of residual activity and the optimum pH for the stability of the enzyme was determined (18).

**2.8.3. Determination of Optimal Temperature for the Effectiveness of Enzyme**

To find the optimum temperature for enzyme activity, enzyme activity was determined for each temperature. In addition, the relationship between temperature and 0.9 ml of the substrate solution was added to0.1 ml of the pure enzyme.The mixture was incubated for 10 min at different temperatures (20, 30, 37, 40, 50°C), and the enzyme activity was determined to calculate the optimum degree of enzyme activity (19).

**2.8.4. Estimation of Optimal Temperature for Enzyme Stability**

To ascertain the besttemperature for enzyme stability, 0.1 ml of the pure enzyme was incubated atdifferenttemperatures (20, 30, 40, 50, and 60 °C) for 30 min. Afterward, the enzyme activity was calculated, and the residual enzyme activity was plotted against temperature to find the optimum temperature for its stability (20).

## 3. Results

### 3.1. Optimum Temperature for L-Glutamate Oxidase Production

The results revealed that the optimum temperature for the production of the enzyme was 30 °C.As shown in figure 1. Accordingly, the highest specific activity reached4.5 units/mgat the temperature of 30 °C, and the lowest specific activity of the enzyme was 2.7 units/mg at the temperature of 20 °C.

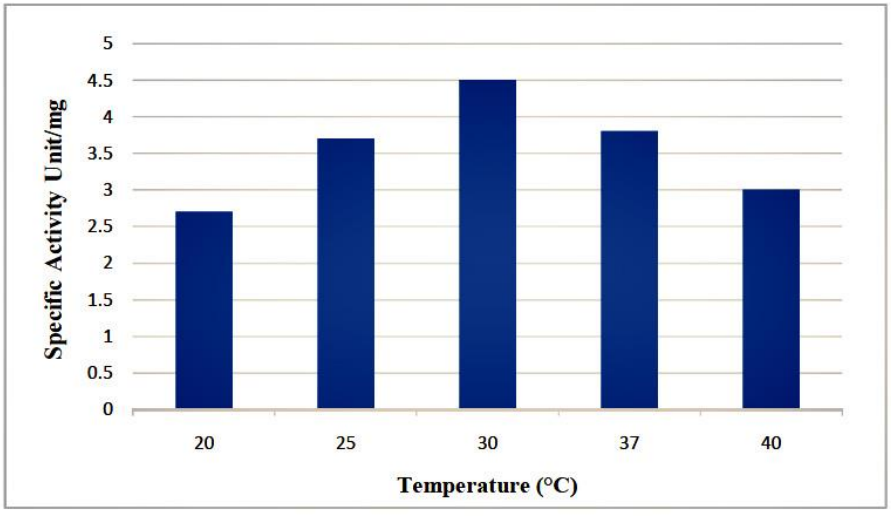


**Figure 1.** Optimum temperature for L-glutamate oxidase production.

### 3.2. Optimum pH for L-Glutamate Oxidase Production

The results revealedthat the highest specific activity was 4.6 units/mg at pH 7.0, while the lowest specific activity was 2.1 units/mg at pH 3.0. Thisindicatesthat 7 is the ideal pH for enzyme productionas illustrated in figure 2.

### 3.3. Enzyme Purification

Ammonium sulfate precipitation, dialysis, ion-exchange chromatography, and gel filtration were used in this study to purify L- glutamate oxidase produced by Streptomyces in the culture broth.

### 3.4. Ammonium Sulfate

Ammonium sulfate does not affect pH and precipitates the largest proportion of proteins present within the enzyme. It also does not affect the enzymes since it does not induce denaturation of many proteins during deposition. The ammonium sulfate at a saturation ratioof 60% was chosen as the optimal ratio for precipitating the crude enzyme extract with a specific activity of 8.25 units/mg, a purification fold of 1.8, and a yield of 20.8%. As shown in table 1.

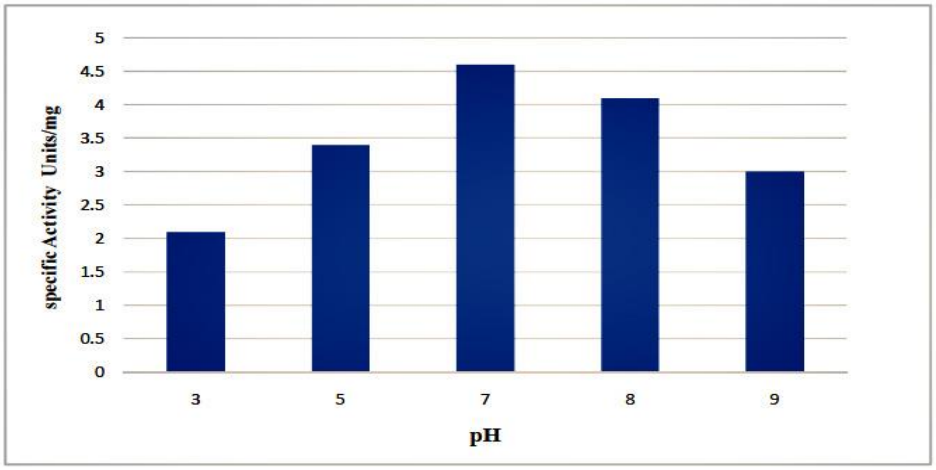


**Figure 2.** Optimum pH for L-glutamate oxidase production.

**Table 1.** Summary of the purification steps of L-glutamate oxidase from *Streptomyces.*

| Steps | Volume (ml) | Enzyme Activity (U/ml) | Protein Concentration (mg/ml) | Specific Activity (U/mg) | Total Activity (units) | Fold of purification | Yield (%) |
|---|---|---|---|---|---|---|---|
| Crude extract | 80 | 26.7 | 5.6 | 4.8 | 2,136 | 1 | 100 |
| Ammonium sulfate precipitation (saturation ratio: 60%) | 15 | 29.7 | 3.6 | 8.25 | 445.5 | 1.8 | 20.8 |
| Purification by dialysis | 15 | 26.5 | 2.8 | 9.5 | 397.5 | 2 | 18.6 |
| Ion exchange chromatography by DEAE-C | 10 | 27.3 | 1.1 | 25 | 273 | 5.2 | 12.7 |
| Gel filtration By Sephadex G200 | 3 | 28.6 | 0.51 | 56 | 85.8 | 11.7 | 4 |

DEAE: diethylaminoethyl cellulose

### 3.5. Purification by Dialysis

Purification by dialysis was used to remove the ammonium sulfate salts and concentrate the enzyme. A volume of 15 ml of the enzyme obtained from the stage of precipitation with ammonium sulfate was taken and placed in dialysis tubes with a diameter of 2.5 cm. The purification results showed an increase in the specific activity of the enzyme reaching 9.5 units/mg of protein, compared to the specific activity after the precipitation step with ammonium sulfate which was 8.25 units/mg. It should be mentioned that the number of purification times in this step was two, and the yield was 18.6%. As shown in Table 1.

### 3.6. Ion Exchange Chromatography

The enzyme solution after the concentration step by dialysis was passed through the DEAE-C ion-exchange column that was already equilibrated with the phosphate buffer (pH 7.4, 0.2 M). Absorbance was calculated for the washing parts (positively charged proteins) at the wavelength of 280 nm. When the absorbance of the line reached zero, the binding protein (negative proteins) was eluted with phosphate buffer (pH7.4) supplement with NaCl (0.15-1M). Figure 3 illustrates the results in two peaks, one in washing and the other in elution. One of them shows an enzyme activity in the elution step. The specific activity in this step was 25units/mg protein with a purification fold of 5.2 and a yield of 12.7%. As shown in table 1.

### *3.7. Gel filtration chromatography*

Gel filtration with Sephadex G200 was used for purification. TheDEAE-Cenzyme fractions were pooled and processed through a gel filtration column. The results shown in figure 4 indicate that the purity of the enzyme increased when using a gel filtration column and that also protein peaks appeared. Moreover, it shows that the enzyme activity was concentrated in one peak recorded from (11-13), and the specific activity reached 56 U/mg with a purification fold of 11.7 and yield of 4%.As shown in table1.

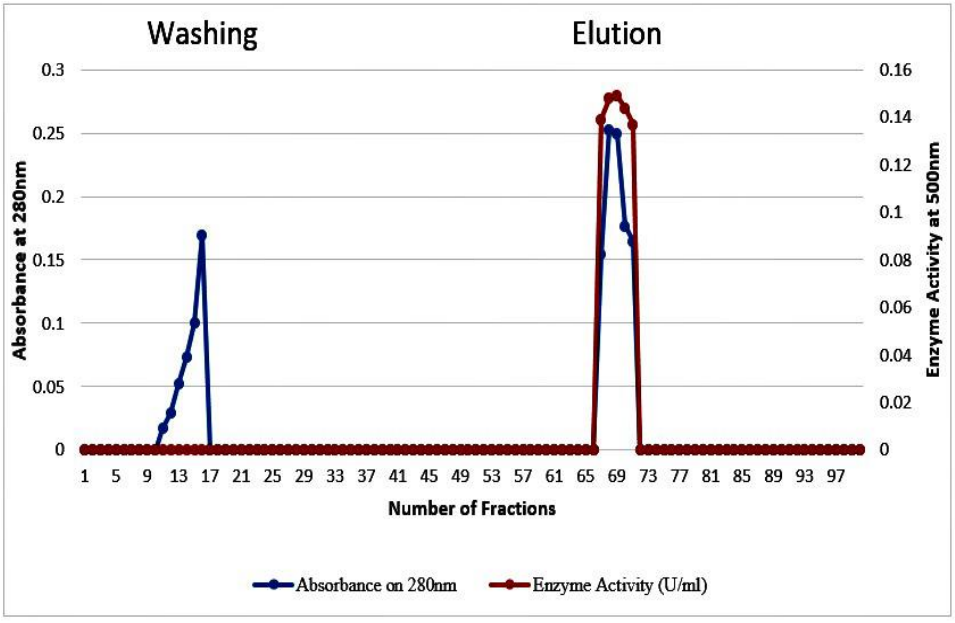


**Figure 3.** Ion exchange chromatography for L-glutamate oxidase purification from *Streptomyces* using DEAE-Column (3×13 cm) equilibrated with phosphate buffer (pH 7.4, 0.2 M), eluted with a phosphate buffer with NaCl gradient (0.15-1) M in phosphate buffer.

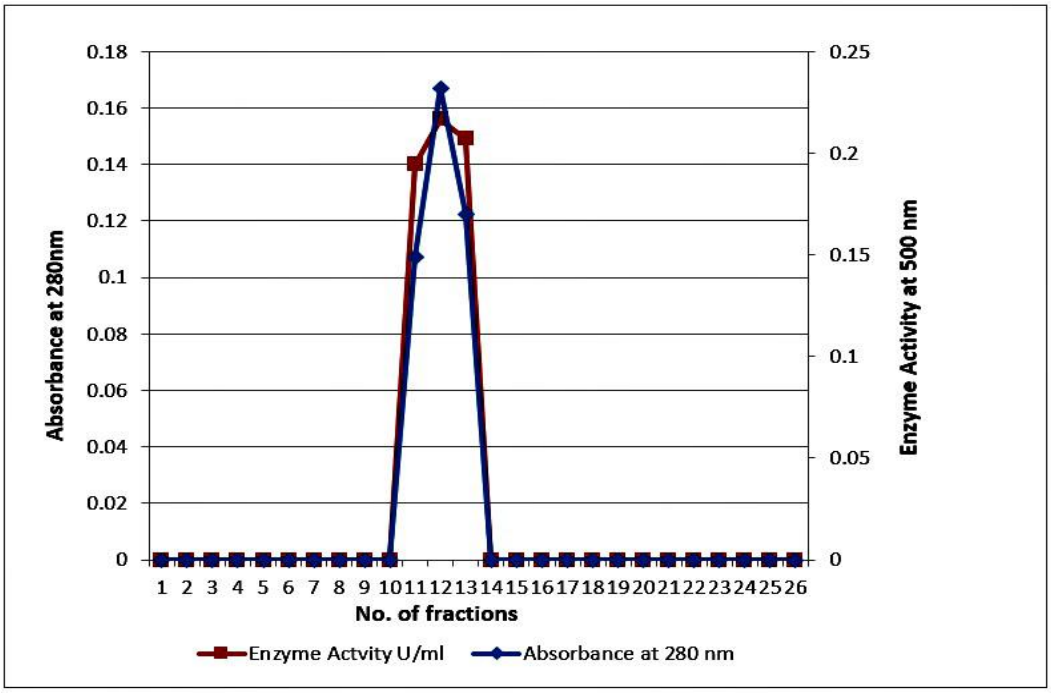


**Figure 4.** Gel filtration chromatography for L-glutamate oxidase purification from *Streptomyces* using Sephadex G200 (21×1) equilibrated with phosphate buffer (PH 7.4, 0.2 M).

### 3.8. Characterization of L-Glutamate Oxidase

#### 3.8.1. Optimal Temperature of Enzyme Activity and Stability

To find the ideal temperature for purified enzyme activity, the enzyme reaction was carried out at a varied temperature range (20-50 ˚C). Results in figure 5 showed an increase in enzyme activity by increasing the temperature and reached a maximum value of 26.7 U/ml at 37 ˚C; therefore, it began to decline with decreasing the temperature until it reached16 U/ml at 20˚C. Furthermore, the remaining activity was calculated at various temperature ranges (20–60 ℃), and the results are shown in figure 6. The enzyme maintained 100% of its activity when incubated at 30-50 ˚C; however, the activity began to decrease above and below this range.

#### 3.8.2. Optimal pH for Enzyme Activity and Stability

The activity was measured at different pH values. It was inferred that the L-glutamate oxidase enzyme had the maximum activity in pH 7.0 which was 27 U/ml as shown in figure 7. It can be concluded that the activity of L-glutamate oxidase was greater at neutral or almost basic pH values, co9mpared to nearly acidic pH values. For determination of the pH stability, the remaining activity was determined. It was observed that the pH rangeof5.0-7.0 was the optimum pH for L-glutamate oxidase stability where the remaining activity was 100% as shown in figure 8. This stability decreased in extreme acidic pH as the enzyme preserved40% and 75% of its activity at pH values of 3and 8, respectively.

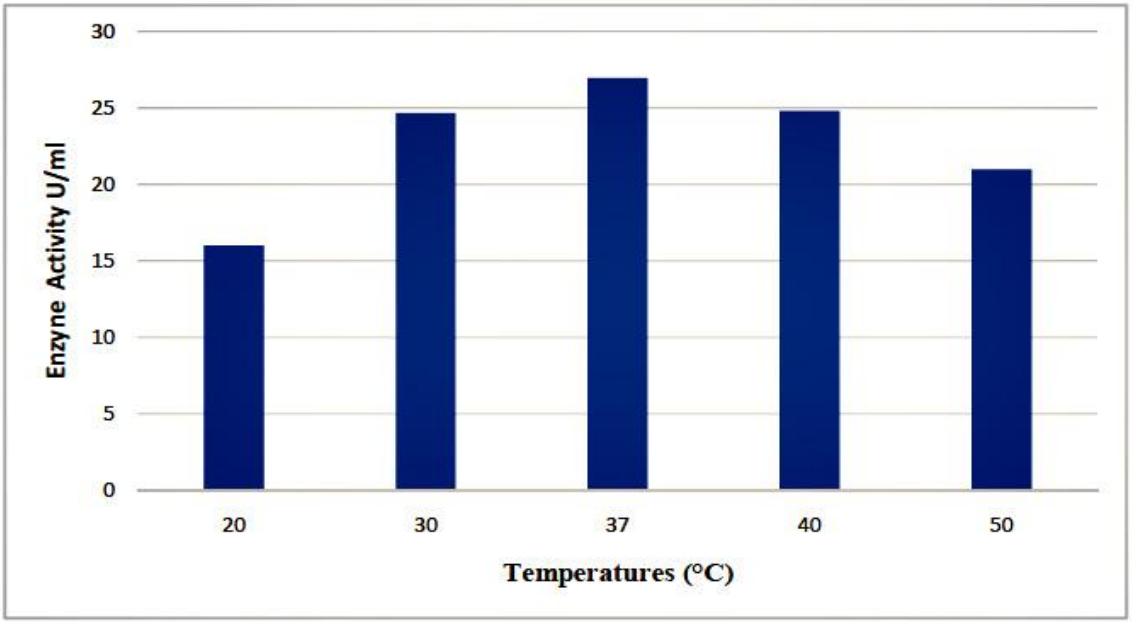


**Figure 5.** Effect of different temperatures (20-50°C) on locally purified L-glutamate oxidase activity from *Streptomyces.*

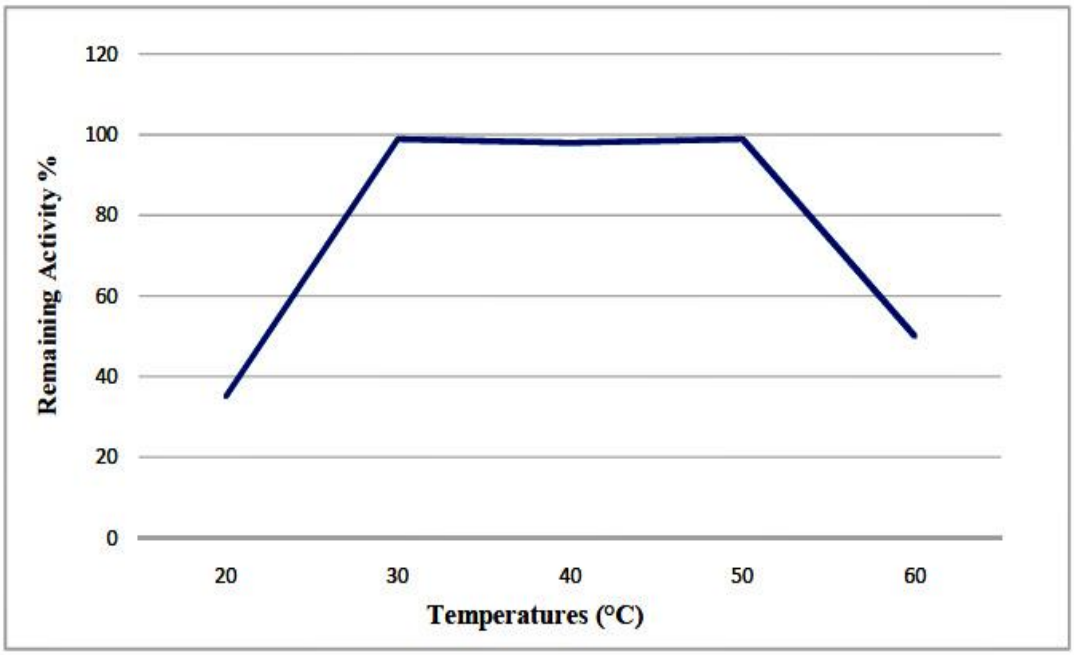


**Figure 6.** Effect of different temperatures (20-60 °C) on locally purified L-glutamate oxidase stability from *Streptomyces.*

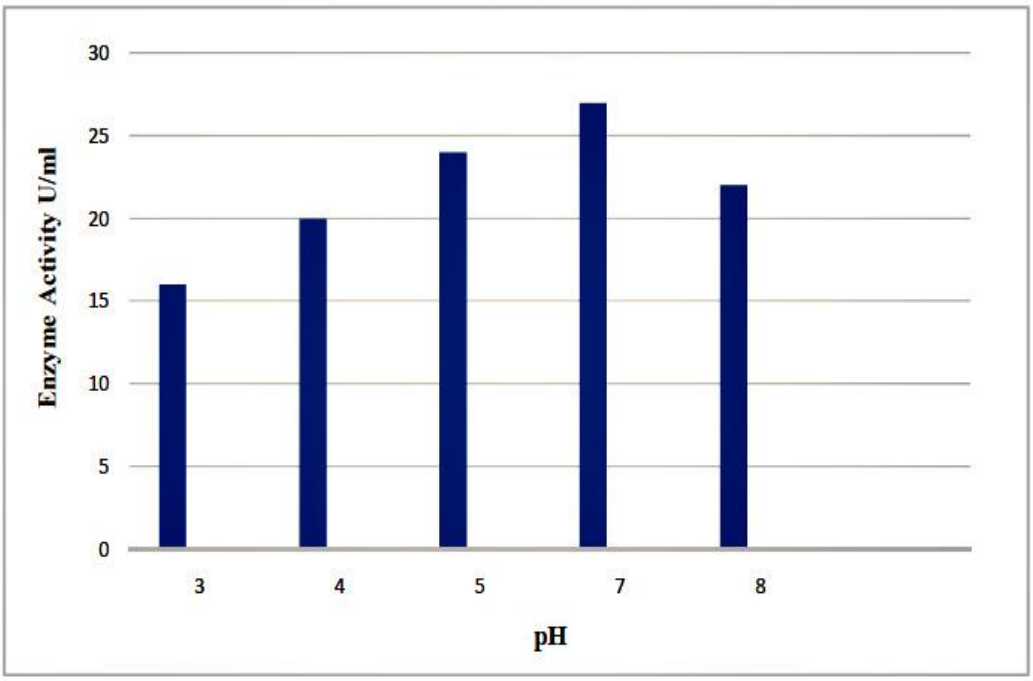


**Figure 7.** Effect of different pH values (3.0-8.0) on locally purified L-glutamate oxidase activity from *Streptomyces.*

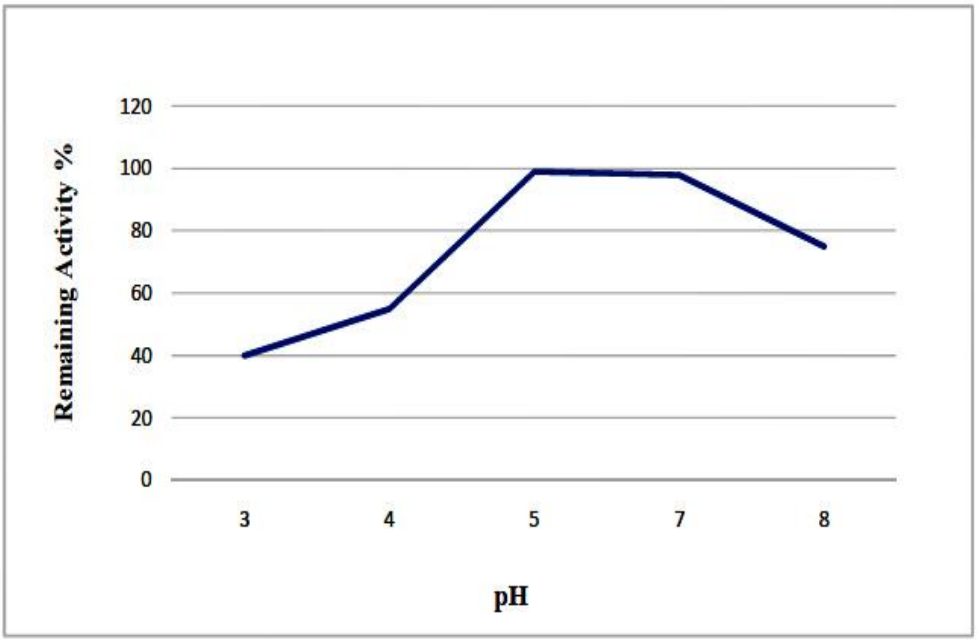


**Figure 8.** Effect of different pH values (3.0-8.0) on locally purified L-glutamate oxidase stability from *Streptomyces.*

## 4. Discussion

The experimental data of this research showed that the extracellular L-glutamate oxidase can be easily purified by the protocol used to purify proteins. One of the successful methods used to purify enzymes was the precipitation with ammonium sulfate. In this study, ammonium sulfate with a 60% saturation ratio was used, and this percentage was favorable and close to the one found by Kusakabe, Midorikawa in their study (21) who used a 50% saturation ratio. After that, ion exchange chromatography and gel filtration were used, and the results of these two steps reflected the difference between the previous steps in purification. This was due to the fact that the highest values of specific activity and high purity of the enzyme were obtained in these two steps.

Since enzymes can be used for different applications, it is necessary to identify the thermal stability and activity in a wide range of pH values. The results showed that the optimum temperature for L-glutamate oxidase activity was 37 °C and that thermal stability was within the range of 30-50 °C. This result is almost in line with those of the research performed by BÖHMER, MÜLLER (22). They found that the enzyme was stable within the temperature range of30 to 50-55 °C. This difference in the effect of temperature is due to the thermal effect on the enzyme structure followed by denaturation. This is because the temperature affects the protein structure by breaking the bonds that stabilize the secondary and tertiary structures of the protein, leading to denaturation, and thereby the loss of enzymatic activity (23).

In this study, the optimal pH for enzyme activity and stability was determined; accordingly, the findings revealed that the optimum pH for L-glutamate oxidase activity was 7.0. This is consistent with the results of the study conducted by Wachiratianchai and Bhumiratana (6). They reported that the optimum pH was within the range of 7.0-7.4, while the stability ranged from 5.0 to 7.0. The reason for the decrease or increase in the activity of the enzyme at different pH values was a change in the protein nature of the enzyme due to a variation in the ionic state of the amino acid side chains which is necessary for the maintenance of the 3D structure of the enzyme. It should also be noted that the pH affects the ionic groups of the active site in the enzyme. The optimum pH for enzyme stability is necessary to provide the appropriate environment for enzyme storage (24).

The results of this work showed that the L-glutamate oxidase enzyme can be obtained after 60 h of incubation of *streptomyces* in a wheat bran medium. The optimal temperature and pH for enzyme production were also investigated, with the results revealing that the best pH and temperature for enzyme production were 7.0 and 30 °C, respectively. Furthermore, ammonium sulfate precipitation, an ion-exchange column, and a gel filtration column can all be used to purify the L-glutamate oxidase enzyme. Gel filtration chromatography was the best approach to purify the enzyme isolated from *Streptomyces.* The appropriate temperature and pH for the activity and stability of the enzyme were evaluated to store the enzyme and preserve its activity and stability. According to the findings, the maximum temperature for the L-glutamate oxidase enzyme activity was 37 °C. Moreover, the temperature range for its stability was 30-50°C while the pH values for the efficiency and stability of the enzyme were 7.0 and 5.0-7.0.

### Authors' Contribution

Study concept and design: D. H. A. H.
Acquisition of data: D. H. A. H.
Analysis and interpretation of data: E. H. A.
Drafting of the manuscript: D. H. A. H.
Critical revision of the manuscript for important intellectual content: E. H. A.
Statistical analysis: E. H. A.
Administrative, technical, and material support: D. H. A. H. and E. H. A.

### Conflict of Interest

The authors declare that they have no conflict of interest.

## Acknowledgment

The authors would like to express their sincere gratitude to the Department of Applied Science Laboratories at the University of Technology in Baghdad, Iraq, for their assistance and provision of facilities during various stages of this work.

## References

1. Cooper J, Pritchard D. Biomolecular sensors for neurotransmitter determination: electrochemical immobilization of glutamate oxidase at microelectrodes in a poly (o-phenylenediamine) film. J Mater Sci Mater Electron. 1994;5(2):111-6.
2. Valero E, Garcia-Carmona F. A Continuous Spectrophotometric Method Based on Enzymatic Cycling for Determiningl-Glutamate. Anal Biochem. 1998;259(2):265-71.
3. Shi R, Stein K. Flow injection methods for determination of L-glutamate using glutamate decarboxylase and glutamate dehydrogenase reactors with spectrophotometric detection. Analyst. 1996;121(9):1305-9.
4. Kusakabe H, Midorikawa Y, Fujishima T, Kuninaka A, Yoshino H. Purification and properties of a new enzyme, l-glutamate oxidase, from Streptomyces sp. X-119-6 grown on wheat bran. Agr Biol Chem. 1983;47(6):1323-8.
5. Ling D, Wu G, Wang C, Wang F, Song G. The preparation and characterization of an immobilized L-glutamic decarboxylase and its application for determination of L-glutamic acid. Enzyme Microb Technol. 2000;27(7):516-21.
6. Wachiratianchai S, Bhumiratana A, Udomsopagit S. Isolation, purification, and characterization of L-glutamate oxidase from Streptomyces sp. 18G. Electron J Biotechnol. 2004;7(3):09-10.
7. Qingshan L, Lijun W, Yourong L. Color development with rational screening method for improved L-glutamate oxidase-producing strains. Enzyme Microb Technol. 1996;18(1):7-9.
8. Sukhacheva M, Netrusov A. Streptomyces sp. Z-11-6, a novel producer of extracellular l-glutamate oxidase. Microbiology. 2000;69(1):13-6.
9. Classics Lowry O, Rosebrough N, Farr A, Randall R. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-75.
10. Kuddus M, Joseph B, Ramteke PW. Production of laccase from newly isolated Pseudomonas putida and its application in bioremediation of synthetic dyes and industrial effluents. Biocatal Agric Biotechnol. 2013;2(4):333-8.
11. Pointing S, Jones E, Vrijmoed L. Optimization of laccase production by Pycnoporus sanguineus in submerged liquid culture. Mycologia. 2000;92(1):139-44.
12. Abood S, Hakeem I. Purification and characterization of salivary amylase inhibitor extracted from barley. Iraqi J Agric Sci. 2016;47(4.(
13. Al-easawi M. Extraction and Purification of Beta-Galactosidase from Local Almond and Its Use for Lactose Intolerance Treatment. Iraqi J Agric Sci. 2020;51(3):767-76.
14. Whitaker JR, Bernhard RA. Experiments for: An Introduction to Enzymology. 1972.
15. Erum S. Production of Peroxidases from Indigenous Fungal Strains and Their Application for Azo Dye Decolorization: Quaid-i-Azam University Islamabad, Pakistan; 2010.
16. Younis R. Production, purification and inhibition of alginate ly-ase from local isolate of pseudomonas aeruginosa na11. Iraqi J Agric Sci. 2020;51(6):1726-39.
17. Boeckx J, Hertog M ,Geeraerd A, Nicolai B. Kinetic modelling: an integrated approach to analyze enzyme activity assays. Plant Methods. 2017;13(1):1-12.
18. Noori H, Aziz G. Purification, Characterization, and Evaluation of Fibrinolytic Activity of Staphylokinase from Locally Isolated Staphylococcus aureus GH38. Iraqi J Agric Sci. 2020;51(4):1195-203.
19. Daniel RM, Danson MJ, Eisenthal R, Lee CK, Peterson ME. The effect of temperature on enzyme activity: new insights and their implications. Extremophiles. 2008;12(1):51-9.
20. Almeida VM, Marana SR. Optimum temperature may be a misleading parameter in enzyme characterization and application. PloS one. 2019;14(2):e0212977.
21. Kusakabe H, Midorikawa Y, Kuninaka A, Yoshino H. Occurrence of a new enzyme, l-glutamate oxidase ina wheat bran culture extract of Streptomyces sp. X-119-6. Agr Biol Chem. 1983;47(1):179-82.
22. Böhmer A, Müller A, Passarge M, Liebs P, Honeck H, Müller HG. A novel l-glutamate oxidase from Streptomyces endus: Purification and properties. Eur J Biochem.1989;182(2):327-32.
23. Whithaker J. Principles of enzymology for the food science. California: Marcel Dekker; 1994.
24. Kennelly P, Rodwell V. Enzymes: Mechanism of action. 28th ed: Mc Graw Hill Lange; 2009. 51 p.