



Obtaining L - Glutamate Oxidase Enzyme from *Streptomyces* and Estimation Its Toxicity on Blood Components

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Abstract

Streptomyces was isolated from the soil and then tested to diagnose morphologically and microscopically. Also, cultivated in the production medium of the L - glutamate oxidase enzyme, for which the wheat bran culture media was used, and then the extracellular L - glutamate oxidase enzyme was extracted using a cooling centrifuge at 8000 rpm for 20 minutes. The optimal temperature and pH for the production of the enzyme's conditions were studied. Research results revealed that the optimum temperature for enzyme production was 30°C, and the best pH for the enzyme's production was 7.0 later on, the enzyme was purified using common purification techniques represented by precipitation with ammonium sulfate as a preliminary purification step, followed by dialysis to remove salts, ion-exchange chromatography and gel filtration chromatography were used to finish purification process. The effect of the enzyme toxicity on the human blood components was evaluated. The enzyme had no harmful effect on the blood cells, with Red Blood Cells reaching 4×10^{12} , White Blood Cells 7.5×10^9 and Platelets 293×10^9 .

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1. Introduction

Enzymes are protein molecules that function as biological catalysts, speeding up chemical processes without being consumed, and they mediate nearly all reactions in the body. Enzymes are made from live cells and have the advantage of being able to function since biological catalysts stay active even after being removed or separated from the cells. They also exhibit a high degree of selectivity in the reactions they catalyze as well as in their interactions with the substrate. Enzymes are always catalysts, and a tiny quantity of enzymes in comparison to the substrate is enough to accelerate the process. Where the mechanism of action of enzymes is based on a chemical reaction in which the enzyme binds to the substrate and creates an enzyme-substrate complex. At the active site of an enzyme, a relatively tiny location is known as the active site [1]. The enzyme is affected by temperature and pH. The temperature has a large influence on enzyme reaction rates, as it impacts them through two opposing processes. 1) Activation: when the reaction speed increases as the temperature rises. 2) Denaturation: Denaturation occurs at high temperatures ranging from 50 to 60 degrees Celsius, except for thermophilic enzymes, wherein denaturation occurs at temperatures over 60 degrees Celsius. The optimum temperature for the enzyme, which can vary from 25-40 °C, is known as the best temperature at which the enzyme is most active and converts the largest amount of the substrate into products [2]. While pH alters the enzymatic activity due to its effect on the side chains of proteins. Each enzyme has an ideal pH number and one for its effectiveness, but some enzymes have more than that, and this number is usually in a very narrow range, but some enzymes show a wide range for it [3].

Streptomyces is the most abundant genus of actinomycetes accounting for half of all soil actinomycetes. *Streptomyces spp.* also produces a wide variety of novel antibiotics than any other actinomycetes genera. Different species of *Streptomyces* produce around 6000 different antibiotics, many of which are commercially available as anti-infective (antibiotics, antifungal, and antiparasitic), anticancer, or immunosuppressant agents [4]. They may be found in nearly all habitats, and they are gram-positive, aerobic, non-motile, and filamentous. *Streptomyces* have genomes with high (G+C) content of 69-78% and they are spore-forming microbes [5]. These bacteria produce aerial hyphae that get divided, resulting in spores that can withstand harsh circumstances and spread quickly to new habitats or sources of food. *Streptomyces* produce secondary metabolites during the growth phase, which are chemicals that are not essential for growth or reproduction but might provide the organism with competitive benefits [6]. The colonies are slow-growing and relatively soft which developed later on into a granular or velvety appearance. It often has a soil-like odor due to its production of a secondary metabolic compound known as Geosmin [7].

Streptomyces strains such as *S.somaliensis* and *S.sudanensis* cause infectious human diseases such as mycetoma. It is an inflammatory disease that causes the skin, subcutaneous tissue, and muscles of the body to gradually become inflamed. Furthermore, some species do not exist in the soil but coexist with plant roots and can cause disease. For example, potato scab disease is caused by the bacteria *Streptomyces scabis* [8]. L-glutamate oxidase (LGOX) [EC 1.4.3.11] from *Streptomyces* It has a molecular weight of 150 KDa and has a hexamer structure consisting of $\alpha_2\beta_2\gamma$. L – glutamate oxidase enzyme induces the oxidative deamination of an L-glutamate to an α -ketoglutarate, ammonia, and hydrogen peroxide. This enzyme is a valuable analytical tool for measuring L-glutamate levels in food and during the fermentation process. It also gained a lot of interest because of the possibility of using it to diagnose liver function. In the clinical laboratory, it might be used to evaluate serum Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT) levels. The enzyme L-glutamate oxidase extracted from the genus *Streptomyces* is an exoenzyme that is stable in temperature and acidity. It is also classified as one of the flavoproteins because it includes a flavin group that is not covalently bound as a cofactor [9].

This work aimed to expand the studies of the enzyme and giving the facts that the researches on L - glutamate oxidase enzyme production and application in Iraq have not been conducted so far, this may be the first study on it. This study was carried out to identify the optimal conditions for enzyme production as well as to determine whether the locally purified enzyme from *Streptomyces* has any toxic or harmful effects when mixed with human blood. Additionally, this study focused on comparing its toxicity to that of toxic hydrogen peroxide on human blood components.

2. Materials and Methods

2.1 Sample Identification

Streptomyces bacteria were isolated from the soil and cultivated on nutrient agar medium and starch casein agar for 48 hours at 30°C, after that they were identified by morphological and microscopic characteristics and biochemical tests [10].

2.2 Enzyme Production

To produce the L-glutamate oxidase enzyme, bacteria were implanted in the enzyme production media for 60 hours in a shaking incubator, and because the enzyme is extracellular, thus it was centrifuged for 20 minutes at 8000 rpm in a cooling centrifuge. The filtrate that containing the enzyme was collected and the precipitate was discarded, after that the enzyme was purified to get it singly and purely to be used in various applications [11].

2.3 Determination of Optimum pH for L- Glutamate Oxidase Production

To determine the optimum pH for enzyme production, the pH of the prepared culture medium was changed to (3, 5, 7, 8, 9) which contains 1 ml of *Streptomyces* culture and the age of culture was 48 hours in the shaking incubator at 60 hours. After that the activity of the enzyme produced by bacteria was measured after extraction to determine the optimum pH for production [12].

2.4 Determination of Optimum Temperature for L- Glutamate Oxidase Production

To determine the optimum temperature for enzyme production, the prepared culture medium which contains 1 ml of culture the age of culture was 48 hours were incubated at different temperatures (20, 25, 30, 37, 40)°C in the shaking incubator for 60 hours, then the activity of the enzyme produced by bacteria was measured after extraction to find the optimum temperature for production[12].

2.5 Evaluation of the Toxic Effect of Enzyme on Human Blood Components

This experiment aimed to distinguish whether the enzyme has any toxic effect on human blood cells by automated analysis utilizing a hematological apparatus (Mindray) which is a novel hematological analyzer specifically designed for complete blood count [13]. Random blood samples were obtained at a rate of 3 ml from healthy persons in specialist laboratories, then 1 ml was placed in tubes containing EDTA, where these samples were treated once with the enzyme L-glutamate oxidase and once with hydrogen peroxide, where 10 microliters of each substance were added to the blood tubes, then the tubes were incubated for an hour, after which the result was read and the results were compared with the control [14]

Number of samples	Ages	Enzyme (Microliter)	Hydrogen peroxide (Microliter)	Control (Blood only)
5 Males 5 Females	25-30	10	10	1 ml

3. Results and Discussion

3.1 Identification of Bacteria

The process of identifying bacteria began with cultivating the samples and incubating them for 48 hours at 30°C, followed by a biochemical test to confirm that the isolate belonged to *Streptomyces*. The results obtained are in agreement with the description in (Rong and Ming 1995) [15].

Table 1. Characteristics of *Streptomyces*.

Characteristics of <i>Streptomyces</i>	Results
Cultural Characteristics	On starch casein agar as its diagnostic medium Shape: filamentous and round Chromogenesis: White and cream Surface: powdery Consistency: dry
Biochemical Tests	Oxidase: positive Catalase: positive
Gram Stain	Positive Purple filamentous



Figure 1. *Streptomyces* bacteria on starch casein agar.

3.2 Optimum Temperature for Enzyme Production

The results revealed that the optimum temperature for the production of the enzyme is 30°C, as shown in Figure (2), with the highest specific activity reaching to 4.5 units per milligram protein at the temperature 30°C, and the lowest specific activity of the enzyme was 2.7 units per mg/protein at the temperature 20°C, based on these findings the temperature of 30 °C was chosen as the ideal temperature for the enzyme. This is consistent with [16], which showed that the best temperature for the production of the L- glutamate oxidase enzyme from bacteria is 30 ° C.

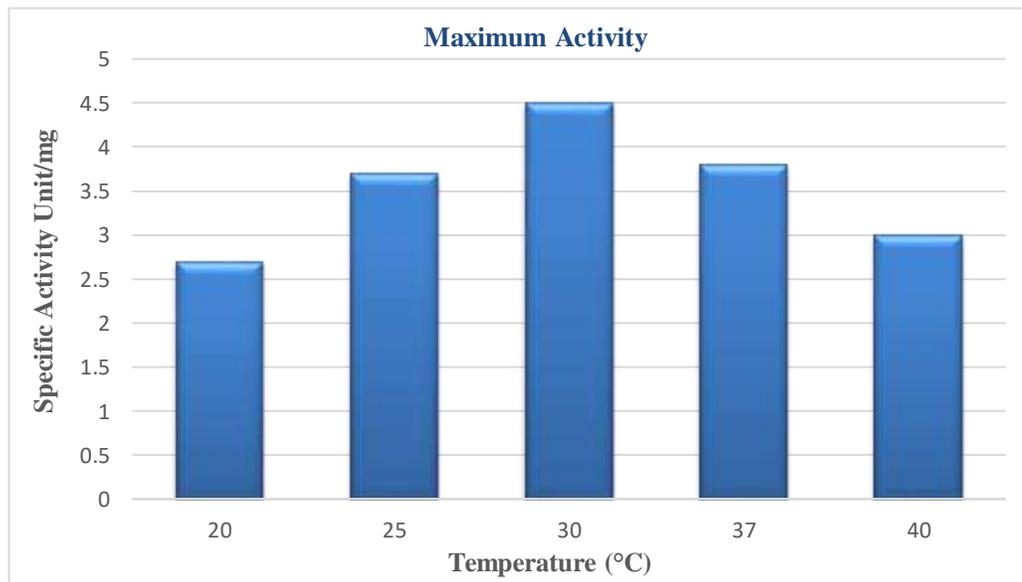


Figure 2. Effect of different ranges of temperatures (20-40) °C on locally isolated L-glutamate oxidase production from *Streptomyces* bacteria.

3.3 Optimum pH for Enzyme Production

In order to study the effect of pH on L- glutamate oxidase production, a production medium was prepared in diverse pH values consisting of (3.0, 5.0, 7.0, 8.0, and 9.0). As in figure (3), the results showed the specific activity of L-glutamate oxidase enzyme synthesis by *Streptomyces* bacteria increased at pH 7.0, reached 4.6 units per milligram protein while the lowest specific activity was 2.1 units per mg/ protein at pH 3.0 this indicates that the pH 7.0 is the optimal pH for enzyme production [16]. This indicates that the bacteria grow well at this pH and are able to produce the enzyme. Based on these results, the pH of the production medium was adjusted to 7 in the later stages of the study.

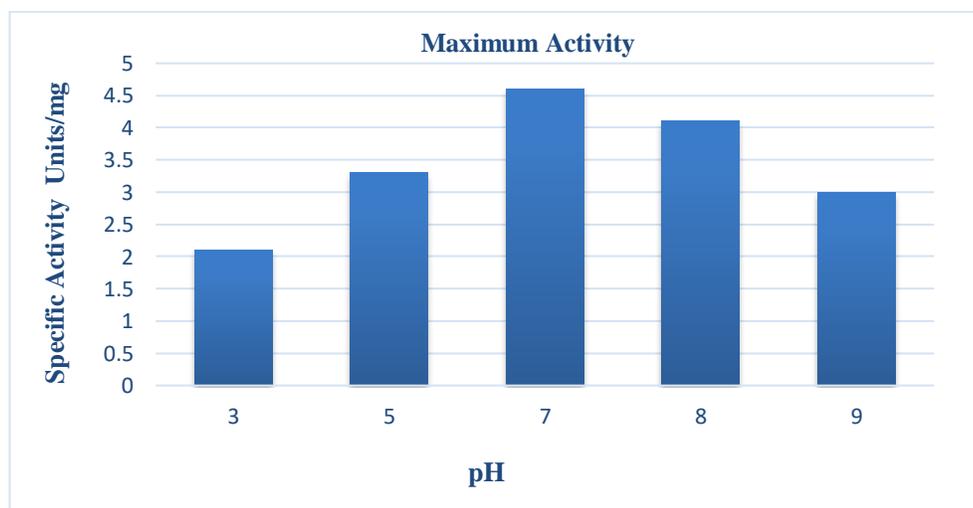


Figure 3. Effect of different range of pH values (3.0-9.0) on locally isolated L-glutamate oxidase production from *Streptomyces* bacteria.

3.4 Determination of the effect of the enzyme on blood components

Male and female blood samples were taken and treated with enzyme and hydrogen peroxide (H_2O_2). The results in figures (4,5,6) revealed that:

- 1) The enzyme's influence on blood components as indicated in the diagrams where it had been it's important to note that the enzyme had very little effect. Therefore, it can be concluded from these results that the enzyme is safe and can be applied and used in the future in various medical applications.
- 2) If the results obtained were compared according to the effect of the enzyme with the effect of hydrogen peroxide, the results showed that the hydrogen peroxide exhibited a simple decomposition in the blood components. Due to the facts, hydrogen peroxide causes toxicity in three main ways: corrosive damage, oxygen gas formation, and lipid peroxidation. So, concentrated hydrogen peroxide is considered hazardous, which can lead to local tissue damage. Ingestion of strong hydrogen peroxide (>35 percent) can also result in the production of large amounts of oxygen. When the amount of oxygen released exceeds its maximum solubility in blood, a venous or arterial gas embolism might happen [17].

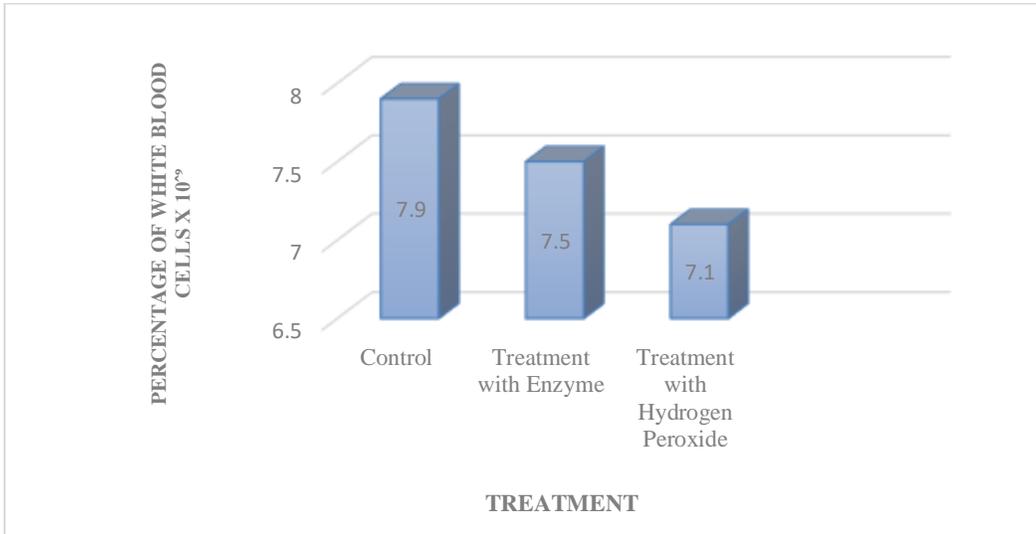


Figure 4. The effect of the studied treatments on the percentage of white blood cells.

Note* Studied treatment means

Control: Only human blood without any addition.

Treatment with enzyme means the human blood after adding 10 microliters of the enzyme to it. Treatment with hydrogen peroxide means the human blood after adding 10 microliters of hydrogen peroxide to it.

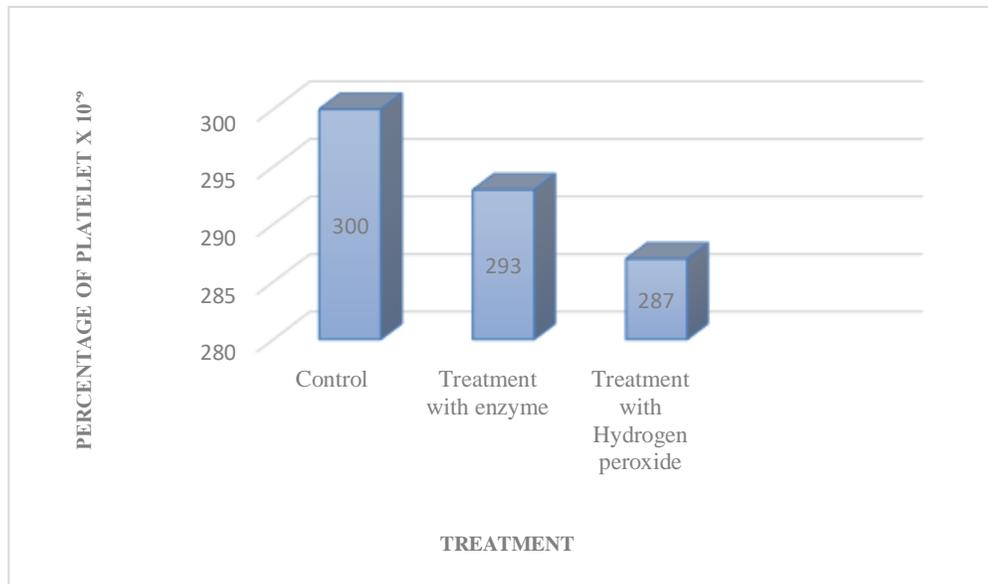


Figure 5. The effect of the studied treatments on the percentage of platelets.

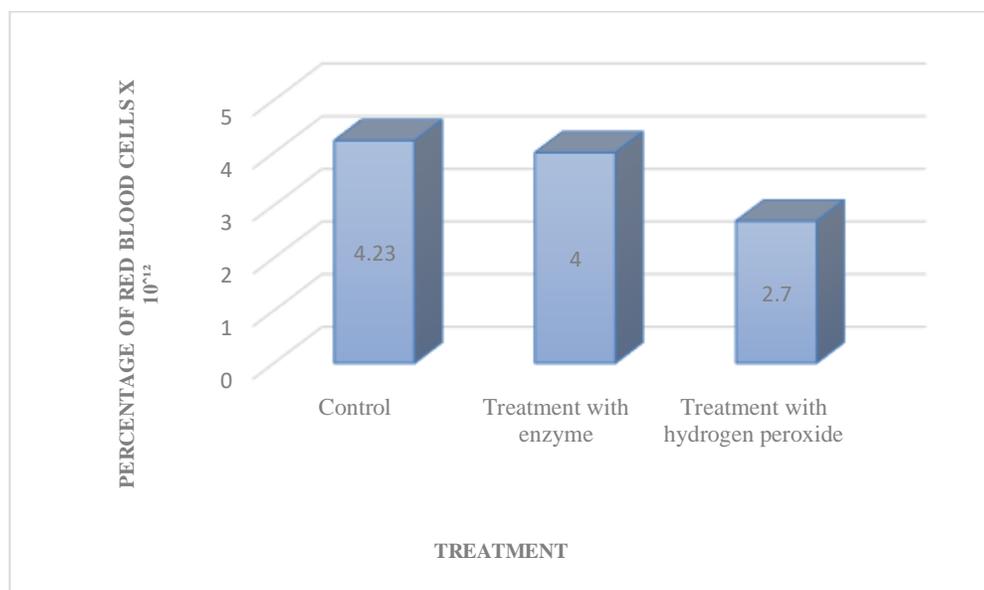


Figure 6. The effect of the studied treatments on the percentage of red blood cells.

4. Conclusions

The enzyme L- glutamate oxidase can be easily extracted after 60 hours of incubation of *Streptomyces* bacteria in a wheat bran medium. The effect of both temperature and pH on enzyme activity was studied to determine the favorable conditions for enzyme production. The results revealed that the best temperature and pH were at 30°C, 7.0 respectively. So, because the enzyme has no effects when mixed with human blood. Studies on it might be developed in the future to use the enzyme in a wide range of medicinal applications. Future studies on the L- glutamate oxidase enzyme could also be expanded since it can also be used in various medical and analytical applications.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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