Optimization of glucose oxidase produced from a Local isolate of *Pseudomonas aeruginosa.*

Baraa A. Al-sultany* , Asmaa A. Hussein

Molecular and Medical Biotechnology Department, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq

*Correspondence email: baraa98ali@gmail.com

Received: 03/06/2024
Accepted: 31/07/2024
Online: 14/12/2025

ABSTRACT

Background: Glucose oxidase (GOx) is an oxidoreductase enzyme that plays a wide range of significant roles in biological processes as well as a variety of industrial and commercial applications. In food processing, for example, it is utilized as a natural preservative, antioxidant, and modifier that are relatively stable and inexpensive. Although GOx has been isolated from various microorganisms, particularly fungi. There is limited research on isolating it from bacteria, so it's important to study GOx from novel microbial strains, such as *Pseudomonas aeroginosa,*which is among the most commonly isolated Gram-negative pathogens. It can be found in most settings, including soil, plants, and mammalian tissues. **Objective:** Evaluation of the optimum conditions of GOx production by a clinical sample of *P. aeruginosa*. **Methodology:** 150 clinical samples were used to isolate *P. aeruginosa* , which were characterized by biochemical and microscopic analyses with verification through the use of the VITEK2 system. Selected isolates were examined for optimal GOx production conditions: carbon and nitrogen sources, pH, temperature, and incubation period. **Results:** A total of 35 *P. aeruginosa* isolates were identified; only 10 were GOx producers. Optimization conditions for enzyme production were achieved for the highest GOx producer isolate when the medium was supplemented with peptone 1% w/v, glucose 2% w/v, pH 7, temperature 37°C, and incubation for 24 hrs., which raised the specific activity to (1, 3, 3.5, 3.2, and 3.5U/mg protein), respectively**. Conclusion**: The present investigation's results indicated that P. aeruginosa is capable of producing the enzyme glucose oxidase, and the optimum conditions were determined by supplementing BSM medium with 2% glucose and 1% peptone at pH 7 and incubating at 37 °C for 24 hours.

Keywords: Glucose oxidase production, Optimization, *Pseudomonas aeruginosa.*
https://doi.org/10.24126/jobrc.2025.19.3.865

INTRODUCTION

Enzymes function as biocatalysts, accelerating chemical reactions to yield valuable products. Enzyme preparations derived from microbes are currently utilized in numerous industrial, agricultural, and medical domains (1). Many plant and animal enzymes have been replaced in recent decades by microbial enzymes. The reason for this substitution is that microbial enzymes may be manufactured cheaply and readily in short fermentation times using low-cost media (2). Glucose oxidase (GOx) is an enzyme that has a tendency to catalyze the oxidation of β-D-glucose to produce H_2O_2 and D-glucono-δ-lactone. This substance has widespread applications in the fields of food, pharmaceutical, beverage, and enzymatic fuel cell (EFC) technology. It may also be used in biosensor devices, such as blood glucose monitors, and renewable energy sources (3). Glucose oxidase is produced by a wide range of microorganisms, including fungi, bacteria, particularly those belonging to the *Aspergillus* and *Penicillium* species, mammals, plants (such as insects), and citrus fruits (4). Research on (GOx) from novel microbial strains is important,

along with purifying it from sustainable sources. (GOx) from *Actinomyces* and *Pseudomonas* species was examined for its characteristics and functions (5). *Pseudomonas aeruginosa* is a frequently isolated Gram-negative bacterial pathogen that can cause serious infections in patients with compromised immune systems, including those with HIV/AIDS, cancer patients undergoing chemotherapy, and patients receiving hematopoietic stem cell transplantation (HSCT) who are immunosuppressed (6). Many parameters, including inoculum size, pH, temperature, the presence of inducers, aeration, medium additives, and growth period, need to be controlled to maximize the productivity of microorganisms that produce enzymes (7). Changing one factor at a time (OFAT), while maintaining the other factors constant, is the traditional optimization approach (8). Therefore**,** this study aimed to determine the optimal conditions for glucose oxidase production from locally isolated *Pseudomonas aeruginosa* as one of the medically important enzymes in the future.

METHODOLOGY

Samples

From September to November 2023, 150 clinical specimens were obtained from patients presenting to AL-Yarmouk Teaching Hospital and Medical City Hospital in Baghdad, Iraq. The specimen included burns, sputum, wounds, and the ears of patients suffering from otitis media. The study was conducted following the moral principles outlined in the Declaration of Helsinki. Before sample collection, the patient provided verbal and analytical consent. The study protocol, subject information, and consent form were reviewed and approved by a local ethics committee, as documented in approval number 21 dated September 11, 2023.

Isolation and identification of *Pseudomonas aeruginosa*

After collecting swaps, specimens were grown in brain heart infusion broth medium at 37 °C for 24 hours. Then each specimen was streaked on (Blood agar, MacConkey agar, and Cetrimide agar) and incubated at 37 °C for 24 hours. Morphological analysis (size, margin, color, shape, and consistency of colonies), microscopical characteristics, biochemical testing, and the VITEK 2 system were used to detect bacterial growth.

Preparation of inoculum

Thirty-five isolates of *P. aeruginosa* were used to inoculate the modified basal salt medium, containing $(NH_4)_2HPO_4$, 0.4; Peptone, 10; Sucrose, 70. KH_2PO_4, 0.2; $MgSO_4$ $7H_2O$, 0.2, adjusted at pH 7 and incubated at 37 °C for 24 hrs. (9)

Glucose oxidase extraction

Each one of the 35 *P.aeruginosa* isolates was inoculated in 250 mL of modified (BSM) medium at 37 °C for 24 h. Then centrifuged for 15 minutes at 10,000 rpm. The supernatant was used as a crude GOx enzyme.

Enzyme activity assay

The (GOx) activity was determined by measuring the decrease in glucose concentration, using glucose as a substrate according to the method described by Miller (1959) (10). 0.1 mL of crude extract was incubated for 30 min at 80°C with (0.1) mL of 1 gm glucose as substrate dissolved in 10 mL of 0.1M sodium acetate buffer pH (5) centrifugation for 10 min. in a cold centrifuge, the enzyme reaction was stopped by 0.2 mL of 3, 5- Dinitrosalicyclic acid (DNS) reagent keeping at 100 °C for 5 min. The absorbance was measured at 575 nm by a UV-visible spectrophotometer. The blank was prepared by adding 0.1 mL of D.W to 0.1 mL of (1 g of glucose dissolved in 10 mL of 0.1M sodium acetate).Standard curve prepared by glucose (11). GOx activity was calculated as µmol glucose min^{-1} using the following equation: (GOx) Activity =(C0 – C).v/t (12).

Where :C0: Initial glucose concentration (mM) /C: Last glucose concentration (mM)

V: Reaction volume (mL) /t: Reaction time (min)

Protein concentration was measured according to Bradford (13).

Specific activity of glucose oxidase

Specific activity of the enzyme was calculated according to (14):

Specific activity (U/mg protein) = (Enzyme activity (U/mL))/ (Protein concentration (mg/mL)

Optimum conditions for glucose oxidase production

Enzyme production was optimized by changing one factor at a time.

Optimum nitrogen and carbon sources

Five nitrogen sources: Peptone, Casein, NaNO3, Yeast extract, and Ammonium sulfate, and five carbon sources: Glucose, Maltose, Glycerol, and Starch were separately added to the BSM medium as a first step. Each Carbone source was included in the medium separately at a final concentration of 2% (w/v), while the nitrogen sources were at 1% (w/v), and the medium was incubated for 24 hours at 37°C.; Glucose oxidase activity, protein concentration, and specific activity were determined.

Optimum Temperature

After inoculating the production medium with the chosen *P. aruginosa*, it was incubated at various temperatures (27, 32, 37, and 42 °C). In the subsequent optimization experiment, the optimum temperature for the synthesis of (GOx) was employed.

Optimum pH

The optimum pH for the production of (GOx) was determined by adjusting the pH of the optimized production medium at different values (4, 5, 6, 7, and 8), then incubating for 24 hrs. At 37°C. Glucose oxidase activity, protein concentration, and specific activity were determined.

Optimum incubation period

By incubating the production medium for (12, 24, and 36 hours), researchers were able to determine the optimum incubation period for *P. aruginosa's* synthesis of glucose oxidase.

RESULTS

Isolation and identification of *Pseudomonas aeruginosa*

Sixty isolates from 150 samples grew on MacConkey agar, appeared as colorless or transparent bacteria,and were non-lactose fermenters. *P. aeruginosa* colonies showed β-haemolysis on blood agar; only thirty-five isolates were able to grow on the selective media, cetrimide agar, and produce yellowish-green fluorescent pigment. Microscopic analysis revealed that isolates were single cells, Gram-negative, non-spore-forming, and rod-shaped. All these 35 isolates gave (oxidase , catalase , citrate) positive , while (urease , MR_VP) negative results, as shown in (Table 1). However, the VITEK2 system indicated that the isolate was 91% identical to *P. aeruginosa* (Table 2).

Table (1): Primary biochemical tests of *P.aeruginosa's* isolates

Biochemical tests	Result
Oxidase	+
Catalase	+
Citrate	+
Urease	-
MR_VP	-

Table (2): Biochemical tests for identification *P. aeruginosa* isolates by VITEK 2

Test	Result	Test	Result	Test	Result	Test	Result	Test	Result	Test	Result
APPA	-	ADO	-	PyrA	-	IARL	-	dCEL	-	BGAL	-
H2S	-	BNAG	-	AGLTp	+	dGLU	+	GGT	+	OFF	-
BGLU	-	dMAL	-	dMAN	-	dMNE	+	BXYL	-	BALap	+
ProA	+	LIP	+	PLE	-	TyrA	+	URE	+	dSOR	-
SAC	-	dTAG	-	dTRE	-	CIT	+	MNT	+	5KG	-
ILATK	+	AGLU	-	SUCT	+	NAGA	-	AGAL	-	PHOS	+
GlyA	-	ODC	-	LDC	-	IHISa	-	CMT	+	BGUR	-
O129R	+	GGAA	+	IMLTa	-	ELLM	-	ILATa	+		

Specific activity of glucose oxidase

Of the thirty-five *P. aeruginosa* isolates, ten produced GOx with variable degrees, and *P. aeruginosa* P6 showed the maximum specific activity (0.91 U/mg); therefore, it was chosen for further study (Table 3).

Table (3): The specific activity of Glucose oxidase production by *P. aeruginosa* clinical isolates after 24 hrs. at 37°C incubation temp.

No. of Isolate	Specific activity (U/mg)
P1	0.6
P2	0.8
P3	0.6
P4	0.8
P5	0.85
P6	**0.91**
P7	0.88
P8	0.86
P9	0.8
P10	0.9

Optimum carbon and nitrogen sources for glucose oxidase production

Among different tested nitrogen sources, peptone was the best for producing GOx from *P. aeruginosa* (**P6)** (Figure 1) with a specific activity reached (1U/mg protein).

When the medium was supplemented with each carbon source individually at 2% w/v., the results showed that, the maximum production of GOx was obtained by using glucose as a carbon and energy source, which produced the highest GOx specific activity of (3 U/mg) followed by sucrose with specific activity of (2.6 U/ mg), maltose (2.2 U/ mg), glycerol (1.7 U/mg), starch (1.5 U/mg) (Figure 2).

Optimum temperature

Incubation temperatures of 27, 32, 37, and 42 °C were used for further optimization of the growth conditions for GOx production from *P. aeruginosa* **P6** The enzyme was found to show a maximum activity at 37 °C with specific activity 3.2 U/mg as shown in (Figure 3).

Optimum pH

P. *aeruginosa* ***P6*** was used to study the impact of starting medium pH on glucose oxidase synthesis. (Figure 4) shows that the production medium pH must be increased to 7 to achieve the highest level of GOx synthesis. At this point, the enzyme's specific activity was 3.5 U/mg protein.

Optimum incubation period

After 12 hrs of incubation, Gox-specific activity was 1.7 U/mg protein. 24 hrs later, it increased to 3.5 U/mg protein, then decreased to 2.8 U/mg specific activity at 36 hrs , as illustrated in (Figure 5).

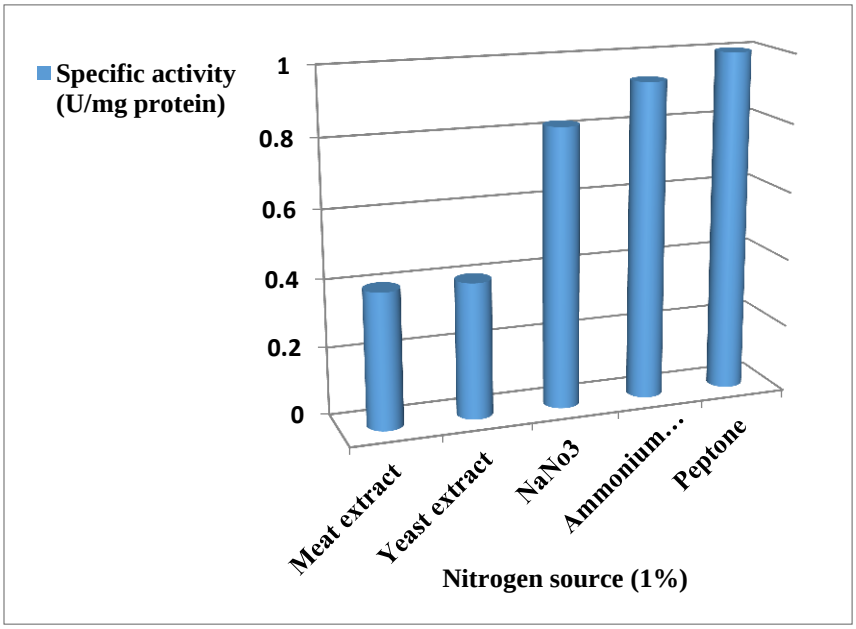

Figure (1): Optimal nitrogen source for Gox production from *P.aeruginosa P6* after incubation for 24 hrs at 37°C.

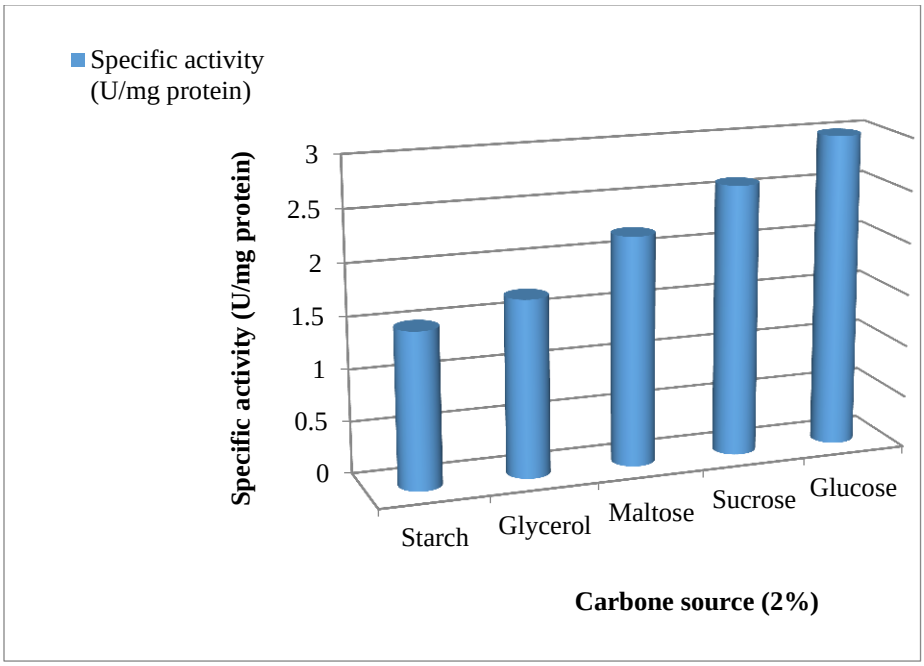

Figure (2): Optimal carbon source for Gox production from *P.aeruginosa P6* after incubation for 24 hrs at 37°C.

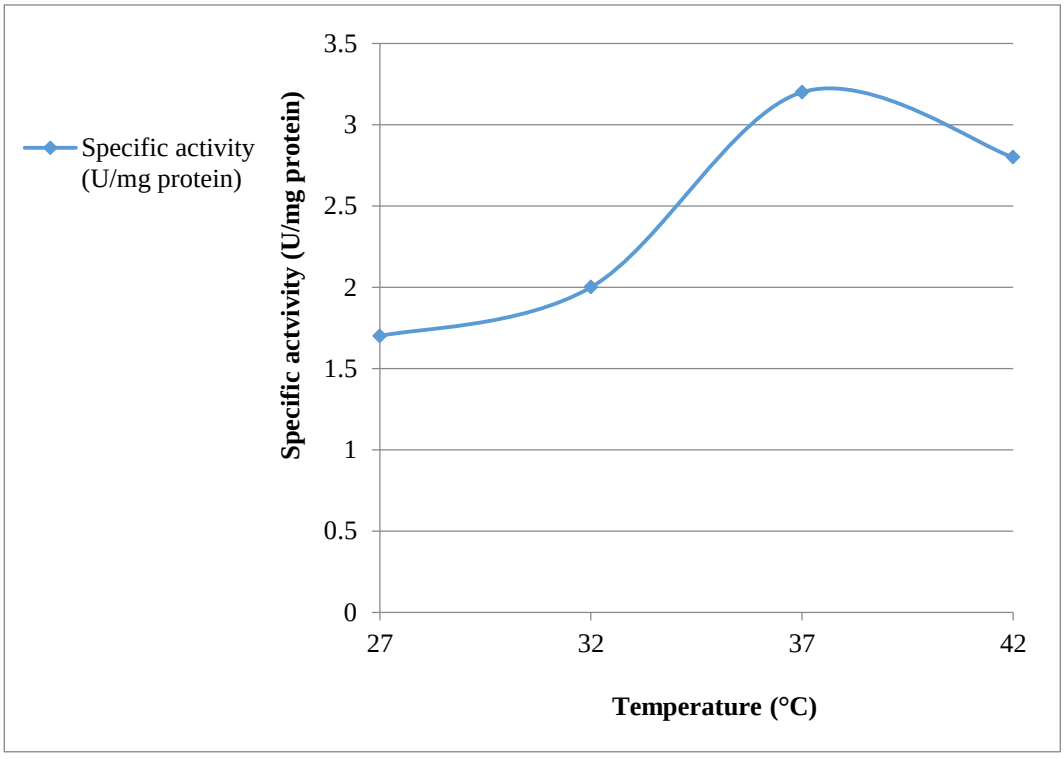

Figure (3): Optimal incubation temperature affected Gox production from *P.aeruginosa* P6 after incubation for 24 hrs at 37°C.

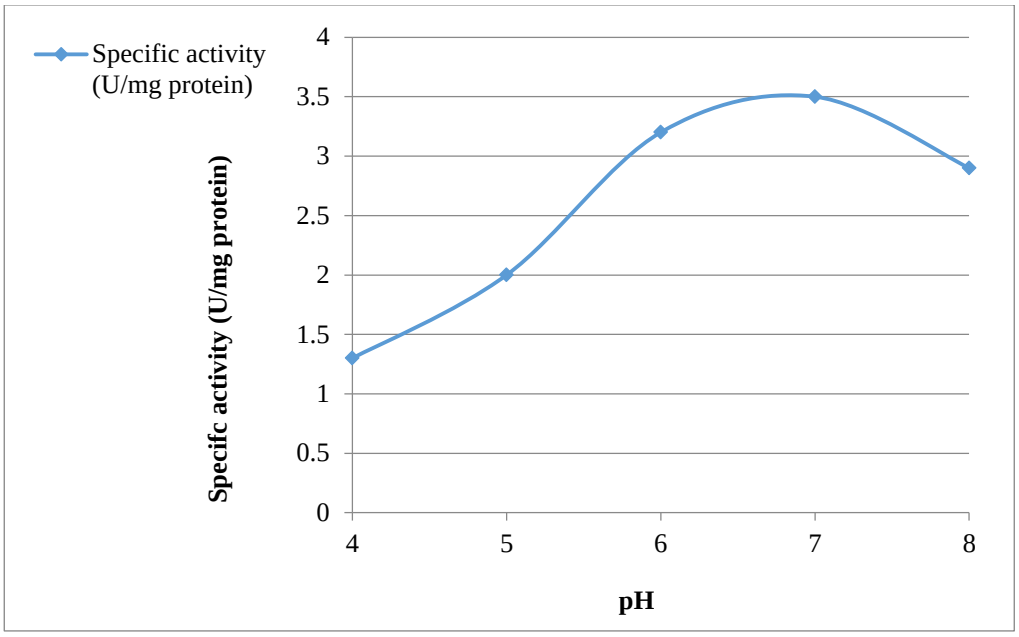

Figure (4): Optimal pH for Gox production from *P.aeruginosa* P6 after incubation for 24 hrs at 37°C.

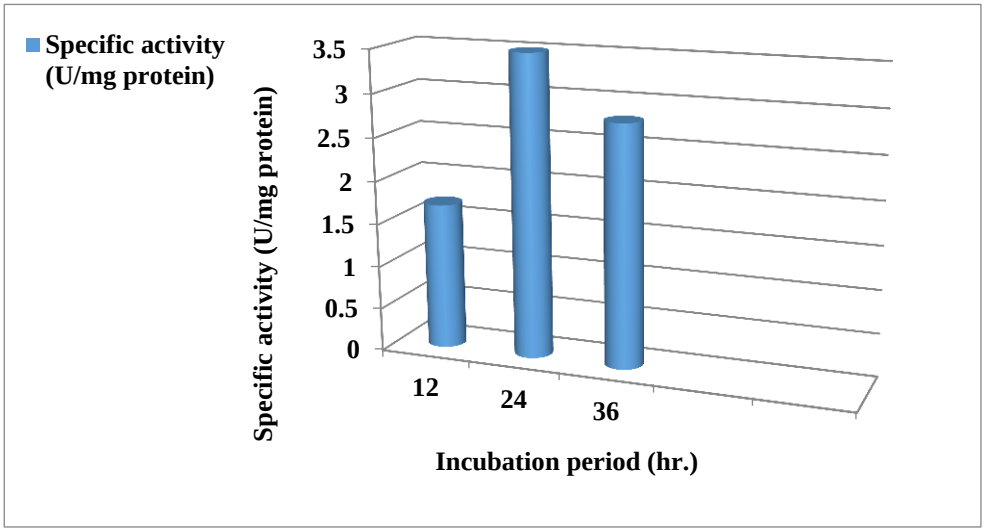

Figure (5): Optimal incubation period for *P.aeruginosa P6* producing Gox at 37 °C

DISSCUSION

P. aeruginosa is the most commonly encountered Gram-negative species that is not a member of the family Enterobacteriaceae (15). *P. aeruginosa* is thought to be a nosocomial infection and an opportunistic pathogen that causes a wide range of illnesses and can develop antibiotic resistance (16,17).

Glucose oxidase is an oxidoreductase enzyme with industrial and diagnostic applications. the study of various aspects of Gox in biotechnology and bioprocessing is crucial(18).

The GOx enzyme has a high specificity to glucose, when compared to other sources, giving the specific activity of (3 U/mg), followed by sucrose. The current study was correlated with (19), which reported that glucose produced the highest GOx, followed by sucrose. All carbon sources supported the growth of *Aspergillus spp*., yet

sucrose and glucose produced a considerable amount of GOx. Another study reported that glucose is the primary inducer of the GOx gene transcription (20).

After carbon, nitrogen is essential for microbial cell growth. Nitrogen is a necessary component of proteins, nucleotides, enzymes, and a cofactor vital to cellular metabolism (21). The study reported in (22) found that peptone was a useful nitrogen source for GOx synthesis, exhibiting the highest activity (1231 µmol/min) at a concentration of 3 g/L.

The organism's development and metabolic processes are impacted by pH levels. Essential active-site amino acid residues involved in substrate binding and catalysis are affected by pH changes in terms of their ionization. Maximum Gox production was achieved when the pH of the production medium was increased to 7. These residues' ionization may change the active-site cleft, which could have an indirect effect on the enzyme (23). The ideal pH range for growth and enzyme synthesis is 6 -7 for the majority of the strains utilized commercially to produce GOx (24).

On the other hand, the microorganism's internal temperature must match that of its environment, as microbial activity is temperature-sensitive. With respect to the organism’s growth, temperature has an impact on GOx production. Studies on the GOx production have mainly focused on mesophilic fungi that grow optimally at temperatures between 25 and 37 °C. At 27–37 °C, *Aspergillus niger* produced the highest yields of GOx (25). The enzyme exhibited maximum activity at 37 °C. After 24 hours of incubation, the amount of Gox was maximal; thereafter, the enzyme's production declined (26). It was shown that the amount of GOx produced over 12-48 hours peaked at 48 hours and then declined. While another study reported that after 36 hours of fermentation, the highest GOx yield was achieved (27).

CONCLUSION

The present investigation's results indicated that *P. aeruginosa* is capable of producing the enzyme glucose oxidase, and the optimum conditions were determined by supplementing BSM medium with 2% glucose and 1% peptone at pH 7 and incubating at 37 °C for 24 hours.

REFERANCES

1. Jankorazov A., Xolmamatova D., Murodboyeva M. "Enzymes and their industrial application methods". International Bulletin of Engineering and Technology. (2023); 3(3): 102-107.

2. Ruiz-Villafán B., Rodríguez-Sanoja R., Sánchez S. "Useful microbial enzymes—an introduction". In Biotechnology of Microbial Enzymes .(2023); (pp. 11-23). Academic Press.

3. Kurniatin P.A., Ambarsari L., Khanza A.D., Setyawati I., Seno D.S., Nurcholis W. "Characteristics of glucose oxidase gene (GGOx) from Aspergillus niger IPBCC 08.610". Jurnal Kimia Valensi. (2020); 6(1):10-19.

4. Kornecki J.F., Carballares D., Tardioli P.W., Rodrigues R.C., Berenguer-Murcia Á., Alcántara A.R., Fernandez-Lafuente R." Enzyme production of D-gluconic acid and glucose oxidase: successful tales of cascade reactions". Catalysis Science & Technology.(2020); 10(17): 5740-5771.

5. Singh S.P., Modi D.R., Tiwari R.K. "Biochemical, Thermodynamic and Kinetic Characterization of Glucose Oxidase Purified from *Pseudomonas* and *Actinomyces spp*. from Natural Sources". Journal of Pure & Applied Microbiology. (2019); 13(4).

6. Wood S.J., Kuzel T.M., Shafikhani S.H." *Pseudomonas aeruginosa*: infections, animal modeling, and therapeutics". Cells. (2023); 12(1): 199.

7. Islam M., Sarkar P.K., Mohiuddin A.K., Suzauddula M. "Optimization of fermentation condition for cellulase enzyme production from Bacillus sp".Malaysian Journal of Halal Research. (2019); 2(2): 19-24.

8. Domínguez-Gómez C.X., Nochebuena-Morando L.E., Aguilar-Uscanga M.G., López-Zamora L. "Statistical optimization of dilute acid and H2O2 alkaline pretreatment using surface response methodology and tween 80 for the enhancement of the enzymatic hydrolysis of corncob". Biomass Conversion and Biorefinery. (2023); 13(7): 6185-6196.

9. Bankar S.B., Bule M.V., Singhal R.S., Ananthanarayan L. "Optimization of Aspergillus niger fermentation for the production of glucose oxidase". Food and Bioprocess Technology.(2009); 2: 344-352.
10. Miller G.L." Use of dinitrosalicylic acid reagent for determination of reducing sugar". Analytical chemistry. (1959); 31(3): 426-428.
11. Poly N.Y., Mamtaz S., Khan M.M., Hoque M.N., Azad A.K., Hasan M. "Isolation, documentation, and biochemical characterization of cellulolytic bacteria from rumen fluid of cattle". Int. J. Adv. Biotechnol. Res. (2022); 5: 433-444.
12. Ozyilmaz G. "Glucose oxidase applications and comparison of the activity assays". Natural and Engineering Sciences". (2019); 4(3): 253-267.
13. Bradford M.M. "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding". Analytical biochemistry. (1976) ; 72(1-2): 248-254.
14. Whitaker XX. J.R, Bernard, R.A. "Experiment For Introduction To Enzymology. (1972).
15. Hasan J.M., Abu-risha R.A., Flayyih M.T. "Determination the titer antibodies against LPS extracted from Pseudomonas aeruginosa isolated from eye infection". Journal of Biotechnology Research Center. (2012); 6(2): 57-66.
16. Zeshan B., Karobari M.I., Afzal N., Siddiq A., Basha S., Basheer S.N., Peeran S.W., Mustafa M., Daud N.H., Ahmed N., Yean C.Y. "The usage of antibiotics by COVID-19 patients with comorbidities: the risk of increased antimicrobial resistance". Antibiotics. (2021); 11(1): 35.
17. Sheet A.H., AL-Azawi H.A. "Antibiotic susceptibility and biofilm formation of *Pseudomonas aeruginosa* isolated from clinical and environmental hospital samples". Biochemical and Cellular Archives. (2022); 22: 3501-3509.
18. Khatami S.H., Vakili O., Ahmadi N., Soltani Fard E., Mousavi P., Khalvati B., Maleksabet A., Savardashtaki A., Taheri-Anganeh M., Movahedpour A. "Glucose oxidase: Applications, sources, and recombinant production". Biotechnology and Applied Biochemistry. (2022); 69(3): 939-950.
19. Jagathy K., Kalpana K., Rajeshkumar S." Production, optimization, Characterization and Immobilization of Glucose oxidase from Aspergillus species". Research Journal of Pharmacy and Technology. (2017); 10(6): 1924-1928.
20. Moorthi P.S." Optimization characterization and applications of glucose oxidase produce from *Aspergillus* awamori MTCC 9645 for food processing and preservation". A PhD thesis submitted to Educational and Research Institute University, Chennai. (2009).
21. Al-anbakey Y.H., Muslim S.N., Hamzah I.H. "Detection and Environmental Optimization of dextran produced from Lactobacillus spp". South Asian Res J Bio Appl Biosci. (2023); 5(4): 89-98.
22. Eze S.O., Ezugwu A.L., Oparaji E., Okwuenu P., Onosakponome I., Chilaka F.C. "Optimization and production of glucose oxidase from a newly isolated strain of Aspergillus fumigatus. Journal of Enzyme Engineering". (2022); 1(1): 39-48.
23. Bodade R.G., Khobragade C.N., Arfeen S. "Optimization of culture conditions for glucose oxidase production by a *Penicillium chrysogenum* SRT 19 strain". Engineering in Life Sciences. (2010); 10(1): 35-39.
24. Singh J., Verma N. "Glucose oxidase from *Aspergillus niger:* Production, characterization and immobilization for glucose oxidation". Adv. Appl. Sci. Res. (2013); 4(3): 250-257.
25. Bankar S.B., Bule M.V., Singhal R.S., Ananthanarayan L. "Glucose oxidase—an overview". Biotechnology advances. (2009); 27(4): 489-501.
26. Khurshid S., Kashmiri M.A., Quershi Z., Ahmad W. "Optimization of glucose oxidase production by Aspergillus niger". African Journal of Biotechnology. (2011); 10(9): 1674-1678.
27. Hamid H.M., Zia M.A., Asgher M. "Optimization of various parameters for the production of glucose oxidase from rice polishing using *Aspergillus niger*". Biotechnology (Pakistan). (2003); 2(1).

الظروف المثلى لانتاج انزيم كلوكوز اوكسيديز من العزلة المحلية لبكتريا الزائفة الزنجارية

براء علي عبيد ، اسماء علي حسين

التقنيات الحيوية الجزيئية والطبية، كلية التقنيات الأحيائية، جامعة النهرين، بغداد، العراق

الخلاصة

خلفية عن الموضوع : الكلوكوز اوكسيديز (GOx) هو إنزيم مؤكسد يلعب دور مهم في العمليات البيولوجية بالإضافة إلى مجموعة متنوعة من التطبيقات الصناعية والتجارية . في مجال الأغذية، على سبيل المثال، يتم استخدامه كمادة حافظة طبيعية، ومضاد للأكسدة، وبمعدل ثابت وغير مكلف للغاية. من المهم دراسته من سلالات مايكروبية جديدة اذ تم عزله من مجموعة متنوعة من الكائنات الحية المجهرية خاصة الفطريات وهناك دراسات محدودة حول عزله من البكتريا لذا فان عزله من مصادر بكتيرية مثل الزائفة الزنجارية يعد امرا مهما . هذه البكتيريا تعد من اكثر البكتريا السالبة لصبغة غرام مرضية اذ تسبب التهابات مختلفة كما انها متواجدة في معظم البيئات التي تشمل التربة ،النباتات وانسجة الثديات .**الهدف من الدراسة:** تقييم الظروف المثلى لانتاج انزيم الكلوكوز اوكسيديز وعزله من عزلات مرضية من الزائفة الزنجارية. **المواد وطرق العمل:** استخدمت 150 عينة مرضية لعزل عزلات الزائفة الزنجارية التي شخصت باستخدام الفحوصات المجهرية والكيموحيوية مع التحقق منها من خلال استخدام نظام VITEK2. تم دراسة الظروف المثلى لعزلات محددة من الزائفة الزنجارية لتحديد قابليتها على انتاج انزيم ال(GOx) لمصادر الكربون والنيتروجين والأس الهيدروجيني ودرجة الحرارة وفترة الحضانة وتحديد اقصى إنتاج لانزيم الكلوكوز اوكسيديز. **النتائج:** تم عزل خمسة وثلاثون عزلة من الزائفة الزنجارية وكانت 10 عزلات فقط لها القابلية على انتاج الانزيم قيد الدراسة. تم تحقيق الظروف المثلى للعزلة الأكثر انتاجا للانزيم وذلك عند تجهيز وسط الانتاج بالببتون 1 % وزن/حجم، الكلوكوز 2% وزن/حجم، الرقم الهيدروجيني 7، درجة الحرارة 37 درجة مئوية والحضانة لمدة 24 ساعة اذ بلغت الفعالية النوعية 1،3، 3.5، 3.2، و 3.5 وحدة/ ملغ. بروتين على التوالي. **الأستنتاج:** أشارت نتائج هذه الدراسة إلى أن بكتيريا الزائفة الزنجارية قادرة على إنتاج إنزيم أوكسيديز الكلوكوز، وقد تم تحديد الظروف المثلى عن طريق إضافة 2% من الجلوكوز و1% من الببتون الى وسط BSM وحضانة عند 37 درجة مئوية لمدة 24 ساعة.

الكلمات المفتاحية: انتاج الكلوكوز اوكسيديز، الزائفه الزنجارية، GOx .