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Characterization of catalyst enzyme by covalent binding chemical immobilization functions on a novel support surface

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Abstract

Cabbage legs peroxidase was used in this study as an economical peroxidase enzyme. Inorganic low-cost supports surfaces such as black stone BS, sand S and quartz rock QR, were utilized to immobilize the catalyst enzyme. One of the chemical processes' immobilization strategies used was the covalent binding technique. The resulting immobilized enzyme was characterized by SEM, EDS, BET analyses for identification of the main. All supports had their optimal Protein loading, pH, temperature, and reusability evaluated. The results showed that immobilization yield (IY%) was 85, 71, and 60 % for QR, S, BS respectively. The QR support showed an enzyme loading of 12 mg protein / g support, which was the highest capacity, while the S and BS support showed a protein loading of 8 mg protein / g support for each of them. The optimal range for all immobilized biocatalysts was found to be at pH of 6.0. Concerning biocatalyst optimum temperature, the outcome of increased temperature for biocatalyst with QR, S, and BS remains at the same value of 40 °C as the initial optimum temperature of the free enzyme. The biocatalyst enzyme's immobilization within QR showed great potential for chemical applications and problems associated with engineering.

Keywords: Chemical characteristic; Cabbage legs peroxidase; Covalent binding technique Immobilization; Biocatalyst enzymes; Sold supports.

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1- Introduction

Enzymes known as biocatalysts (EC 1.11.1.7) oxidize a variety of substrates that are dependent on H_2O_2. Among many plant enzymes, this one is the subject of the greatest research due to its variety of applications, including bioremediation, biosensors, and diagnostic kits [1].

Additionally, the removal of contaminants from paper, textiles, cosmetics, pharmaceutical, chemical, and detergent industries is accomplished by enzymes [2, 3]. Peroxidase also exhibits a high degree of stability. On the other hand, its high commercial cost could prevent widespread use. Finding plant peroxidases that are similar to CLP and capable of performing the same tasks is one way to overcome this obstacle. Consequently, several studies have been carried out to discover applications for substitute peroxidases, the utilization of turnip peroxidase in analytical and diagnostic kits, as well as guinea grass leaf peroxidase as a biosensor, has been observed [4, 5].

Rapid growth, few requirements to grow this plant, and great resistance to external environmental conditions are all characteristics of this type of plant, which provides a sustainable source of the peroxidase enzyme. One important source of the peroxidase catalyst enzyme is plant cabbage legs, which may be found in local markets at reasonable prices or even salvaged from restaurant waste. It has been demonstrated that the peroxidase enzyme (EC 1.11.1.7) is an effective, economical, and ecologically benign catalyst [6]. Enzyme in free form has limited commercial applications since they are unstable and cannot be reused at medically inappropriate temperatures and pH levels. They also lose their stability in organic solvents and during storage [7].

Another method for cutting costs in the synthesis of biocatalysts is the extraction of enzymes from agro-industrial waste. As a low-cost alternative and a high source of peroxidases, cabbage is a by-product of the processing industry [8].

The catalyst may become more stable by enzyme immobilization. Using support with advantageous chemical and physical properties (specific area, internal morphology, pore size, active groups for enzyme interaction) to intensify the protein/support interactions is one method to accomplish this aim. Enzyme structure must be preserved or changed conformational to a more active state for the immobilization procedure to be successful. As a result, it is best to maintain or even boost catalytic activity [9].

Enhanced durability against different denaturing conditions, increased catalytic activity, simpler recovery of products and enzymes, continuous enzymatic activities, and reusability are just a few of the benefits that immobilized enzymes have over free enzymes. There are many kinds of immobilization techniques, which can be divided into the "Physical Method" such as Entrapment, Adsorption, Encapsulation and "Chemical Method "such as Covalent bonding, Ionic binding, Cross-linking. One of

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the efficient immobilizing enzyme methods is covalent bonding, which involves attaching the enzyme to a matrix through chemical reactions like 3-aminopropyltriethoxysilane (APTS), peptide, thiol-disulfide, and alkylation reactions. This approach results in minimal changes to the enzyme's structure and enhances its resistance to harsh physical and chemical conditions, also this method is suitable for continuous reaction with high thermal stability, high efficiency, and no enzyme leakage [10].

The use of enzymes as catalysts in many industrial areas has increased significantly due to the need for sustainable operations. Furthermore, the rapid advancements in protein engineering and extraction/purification technologies have made it possible to efficiently produce specific enzymes with highly customized and optimized properties at an analytical purity level. To be suitable for an immobilized enzyme to perform effectively support materials must be cost-effective, have a large surface area, and have the least amount of diffusion restriction in the transfer of substrate and product. [11-13].

The aim of this work is to find a reasonable and readily available source of peroxidase enzyme and immobilize it in an efficient manner on inexpensive and solid supports (QR, S, and BS) that can enhance the operational conditions of treating pollutants in order to protect the enzyme from denaturation, leakage, and damage.

The aforementioned substance has been utilized as a suitable inorganic matrix for the purpose of immobilizing enzymes due to its abundant availability, cost-effectiveness, and lack of toxicity. Enzyme immobilization was achieved through the utilization of inorganic support characterized by their stable chemical properties.

Furthermore, an investigation was conducted to examine the physical, chemical, and morphological properties.

2- Methods and chemicals

2.1. Materials

The biocatalyst enzyme has been extracted from cabbage trash. It came from the local vegetable market. 3-aminopropyltriethoxysilane (APTS), bovine serum albumin, Sephadex G-150, pyrogallol, CBBG, and Tris-HCl were purchased from USA (Sigma-Aldrich). S, BS, and QR are low-cost solid supports that can be found in local markets and natural areas. QR, S, and BS grind well to produce particles with size (1mm) as shown in Fig. 1. Then, pass it through a sieve mesh 18–20, stopping at mesh 20, wash it with distilled water, and dry it in an oven set to 120 ºC.

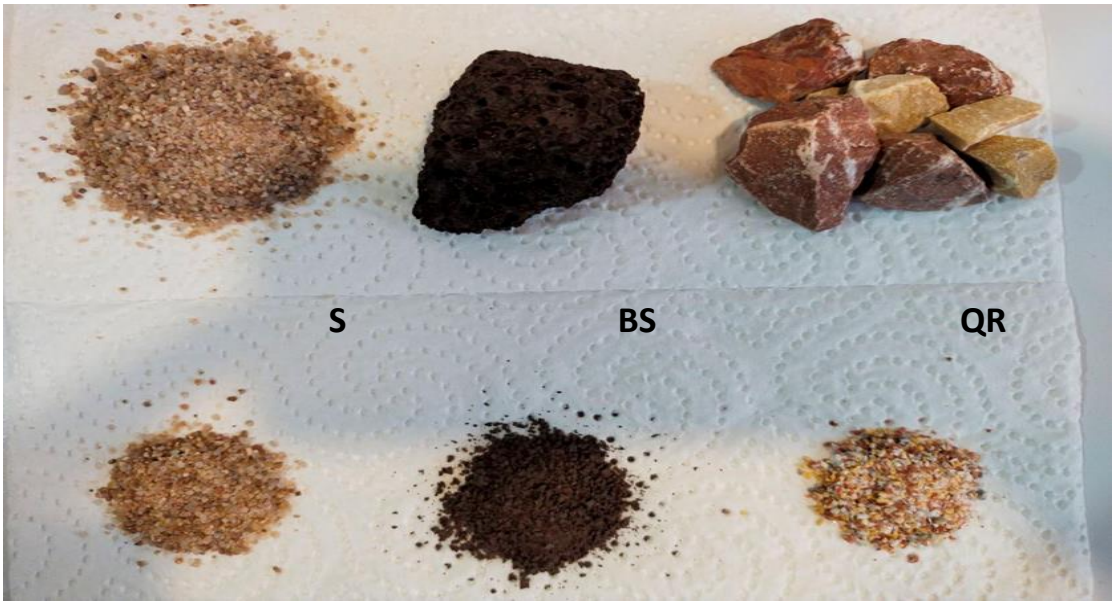

Fig. 1. Sand (S), Black Stone (BS), and Quartz Rock (QR)

2.2. Methodology

2.2.1. Bioenzyme plant extracted

From cabbage trash, the biocatalyst Peroxidase was derived with slightly modified version of [14]. For 20 minutes, phosphate buffer by an amount 200 ml at pH 6.5 with concentration 0.2 M was used to homogenize waste cabbage legs weighing 50 g crush by mortar. A Cole-Palmer VS-13000 centrifuge was used to filter the homogenate using two layers of clean cheesecloth, and the filtrate was then centrifuged for 15 minutes at 8000 rpm. Carefully gathered, the supernatant was then filtered through filter paper. The substance was extracted and kept at 4°C as a crude enzyme in preparation for further analysis.

2.2.2. Estimation the enzymatic activity

Enzyme activity was computed by employment pyrogallol as a substrate in the presence of H_2O_2, Brownish-orange purpurogallin is produced when

peroxidase assists in the oxidation of pyrogallol. spectrophotometer was used to assess peroxidase activity at room temperature 25 °C, at (λ max = 420 nm, ϵ = 4400 $M^{-1}cm^{-1}$).

Oxidation reaction mixture contained from 3.6 ml as flow:

- 1.5 ml of phosphate buffer at pH 6.5
- 0.3 ml of peroxidase enzyme
- 1.4 ml of 50 mmol l^{-1} pyrogallol
- 0.4 ml of 25 mmol $^{-1}$ H_2O_2

Using (UV-9200, BIOTECH ENGINEERING) as a spectrophotometer instrument, at 420 nm the change in absorbance was estimated every minute and accorded to Eq. 1 enzyme activity was expressed.

One unit of enzymatic activity $U.ml^{-1}$, has been given to an enzyme that can catalyzed 1.0 µmol of H_2O_2 per minute at 25 ºC. [15,16].

$$Activity\ \left(\frac{U}{mL}\right) = \frac{\left(\frac{dA}{dt}\right)\times AV\times DF}{EV\times\epsilon_{420}} \quad (1)$$

Where:

$\frac{dA}{dt}$: $changing\ in\ absorbency\ per\ minute.\ (min^{-1})$.

AV: $Overall\ volume\ (3.6ml)$. DF: $Dilution\ factor\ (1)$.

EV: $Enzyme\ volume\ (0.3ml)$.

ϵ 420: $Purpurogallin\ absorptivity\ at\ 420\ nm\ (4400\ M^{-1}cm^{-1})$.

2.2.3. Enzymatic protein estimation

The research utilized the Bradford method [17] to quantitatively determine the overall protein concentration, using bovine serum albumin (BSA) as the standard reference. The net absorbance values at a wavelength of 595 nm were used to establish the protein content of the unknown sample by employing a linear equation that represents the link between these values and the concentrations of BSA [18].

2.2.4. Immobilized enzymes by covalent binding chemical method

Biocatalyst peroxidase was immobilized by covalent binding technique according to reference [19] with minor modifications. With protein concentration 4mg/ml the immobilization by covalent linking at 1g for each BS, S and QR supports were applied by series of chemical functions;

A-Silanized with 3-aminopropyltriethoxysilane (APTS) 5% (v/v) under mechanical shaker at least 4 hours at room temperature.

B-The specimen was exposed to a 5 ml volume of a glutaraldehyde solution with a concentration of 2.5% (v/v). Mechanical agitation was applied to the sample for a period of 8 hours at ambient temperature. The activated supports were subjected to a washing procedure involving the use of 10 ml of distilled water, followed by vacuum filtering.

C-The quantity of protein required in the crude enzyme extract was estimated through loading evaluation. This was achieved by employing the covalent binding technique CBT, and the resulting volume was adjusted to 10 ml using phosphate buffer. The mixture underwent continuous stirring for a period of 4 hours at a temperature of 25 °C. Subsequently, the specimen was subjected to static conditions and maintained at a temperature of 4ºC for the duration of the nighttime period.

The immobilized biocatalysts underwent a washing and filtration process to remove the enzymes that were not immobilized. During the immobilization procedures, the filtrates were kept in order to measure peroxidase activity. Eq. 2 was used to compute the immobilization efficiency of peroxidase immobilization yield (IY%) for biocatalysts on BS, S, and QR.

Immobilization yield was estimated using the relation indicated:

$$IY\ \% = \frac{Total\ activity\ of\ immobilized\ enzyme}{Total\ activity\ of\ free\ enzyme} * 100\% \quad (2)$$

2.3. Analytical Statistics

The experiments were conducted in triplicate value and the analysis of variance (ANOVA) and a Tukey post-test $p < 0.05$ were used in the statistical analysis of the data to determine their significance.

3- Results and discussion

3.1. Scanning electron microscopy (SEM analysis)

SEM was used to examine the solid support samples. The surface morphology of the original, altered, and immobilized particles was examined using SEM. Significant changes were noted on the particle surface. The change in morphology indicates the presence of the enzyme-support interaction. In Fig. 2 (A- F) show the superiority of quartz in fixing the enzyme on a surface with more particles than sand and black stone before and after immobilization.

3.2. Energy dispersive spectrometer (EDS analysis)

EDS was employed to analyze the alterations in support characteristics before and after immobilization. Fig. 3 (A, B) illustrates various EDS techniques. The findings indicated that there was an approximate 16 wt% decrease in the weight of element Si, followed by 8 wt% increase in the weight of element O, and the element C decreased about 8 wt% in the region of the initial support when compared to Quartz after immobilization, which confirmed the successful immobilization. In Sand the element S, Ca, decreased about 28 wt% and 20 wt%, respectively between initial and final support after immobilized and the element C decreased about 3 wt% as shown in Fig. 3 (C, D), while in Black Stone the element C decreased about 5 wt% followed by a 4 wt% increase in the weight of element O, between initial and final weight ratio of support after immobilized as shown in Fig. 3 (E, F) as listed in Table 1.

Furthermore, the EDS showed that the elements Na, Mg, K, and Fe were absent from the original supports, but they were discovered following the enzyme's immobilization, confirming the presence of peroxidase on the supports and indicating a successful immobilization process [20].

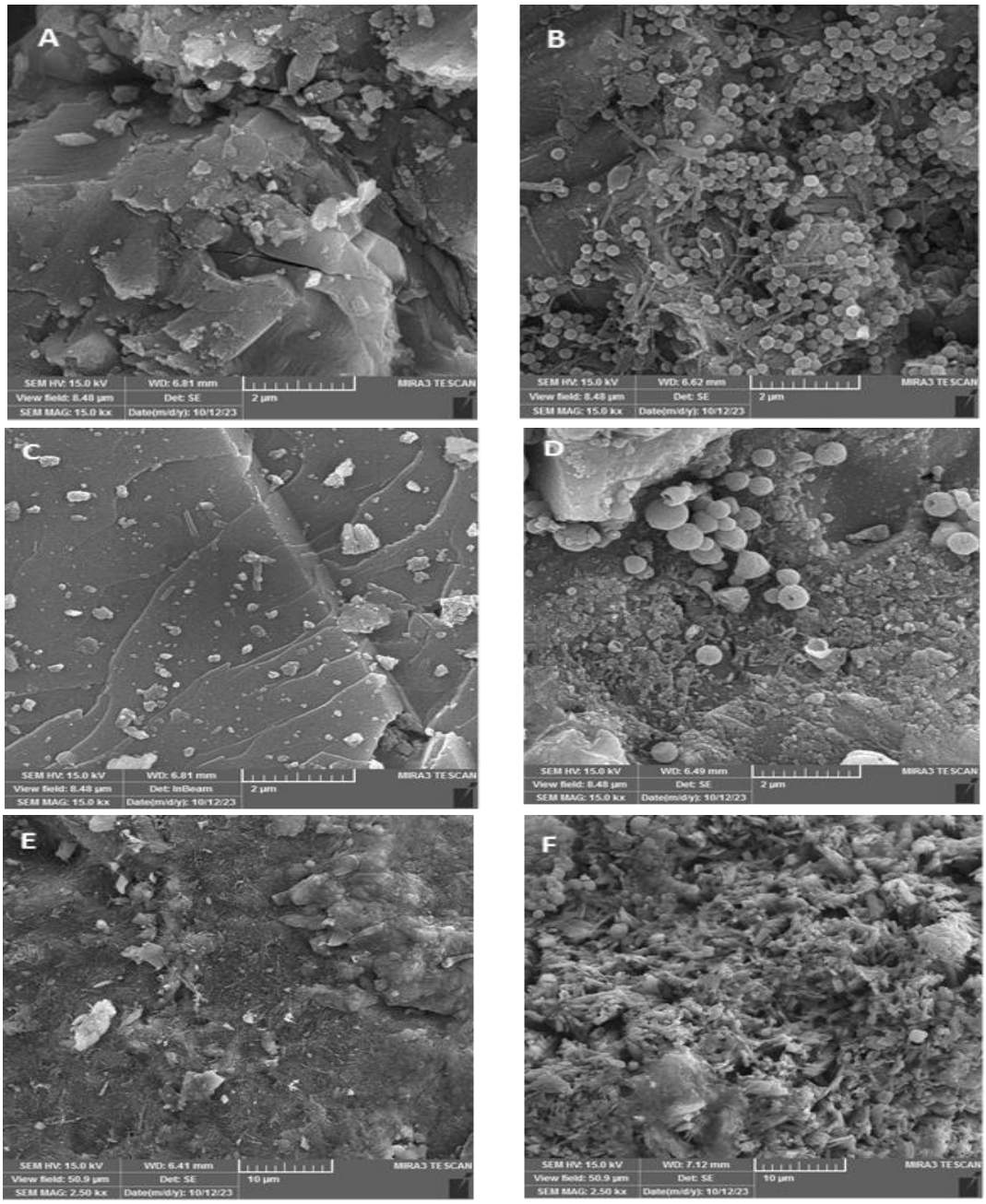

Fig. 2. SEM Images Supports/Immobilized Biocatalysts Before and After Immobilized A, B. (QR) support, C, D.(S) support, E, F. (BS)

Table 1. EDS Weight for Three Type of Support Before and After Immobilized

	Before Immobilization				
Support	C	O	Si	S	Ca
	Wt%	Wt%	Wt%	Wt%	Wt%
QR	12.69	27.71	48.81	10.97	-
S	6.25	24.02	-	37.52	32.21
BS	9.5	30.31	-	-	60.19
	After Immobilization				
Support	C	O	Si	S	Ca
	Wt%	Wt%	Wt%	Wt%	Wt%
QR	4.49	35.32	32.11	12.22	10.1
S	3.01	29.12	40.22	9.03	12.02
BS	4.53	34.21	39.03	-	16.01

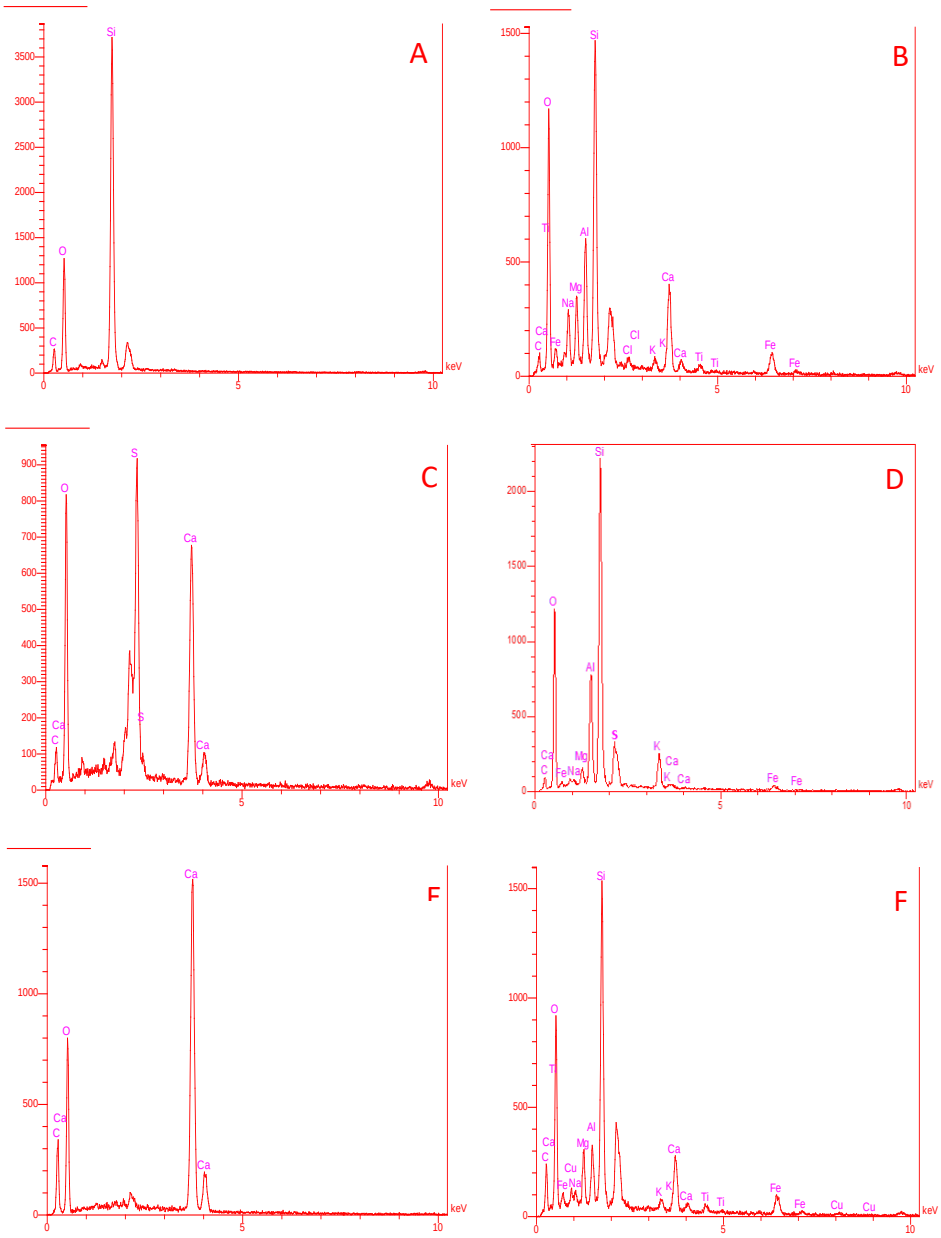

Fig. 3. EDS Spectrum Photographs of Supports A, B. (QR) Before and After Immobilized Enzyme, C, D. (S) Before and After Immobilized Enzyme, E, F. (BS) Before and After Immobilized

3.3. Brunauer-emmett-teller (BET analysis)

The BET analysis is excessively employed in various domains, including catalysis, bioenvironmental science, and materials science, to achieve the physical and chemical characteristics of porous materials, which encompass catalysts, adsorbents, and nanomaterials. It offers necessary information on the surface area, the pore volume, and the pore size distribution [21].

In this study the surface area and void volume of quartz, sand, and black stone particles were calculated by BET analyses at gas pressure of 88 kpa, as shown in Table 2, the results showed that the surface area was (3.38,2.85,1.406) m^2/g respectively while total pore volume was (0.012,0.0092,0.0068) cm^3/g respectively, and this results explain why quartz is superior to CLP immobilization.

Table 2. BET Characterization of Supports

Characteristics	QR	S	BS
Sample weight (g)	0.26	0.28	0.33
Standard volume (cm^3)	10.17	10.17	10.17
Dead volume (cm^3)	21.581	21.331	20.178
Adsorptive	N2	N2	N2
	BET plot		
V_m (cm^3(STP) g^{-1})	0.7775	0.6556	0.323
BET surface area (m^2 g^{-1})	3.3842	2.8534	1.406
Total pore volume(p/p0=0.982) (cm3 g^{-1})	0.012053	0.0092037	0.0068502
Average pore diameter (nm)	14.246	12.902	15.489

3.4. Influence of protein capacity

The evaluation of the impact of enzyme capacity on a support for the purpose immobilization is of particular importance, particularly when examining various supports

and techniques. The impact of protein loading was assessed across a spectrum of 4 to 20 mg protein per gram of support, as depicted in Fig. 4. The statistical analysis revealed that the immobilization efficiencies of the immobilized biocatalysts were found to be statistically significant at a level of $p < 0.05$.

The immobilized biocatalysts QR, S, and BS achieved immobilization yields (IY) of 85 %, 71 %, and 60 %, respectively, when the protein loading was 12, 8, and 8 mg protein/g support Fig. 4 (A, B, C). Despite the introduction of a heightened protein load of 20 mg protein per gram of support. The immobilization of cabbage peroxidase on the QR support using CBT yielded promising results, with an immobilization yield (IY) of 85 %. This is notably higher contrast with the literature, where commercial HRP was immobilized on sugarcane bagasse using the CBT and achieved an immobilization yield of 35% [22].

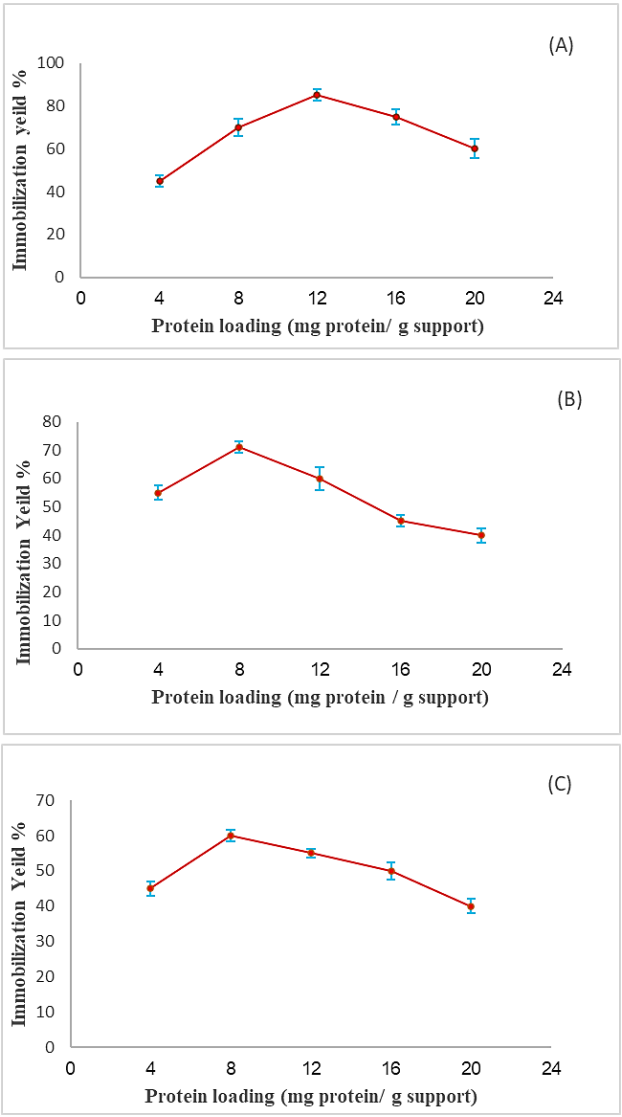

Fig. 4. Effect of Protein Capacity (4 to 20 mg protein/g support) on The Cabbage Enzyme Immobilization by CBT. (A) Quartz Rock, (B) Sand, (C) Black Stone

3.5. Impact of pH on free and immobilized (CLP)

The experiment implicated affecting the pH of the buffer solutions within the range of 4.0 to 9.0, with an increase of half a pH unit. The present research investigated the reaction rates of immobilized and free peroxidase preparations at different pH levels by utilize different buffers at concentration (0.2) M, Tris–HCl buffer pH ranges (8–9), phosphate buffer ranges (6–7.5), and sodium acetate buffer for pH ranges (4–5.5). Both form-free and immobilized enzymes were subjected to a 30-minute incubation period in the respective buffers prior to the measurement of their activity.

The impact of pH on the RA of both free and immobilized CLP was exhibited in Fig. 5 (A- D), which observed that at pH 6, there is a significant increase in activity for all supports. However, in both acidic and basic regions, a decrease in activity is noticed. This can be attributed to the ionic interaction between the (H^+ or OH^-) ions in the buffer and bioenzym. The pH alteration has an impact on the biocatalyst action of enzymes due to the modification of ionic properties within the enzyme, which subsequently affects the formation of the active site [23,24]. The statistical analysis revealed that all RA, including both free and immobilized (CLP), exhibited a significant difference $p < 0.05$.

According to research [25], the pH optima of the immobilized gourd peroxidase preparations were pH 5.0, which was the same as that of their soluble counterparts. In contrast, previous studies have reported different outcomes in terms of pH adjustment for gourd peroxidase, ranging from 5.0 for the soluble form to 4.0 for the immobilized form [26].

The impact of temperature on the performance of the free and immobilized CLP was assessed within a temperature domain from 25 - 80 °C, with each experiment lasting for a duration of 30 minutes. The findings were presented in relation to RA, and all RA values for both free and immobilized CLP demonstrated statistical significance $p < 0.05$. The peroxidase enzyme exhibits stability when free or immobilized using Quartz, Sand, and Black Stone as support materials, across a temperature range of 25-40 °C, while maintaining its relative activity. Subsequently, the enzyme exhibited a decline in its enzymatic activity.

The results presented in Fig. 6 (A- D) indicate that the optimum temperature for free peroxidase activity was found to be 40°C. This finding aligns with the results obtained for immobilized CLP on various supports. It is crucial to acknowledge that the robust interaction formed between the activated support and the enzyme can induce alterations in the conformation of the biocatalyst, potentially affecting the thermal stability of the immobilized biocatalyst either positively or negatively.

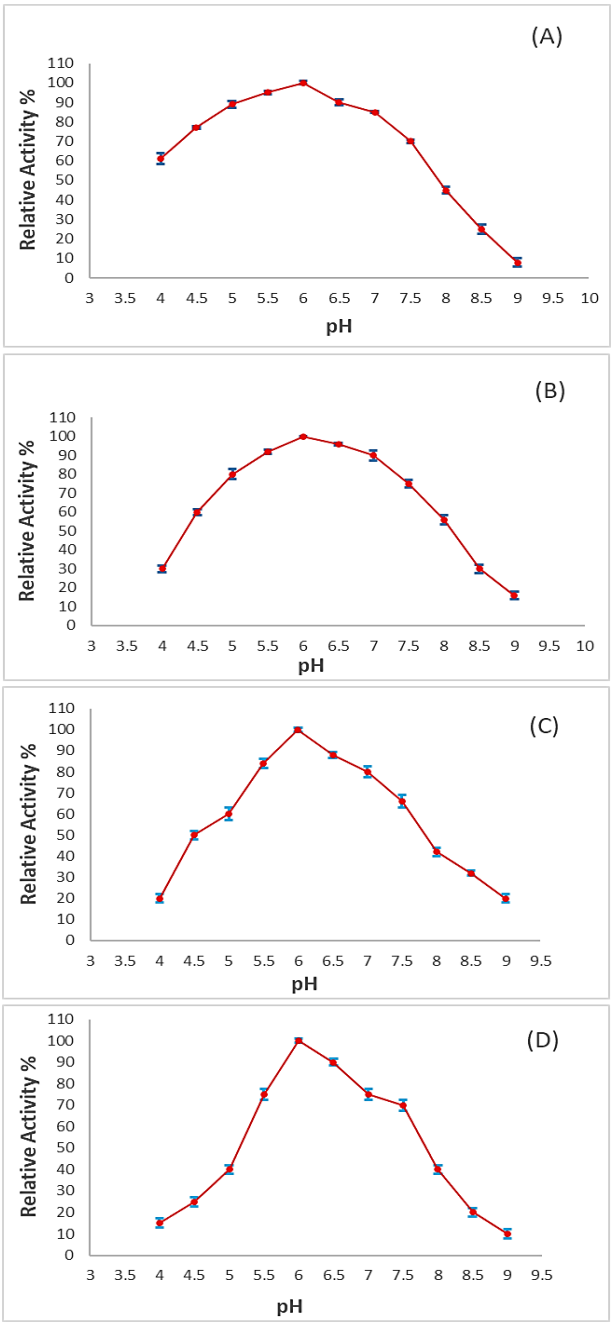

Fig. 5. Impact of pH on the Enzyme Relative Activity. (A) Free Enzyme, (B) Immobilized on QR, (C) Immobilized on S, (D) Immobilized on BS

3-6- Impact of Temperature on Free and Immobilized (CLP)

The immobilized peroxidase CLP with QR exhibited a retention of 45% of its enzymatic action when exposed to a temperature of 80 °C. In comparison, the retention percentages for sand and (BS) were found to be 38 % and 35 % respectively, under the same temperature conditions. The observed decline in free CLP activity as temperature increases can be attributed to the conformational changes, increased mobility, and enhanced vibrational motion of the protein enzyme. These alterations ultimately lead to the disruption of the enzyme's active site and denaturation of its structure. This phenomenon serves as evidence that the enzyme is shielded from the detrimental effects of thermal conditions. This characteristic guarantees that the immobilized enzymes can be employed at elevated operational temperatures, thereby resulting in enhanced reactivity, which is evidently advantageous.

Previous research has also stated an excess in temperature by immobilization of HRP on activated wool according to Report [27].

3.7. Influence of thermal stability temperature

By comparing the characteristics of the free and immobilized peroxidases at various temperatures (25 to 80°C), the temperature of each was assessed, and the enzyme activities were verified. The exhibited responses pertaining to remaining activities are illustrated in Fig. 7 (A- D). According to the study's findings, the immobilized enzyme's residual activity on solid support was much higher than the free enzyme's. When the free form was pre-incubated at temperatures over 40 °C and the immobilized form at temperatures above 50 °C, the activity decreased. The free bioenzyme got completely inoperative at 80°C, while immobilized at Quartz, Sand, and Black Stone showed residual activity 45%, 20%, and 28% respectively.

The findings indicate that the support that was sold exhibited a high level of stability, since there was no evidence of structural distortion even when subjected to elevated temperatures of 80 °C. Additionally, the findings of this study indicate that immobilizing CLP on a solid support serves as an external framework for enzyme molecules, hence safeguarding against temperature-induced structural changes in the enzyme. Consequently, this immobilization technique enhances the thermal stability of the CLP enzyme [28]. In previous studies it has been observed that the activity of chitosan beads encapsulated with HRP decreased at a slower rate than that of the free enzyme. After a 6-hour incubation at 70 °C, the remaining activity of the free enzyme was found to be 3.2%, while the encapsulated enzyme retained 26.4% of its initial activity [29].

3.8. Reusability of enzyme

The reutilization of immobilized enzymes is necessary due to the significant expense associated with soluble enzymes. The decrease in activity that is observed upon repeated use can potentially be ascribed to the inhibition of enzyme activity resulting from the leaching of enzymes during the repetitive washing procedure, as well as the damage inflicted upon the supports. After each assay, the immobilized CLP preparation was subjected to extraction and rinsing using a (0.2 M) phosphate buffer solution with a pH of 6. and subsequently stored at temperature of (4◦C) for the duration of the overnight period. The enzyme that was immobilized and subsequently recovered through this procedure was employed in multiple iterations. The initial activity measurement was designated as the control (100%) for the subsequent calculation of the remaining percentage activity after each subsequent use.

The assessment of operational stability is crucial in determining the suitability of immobilized enzymes for various applications. The potential for reusing materials and resources in bioprocesses serves to reduce overall costs [30]. The reusability cycles are depicted in Fig. 8. The cabbage legs peroxidase, which was immobilized in (QR, S, and BS) by CBT, exhibited a maintenance of 90 %, 85 %, and 75 % respectively, in terms of the initial activity, during double cycle. However, in the fourth cycle, the remaining action decreased to 40 %, 20 %, and 4 % for (QR, S, and BS) respectively.

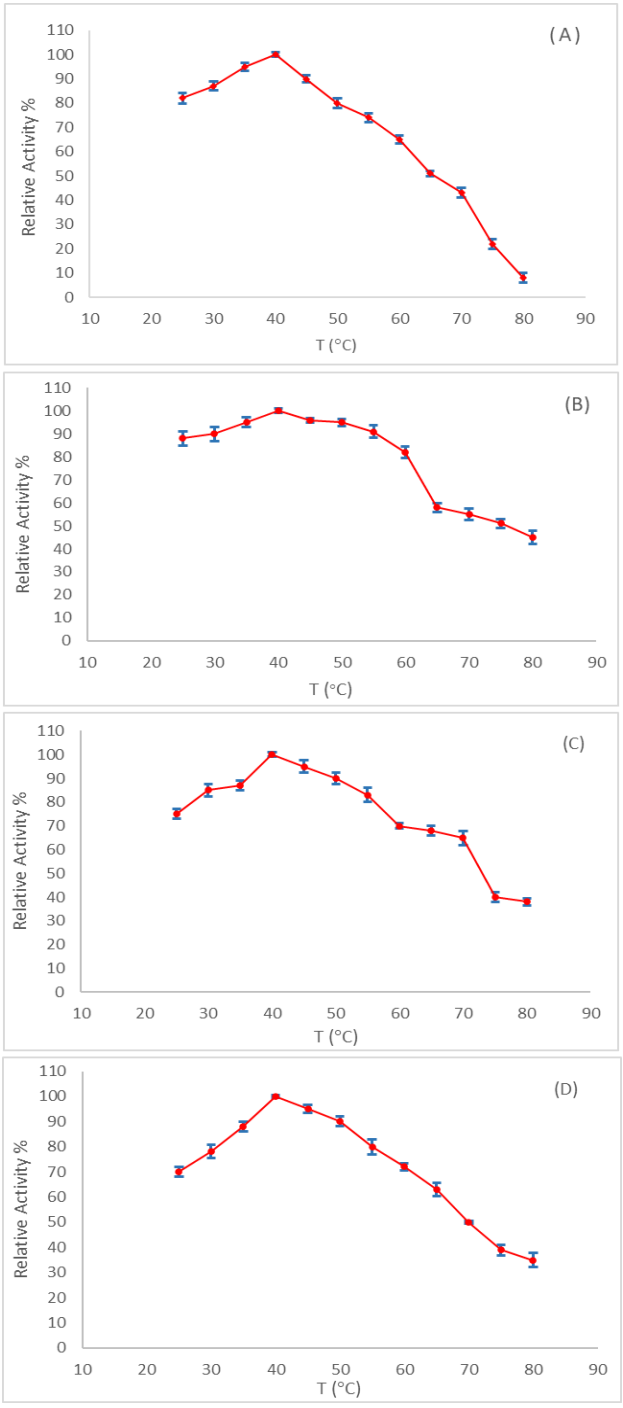

Fig. 6. Impact of Temperature on the Enzyme relative activity. (A) Free enzyme, (B) Immobilized on QR, (C) Immobilized on S, (D) Immobilized on BS

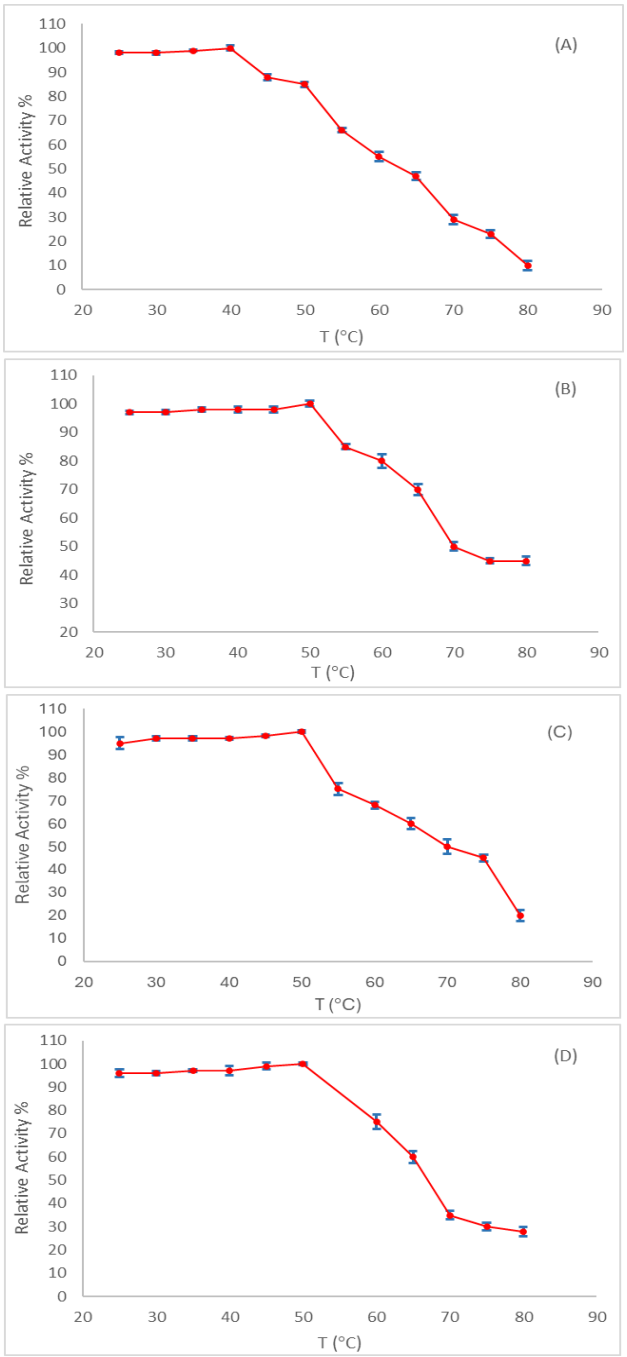

Fig. 7. Impact of thermal stability on the Enzyme Relative Activity. (A) Free enzyme, (B) Immobilized on QR, (C) Immobilized on S, (D) Immobilized on BS

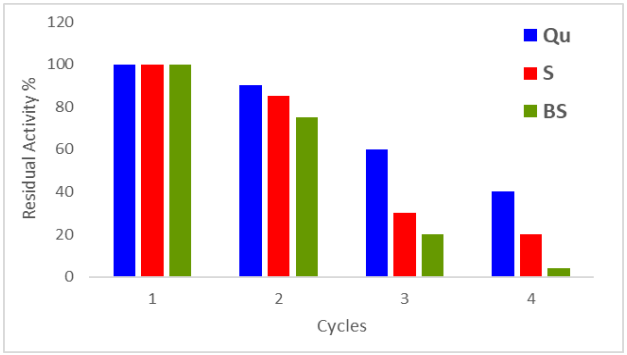

Fig. 8. Reusability Cycles of Immobilized CLP enzyme

4- Conclusion

The free peroxidase enzyme extracted from cabbage legs was immobilized on three solid and cheap supports: QR, BS and S the best support according to the tests was quartz QR with Immobilization yield 85%. The covalent binding chemical technique CBT was evaluated as one of the immobilization methods by a series of chemical functions in three stages. This method was used as the best method of immobilization because it preserves the stability of the enzyme and its internal structure and prevents it from leaking. This was observed in morphological tests. Changing several parameters, such as protein loading, pH, temperature, thermal stability, and reuse, were tested on both the free enzyme and immobilized on each three supports. The results were identical in some parameters and different in others. The QR support illustrates an enzyme loading of 12 mg protein / g support, which was the highest capacity from the others. The pH 6.0 was illustrated to be the optimal range for all immobilized biocatalysts. Concerning biocatalyst optimum temperature, the outcome of increased temperature for biocatalyst with all support remains at the same value 40 ℃ as the optimum temperature of the bioenzyme. When the temperature reached 80°C, the QR support maintained approximately 45% of the original enzyme efficiency Which is the highest remaining percentage of enzyme efficiency than other supports. Concerning biocatalyst optimum thermal stability temperature, the outcome of increased temperature for all biocatalyst remains at the same value 50 ℃ as the optimum thermal stability temperature of immobilized enzyme. When the temperature reached 80C, the QR support maintained approximately 45% of the original enzyme efficiency, which is the highest remaining percentage of enzyme efficiency than other supports. Over the course of four cycles, the immobilized enzyme was reused after washing it with phosphate buffer each time, and the efficiency of the immobilized enzyme on QR, S, and BS supports was 40%, 20%, 4% respectively. The study is an introduction to developing enzyme-immobilized technology, its widespread use in treating chemical industrial pollutants, and many chemical processes in which enzymes play an important role.

Abbreviations

CLP	Cabbage legs peroxidase (Brassica oleracea var.)
QR	Quartz Rock
S	Sand
BS	Black stone
CBT	Covalent binding technique
CBBG	Coomassie Brilliant Blue solution G250
APTS	3-aminopropyltriethoxysilane
BSA	Bovine serum albumin
IY	Immobilization yields
RA	Relative activity
HRP	Horseradish peroxidase

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خصائص الانزيم المحفز المثبت كيميائيا بالروابط التساهمية على أسطح دعامات جديدة

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الخلاصة

تم استخدام بيروكسيديز سيقان الملفوف في هذه الدراسة باعتباره انزيما اقتصاديا. تم استخدام أسطح الداعمات غير العضوية منخفضة التكلفة مثل الحجر الأسود، والرمل، وصخور الكوارتز لتثبيت الانزيم المحفز. إحدى استراتيجيات التثبيت الكيميائي المستخدمة هي تقنية الربط التساهمي. تم تشخيص الإنزيم المثبت على الدعامات عن طريق تحليلات SEM وEDS وBET لتحديد الأنزيم الرئيسي. تم تقييم تحميل البروتين الأمثل لجميع الدعامات ودرجة الحموضة ودرجة الحرارة وقابلية إعادة الاستخدام. كانت نسبة التثبيت (٨٥، ٧١ و٦٠٪) لدعامة الكوارتز والرمل والحجر الاسود على التوالي. أظهرت دعامة الكوارتز تحميلًا إنزيميًا قدره ١٢ ملغ بروتين / جم، وهو أعلى قدرة، بينما أظهرت دعامة الرمل والحجر الاسود تحميلًا بروتينيًا قدره ٨ ملغ بروتين / جم لكل منهما. تبين أن الرقم الهيدروجيني ٦ هو النطاق الأمثل لتثبيت الانزيم على جميع الدعامات. اما فيما يتعلق بدرجة الحرارة المثلى للأنزيم المثبت فتظل عند نفس القيمة ٤٠ درجة مئوية على كل الدعامات وهي مثل درجة الحرارة المثلى الأولية للإنزيم الحر. أظهر تثبيت الانزيم المحفز على دعامة الكوارتز إمكانية كبيرة للتطبيقات الكيميائية والمشاكل الهندسية المرتبطة.

الكلمات الدالة: الخصائص الكيميائية، بيروكسيديز سيقان الملفوف، تقنية التثبيت بالربط التساهمية، الانزيم المحفز، الدعامات.