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Research Article

Comparative study of immobilized enzyme on nano-composite (SCN) and free enzyme of invertase isolated from baker's yeast

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Abstract

The objective of this study was to compare the properties and performance of invertase enzyme isolated from baker's yeast, both in free and immobilized form on a starch-copper nanocomposite (SCN). The SCN was synthesized using starch as a reducing agent for the biological production of copper nanoparticles (CuNPs). The Characterization of SCN was performed using Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction to confirm nanoparticle formation and structural properties. The immobilization of invertase onto SCN was optimized by varying nanoparticle concentration, pH, incubation time, and temperature to maximize enzyme attachment and activity. Enzyme activity was measured for both free and immobilized forms to determine the immobilization efficiency. The study found that the high levels of enzyme immobilization were observed at pH = 9, temperature T = 30, and 3% SCN concentration. For both free and immobilized invertase, the ideal reaction temperatures were 35°C and 40°C, with corresponding pH values of 5 and 4.5. Reusability experiments revealed that the immobilized enzyme retained 49% of its activity after ten cycles, demonstrating improved stability and potential for repeated use. The results suggest that enzyme immobilization on SCN occurs through non-covalent interactions, providing a practical and sustainable approach for biocatalytic applications. This research highlights the potential of starch-based nanocomposites for enzyme stabilization, offering a cost-effective and environmentally friendly solution for industrial and biotechnological applications.

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1. Introduction

Microbiological enzymes, derived from various microorganisms such as bacteria, fungus, and yeasts, are recognized for their exceptional quality and are commonly used in commercial enterprises (Kumar et al., 2017). One of the most basic commercial carbohydrases is invertase (EC 3.2.1.26), also known as β-D-fructofuranoside fructohydrolase, β-fructofuranosidase, sucrase, invertin, and saccharase, it is responsible for hydrolyzing sucrose and related glycosides. The enzymes of economic interest come from Saccharomyces strains, despite their extensive dispersion (Kulp, 1975).

One of the most popular enzymes in the food (confectionery) business, fructose is chosen over sucrose because it is sweeter and does not crystallize as easily when making jams, candies, soft-centered chocolates, and cookies. (Nadeem et al., 2015).

Saccharomyces cerevisiae is one of the yeasts used in the commercial production of extracellular and intracellular invertases because of its high fermentation capacity. (Parapouli et al., 2020) Numerous studies have utilized yeast (*Saccharomyces cerevisiae*) as an alternative to enhance vegetative growth and boost agricultural crop yield (Mohammed et al., 2020). The production of active chemicals (phytohormones, amino acids, vitamins, or NH3), the solubilization of inorganic phosphate or zinc, the siderophores that trap iron, and the inhibition of pathogen colonization could all contribute to the stimulating action of yeasts (Fernandez-San Millan et al., 2020). Studies show that baker's yeast improves plant resilience to various soil contaminants, such as heavy metals and organic pollutants, and allows plants to flourish in contaminated soils, suggesting possible applications in phytoremediation (Remy et al., 2017).

The term "enzyme immobilization" describes the process of physically containing or localizing an enzyme inside a specific area of space while preventing its catalytic activity (Naqash et al., 2019). Since the 1960s, enzyme immobilization has attracted a lot of attention in research (Razzaghi et al., 2022), compared to their free versions, immobilized enzymes are more resistant to environmental changes and are easier to recover or recycle. Protecting the enzymes from adverse environmental factors (such as high temperatures and severe pH values) is the main advantage of immobilization. In addition to being used in water treatment facilities, the immobilized enzymes can be employed in a variety of large-scale businesses, including the food, pharmaceutical, detergent, and textile sectors (Maghraby et al., 2023).

Promising methods for immobilization and subsequently enhanced enzyme functions can be found in nanotechnology, one of the burgeoning fields (Naqash et al., 2019). In practical applications across various scientific domains, nanotechnology refers to the usage of structures and molecules on nanometer scales, typically between 1 to 100 nm (Bayda et al., 2019). Nonetheless, this approach has made it possible to construct enzyme biosensors that are highly reproducible, stable, sensitive, and have fast reaction times (Naqash et al., 2019).

Metal nanoparticles find application in a multitude of fields, including medicine, electronics, fertilizers, insecticides, and environmental problem mitigation (Hartemann et al., 2015). Because of copper's great natural abundance, low cost, and the many easy and practical methods for creating Cu-based nanomaterials, copper nanoparticles are particularly appealing (Gawande et al., 2019). The aim of this research is to investigate the effectiveness of immobilizing invertase on SCN nano-composite in comparison to the free enzyme. This study seeks to evaluate the enzymatic activity, stability, and reusability of the immobilized enzyme under various temperature and pH conditions, The findings could have significant applications in agricultural biotechnology, particularly in improving enzymatic processes for soil health, biofertilizers, and sustainable food production.

2. Materials and Methods

2.1 Extraction of invertase enzyme

Ferreira et al. (2018) presented the autolysis approach used to extract invertase. 40 mL of 100 mM NaHCO3 was added to 10 g samples of lyophilized yeast, and the mixture was stirred for 24 hours at 200 rpm and 35–40°C in an orbital shaker, next, the sample was centrifuged for five minutes at 5000 rpm, the liquid supernatant was named raw extract (RE) because it included invertase that had been liberated by autolysis. The protein content was then measured using Bradford's technique (Pedrol & Tamayo, 2001). RE was kept in storage at -20°C.

2.2 Invertase Activity

Reducing sugars (RS) and 3,5-dinitrosalicylic acid (3,5-DNS, Inlab) were reacted to measure the activity of the invertase enzyme using a transmittance of 490 nm. To create a final reactive combination containing 40 mM sucrose, the reaction medium contained 1.0 mL of extract, 1.0 mL of buffers (pH 5.0), and 1.0 mL of 120 mM sucrose solution as substrate, in the blank test, the substrate was replaced with 1.0 milliliter of water, the reaction was conducted at 25°C and pH 5.0. Using glucose as a standard, 3,5-DNS was used to determine the amount of RS after 10 minutes (Ferreira et al., 2018). An enzyme activity unit is the quantity of enzyme that causes a one-gram rise in glucose concentration of one µg RS.min-1.

2.3 Synthesis of starch-Copper nanocomposite (SCN)

Using starch as a capping agent and copper (II) sulfate pentahydrate as a precursor salt, the Cu nanoparticles were created via a chemical reduction method. The first step in the preparation process is to add 0.1 M copper (II) sulfate pentahydrate solution to 120 mL of 1.2% starch solution, stirring vigorously for 30 minutes. The synthesis solution is mixed continuously and quickly while 50 mL of a 0.2 M ascorbic acid solution is added in the second stage. The resulting solution was then heated to 80°C for two hours while 30 mL of a 1 M sodium hydroxide solution was gradually added and constantly stirred.

The solution changed from yellow to ocher in color. Following the reaction's completion, the mixture was removed from the heat source and let to settle for the entire night before the supernatant solution was carefully disposed of. To remove the excess starch bonded to the nanoparticles, the precipitates were filtered out of the solution and then three times rinsed with deionized water and ethanol (Khan et al., 2016).

2.4 Characterization of CuNPs and CST nanocomposite

The SCN was characterized using several methods. FT-IR spectroscopy was utilized to illustrate how starch contributes to the reduction and capping of particulate matter. The size and surface shape of the produced SCN were examined using an X-ray diffractometer and scanning electron microscopy (SEM).

2.5 Preparation of immobilized invertase enzyme system

Enzyme solution (1µg/µL) was kept in a 30 mL tube with 2 mL of concentrated starch CuNPs to create immobilized enzyme. Using distilled water, the volume was increased to 10 mL, fully mixed, and rotated at a steady 500 rpm. The samples were centrifuged after four hours, and the resulting immobilized enzyme was then rinsed twice with distilled water and stored in a refrigerator at 4°C for additional research (Sachin et al., 2020). In order to compute the immobilization

yield (IY), which was determined by Eq. (1), the free and immobilized invertase were subjected to protein estimation by the Bradford method (Pedrol & Tamayo, 2001);

$$IY(\%) = \frac{C1 - C2}{C1} \times 100 \qquad (1)$$

where C1 represents the protein concentration added during immobilization and C2 represents the protein concentration found in the supernatant following immobilization. also enzyme's activity was measured by the 3,5-dinitrosalicylic acid methods (Miller, 1959).

2.6 Effect of experimental parameters on immobilization efficiency

At a pH of 7 and 30°C, the impact of time on immobilization efficiency was assessed throughout a range of time intervals (2–21 hours), in a buffer with a pH range of 6 to 10, the impact of pH on immobilization efficiency was assessed at 25°C. The 20–60 °C temperature range was used to assess the impact of temperature. By adjusting the concentration between 0.5 and 7%, the impact of SCN concentration on immobility was evaluated. To choose the ideal parameter for the experiment, we rely on the activity of the immobilized enzyme. Following each experiment, we fix the optimal parameter.

2.7 Effect of pH, Temperature and substrate concentration on free and immobilized Enzyme Activity

The impact of pH on invertase activity was assessed at 25°C using sodium acetate buffer (20 mM) in the 3.5–5.5 pH range. All further testing were conducted using the pH that was determined to be ideal. With the use of a temperature-controlled water bath, the impact of temperature on the activity of free and immobilized invertase was assessed between 30°C and 50°C. Using sucrose at concentrations ranging from 3 mM to 11 mM in the reaction medium at the ideal pH and temperature, the impact of substrate concentration on invertase activity was ascertained.

2.8 Reusability of immobilized enzyme

Since immobilized invertase can be utilized repeatedly for many batch processes without significantly losing activity for the initial batches, its reusability was investigated. We assessed the immobilized enzyme's reusability under the best possible circumstances. The immobilized invertase was recovered by centrifugation after each enzyme test cycle, repeatedly cleaned with distilled water, and utilized to measure the activity of the enzyme.

2.9 Data analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD) to ensure accuracy and reproducibility. Statistical analysis was conducted using Excel and Minitab software. Graphs and data visualizations were generated using Origin Pro 2018 to illustrate trends in enzyme activity, immobilization efficiency, and reusability over multiple cycles.

3. Results

3.1 Characterization of SCN

Figure 1A demonstrates the starch-CuNPs samples' FTIR spectra. Broader bands were seen between 3000 and 3700 cm−1, which were associated with intermolecular hydrogen bonds and O–H stretching. A typical C-O stretching peak within the glucose-based ring is located at 1020 cm−1, while the production of CuO was shown by the peaks between 400 and 600.

XRD analysis is used to determine the phase composition and crystal structure of the produced starch-CuNP, as seen in Figure 1B. Peaks at 2-theta values of 43.20, 50.38, and 73.94 are associated with the metallic Cu planes (111), (200), and (220). These three peaks closely matched the standard spectrum of the pure face-centered cubic (fcc) metallic Cu found on JCPDS Card No. 04-0836. Based on Scherrer's equation, the average crystallite size of the powder was determined to be 10 nm.

To determine the form of the copper nanoparticles, SEM technique was used (Figure 1C). That was demonstrated by the production of CuNPs and their morphological traits in the SEM analysis. The shapes of the SCN particles are diverse and asymmetrical.

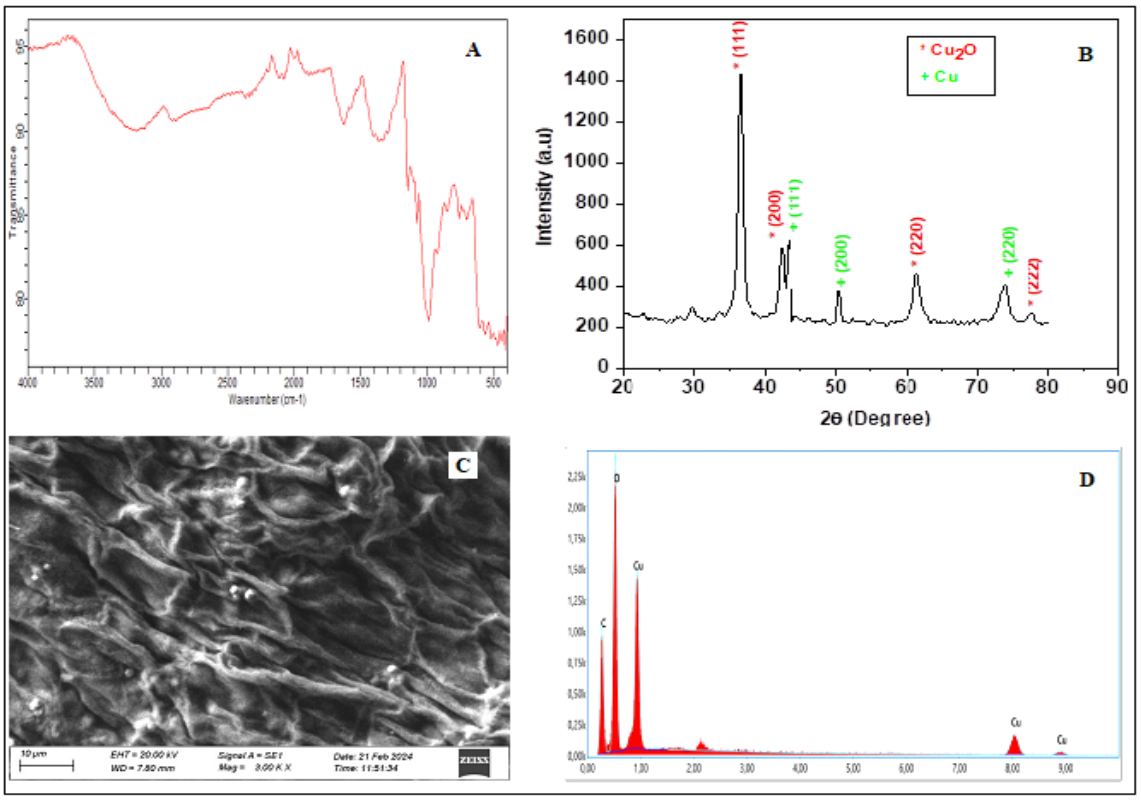

Figure 1. Characterization of SCN, FTIR spectrum (A), X-ray diffractograms (B), SEM image (C) and EDX analysis (D)

3.2 Effect of experimental parameters on immobilization process

3.2.1 Effect of incubation time

Under ideal circumstances, invertase extract was incubated with SCN at various intervals, over time, the invertase enzyme's immobilization on SCN changed. Enzyme binding began during the second hour of incubation, and this was verified by 05% immobilization yield and invertase

activity. The maximum enzyme activity was measured at 12 µmol/min/ml during the fourth hour of incubation. The highest immobilization yield (13%) was obtained after 8 hours of incubation, but Table 1 shows that the enzyme activity began to decline.

Table 1. Effect of incubation time on invertase immobilization

Parameters	2h	4h	8h	18h
Enzymatic activity (µmol/min/ml)	7.91± 0,418	12.86± 0,098	8.33± 0.0148	3.79± 0.00001
Amount of Protein (mg/ml)	0.71±0.453	1.415±0.476	1.845±1.294	1.67±1.002
Relative activity %	55.08 ±0.418	89.55 ± 0.098	58.00 ± 0.0148	26.39± 0.00001
Immobilization yield %	5.09±0.453	10.16±0.476	13.24±1.294	11.99± 1.002

Reaction conditions: solution buffer pH 5 (100 mM citric acid – 200 mM sodium phosphate), temperature T=20°C. Enzyme: 100 uL in 300 uL of reaction medium with 9 mM sucrose.

3.2.2 Effect of temperature and pH

The results presented in Table 2 demonstrate that pH significantly influences the enzymatic activity, protein immobilization, relative activity, and immobilization yield of invertase. The enzymatic activity increased with pH, reaching a maximum at pH 9 (8.02 µmol/min/ml) before decreasing at pH 10 (6.50 µmol/min/ml), whereas lower pH values (pH 6 and 7) resulted in considerably lower activity. Similarly, the immobilization yield followed a comparable trend, peaking at pH 9 (3,14%), before slightly declining at pH 10. The relative activity was also highest at pH 9 (55.90%), whereas acidic conditions (pH 6 and 7) resulted in significantly reduced enzymatic efficiency, this indicates that invertase exhibits optimal catalytic efficiency under mildly alkaline conditions.

Table 2. Effect of pH on invertase immobilization

Parameters	pH=6	pH=7	pH=8	pH=9	pH=10
Enzymatic activity (µmol/min/ml)	1.89± 0.0064	3.13± 0.233	4.01± 0.312	8.02±0.530	6 .50± 2.50
Amount of Protein mg /ml	0.12±0.0002	0.15±0.022	0.38±0.354	0.43±0.205	0.35±0.933
Relative activity %	13.16±0.0064	21.79±0.233	27.92±0.312	55.90 ±0.530	45.26 ± 2.50
Immobilization yield %	0.86±0.00003	1.09±0.0212	2.78±0.354	3.14±0.205	2.55±0.933

Reaction conditions: incubation time ; 4 hours**,** temperature; T=20 °C. Enzyme: 100 uL in 300 uL of reaction medium with 9 mM sucrose.

Table 3 shows that The enzymatic activity was highest at 20°C (8.02 µmol/min/ml) and slightly decreased at 30°C (7.79 µmol/min/ml). However, a further increase in temperature led to a significant decline in activity, dropping to 6.11 µmol/min/ml at 40°C and 5.02 µmol/min/ml at 50°C. The relative activity followed the same trend, with the highest value at 20°C (55.90%), slightly decreasing at 30°C (54.27%), and significantly dropping at 40°C (42.58%) and 50°C (34.97%), indicating that higher temperatures negatively affect enzyme stability.

The immobilization yield was highest at 30°C (6.67%), then decreasing at 40°C (4.94%) and 50°C (0.85%). This findings suggest that high temperatures reduce the enzyme's ability to bind effectively to the support material. These findings indicate that temperatures between 20°C and 30°C are optimal for invertase immobilization, while temperatures above 30°C lead to a gradual

loss of activity and immobilization efficiency, likely due to enzyme denaturation and structural instability.

Table 3. Effect of temperature on invertase immobilization

Parameters	T=20 C°	T=30 C°	T=40 C°	T=50 C°
Enzymatic activity (µmol/min/ml)	8.02± 0.530	7.79± 0.001	6.11± 0.0530	5.02± 0.365
Amount of Protein (mg/ml)	0.43± 0.0004	0.93± 0.002	0.68± 0.0566	+0.11± 0.615
Relative activity %	55.90 ± 0.530	54.27 ± 0.001	42.58±0.0530	34.97 ± 0.365
Immobilization yield %	3.14± 0.002	6.67± 0.005	4.94± 0.0566	0.85± 0.615

Reaction conditions: solution buffer pH 9, Enzyme: 100 uL in 300 uL of reaction medium with 9 mM sucrose.

3.2.3 Effect of concentration of SCN

The optimization of the % SCN for immobilizing the invertase enzyme is presented in Table 4. Invertase was incubated at percent concentrations ranging from 0.5 to 7 (w/v). CuNPs bound to the enzyme were collected at the conclusion of the incubation period, and the enzyme's activity was examined.

The enzymatic activity of immobilized invertase increased slightly as the SCN concentration increased from 0.5% to 3%, reaching a maximum of 9.01 µmol/min/ml at 3% SCN. However, further increases in SCN concentration to 5% and 7% resulted in a decline in enzymatic activity, with the lowest recorded activity at 7% SCN (6.56 µmol/min/ml). The immobilization yield exhibited a significant increase, reaching its highest value at 5% SCN (3.14%), suggesting efficient enzyme attachment at this concentration.

However, beyond that point, the yield decreased at 7% SCN (1.50%), possibly due to enzyme denaturation or excessive crosslinking that hindered enzyme activity. Overall, the results indicate that 3% SCN provides the optimal conditions for achieving high enzymatic activity and relative activity, whereas 5% SCN yields the highest immobilization efficiency. However, excessive SCN concentrations beyond these values appear to reduce enzyme stability and performance.

Table 4. Effect of concentration of SCN on invertase immobilization

Parameters	0.5%	1%	3%	5%	7%
Enzymatic activity (Umol/min/ml)	8.05±0.0997	8.19± 0.146	9.01±0.1171	8.02± 0.530	6.56±0.384
Amount of Protein mg /ml	0.11 ± 0.198	0.16±0.0006	0.19±0.0008	0.43±0.191	0.21±0.141
Relative activity %	56.05±0.0997	57.03 ±0.146	62.74±0.1171	55.84 ± 0.530	45.68 ±0.384
Immobilization yield %	0.79±0.198	1.15±0.002	1.36±0.001	3.14±0.191	1.50±0.141

Reaction conditions: solution buffer pH 9, temperature T=20°C. Enzyme: 100 uL in 300 uL of reaction medium with 9 mM sucrose.

3.3 Effect of pH, temperature and substrate on free and immobilized enzyme activity

In the temperature range of 30 to 50°C, the temperature dependency of the sucrose hydrolysis reaction catalyzed by free and immobilized invertase was investigated. The findings are displayed in (Figure 2A, 2B). The temperature was discovered to have an impact on the enzyme's activity.

It was discovered that 35°C was the ideal temperature for free invertase, and that it increased to 40°C after immobilization. Both enzymes showed a considerable decrease in activity by 50°C as the temperature was raised higher. The free enzyme's activity was much higher than the immobilized enzyme's during the 30–50°C range, and they were roughly equal at 40°C.

Invertase activity is impacted by pH, as seen in (Figure 2C, 2D). It was discovered that the invertase activity changed with pH levels. It was discovered that the ideal pH ranges for the activity of free and immobilized enzymes were 5 and 4,5, respectively. At lower pH values, the immobilized enzyme exhibited increased activity compared to the free enzyme, and conversely, at higher pH values.

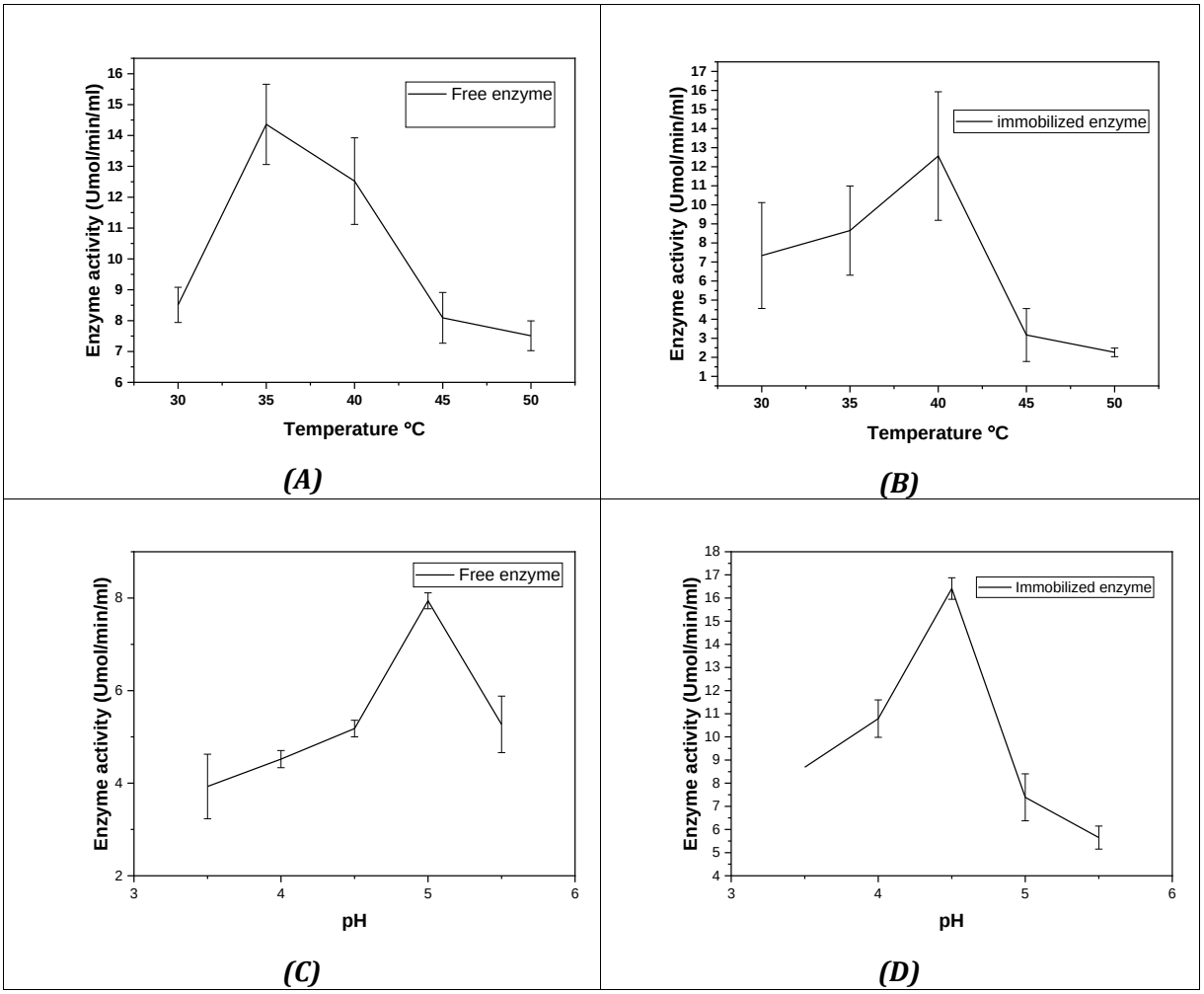

Figure 2. Effect of temperature and pH on free enzyme (A and C) and immobilized enzyme (B and D)

3.4 Reusability of immobilized enzyme

Ten cycles of immobilized invertase activity were measured. The results shown in Figure 3 show that 67% of the enzyme activity was still present after the fifth cycle. However, after the tenth cycle of reuse, the enzyme was able to maintain 49% of its activity.

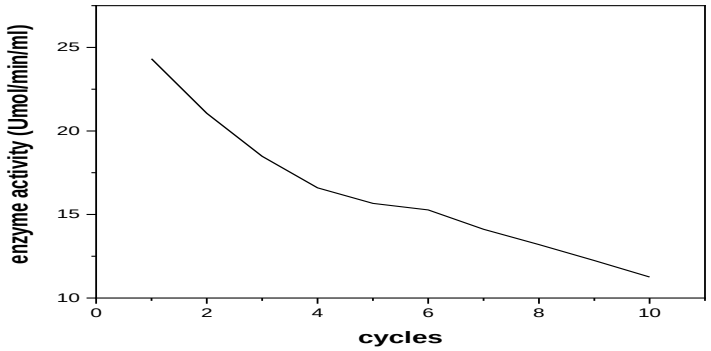

Figure 3. Reusability of immobilized enzyme

4. Discussion

Our results confirm the observations of the creation of copper nanoparticles. The FTIR spectra of SCN revealed peaks at 3452 cm−1, 2940 cm−1, and 1455 cm−1. These peaks are attributable to the —CH2OH moiety's asymmetric stretching and deformation vibration, respectively. It is claimed that the polymeric OH stretching mode corresponds to the band at 3452 cm 1.

The Siam bend, according to methyl C-H, is 1388 cm1. This research implies that OH may be able to organize and control CuNPs in a starch medium. The peaks in our data between 400 and 600 showed where CuO was formed. The main peak, which measured 576 cm-1, is thought to represent a Cu-O stretching (Ouidad et al., 2020).

By using X-ray diffraction (XRD) and SEM scan analysis, the crystallinity and shape of the synthesized particle structures were revealed. Several diffraction peaks were indexed to cuprous oxide (Cu2O), with corresponding peaks to (111), (200), (220), and (222) at 2-theta values of 36.43, 42.26, 61.22, and 77.74, respectively (Martis et al., 2010).

The cuprous oxide XRD peaks were in agreement with the bbc (face centered cubic) cuprous oxide standard powder diffraction card (JCPDS No. 05-667) (Waseda et al., 2011). However, SEM and XRD examination results showed the presence of many highly strong reflections. Similar to the findings of Samir et al. (2022), the XRD diffraction pattern revealed the coexistence of two crystalline phases, namely metallic Cu and Cu2O.

In relation to the impact of experimental parameters on immobilization efficiency, the findings indicate that an 8-hour incubation period provides a high immobilization yield, with a maximum activity reached after 4 hours. Regarding the effect of temperature, the highest activity was observed between 20 and 30°C, but the maximum immobilization yield was observed at 30°C with 16%. This temperature range was also reported by Amaya et al. (2006), who discovered that the maximum immobilization yield of invertase occurs at a temperature of 30°C.

Regarding the pH effect, the results showed that the best immobilization yield and highest activity were found at pH 9, with an immobilization yield of 7%. In contrast, Amaya et al. (2006) reported that invertase exhibited maximum activity when immobilized at pH 5.0 on nylon-6 microbeads. Additionally, Marquez et al. (2008) discovered that a pH of 5.0 was the maximum for invertase immobilization via adsorption in ionic exchange resin. This difference can result from the employment of various immobilization methods and support materials. On the other hand, 3% of starch CuNPs had the highest immobilized enzyme activity, despite 5% having the maximum immobilization yield.

Regarding the ideal pH values for the activity of free and immobilized enzymes, the findings indicate that they correspond to 4,5 and 5, respectively, for these circumstances. At lower pH values, the immobilized enzyme exhibited increased activity compared to the free enzyme, and conversely, at higher pH values. Since enzymes are proteins, pH variations will have a significant impact on the ionic character of amino and carboxylic acid groups on proteins, which will in turn

have a significant impact on the catalytic site and conformation of an enzyme. This helps to explain how pH affects enzymatic activity (Rouzer & Marnett, 2020).

Apart from purely ionic effects, significant denaturation and subsequent inactivation of the enzyme protein can be caused by either high or low pH levels. Furthermore, because many substrates have an ionic nature, an enzyme's active site could need a certain ionic species in order to function at its best. These factors most likely determine the majority of an average enzyme activity-pH relationship (Aburigal et al., 2014). The results show that, when compared to the free enzyme, the usage of starch CuNPs in conjunction with invertase can enhance the activity of immobilized invertase at acidic pHs and demonstrate good stability of invertase at this pH range.

The temperature was discovered to have an impact on the enzyme's activity. It was discovered that 35°C was the ideal temperature for free invertase, and that it rose to 40°C after immobilization. A decrease in activity may result from an enzyme's inactivation at low temperatures and thermal denaturation at high temperatures (Naqash et al., 2019).

In line with our findings, Hakkoymaz and Mazi (2020) reports that the optimal temperature for immobilized invertase was determined to be 70°C, while the maximal activity of free invertase was reported at 55°C. that there was no difference in the ideal temperature of 55°C and 60°C for free and immobilized invertase, respectively.

Fructose and glucose are primary sources of energy for plant metabolism, playing crucial roles in cellular respiration, osmotic regulation, and biosynthesis of essential biomolecules. These sugars also influence root exudation, which can enhance nutrient uptake and promote beneficial microbial activity in the rhizosphere.

Ten cycles of immobilized invertase activity were measured, the findings showed that 67% of the enzyme activity remained after the fifth cycle and 49% after the tenth cycle of reuse. The reduction of support strength, which caused enzyme leakage from the starch-CuNPs, was the cause of the drop in activity with subsequent cycles. In the work of Malhotra and Basir (2020) after 9 batches of reusing invertase immobilized on chitosan, there was a 70% retention in activity.

Unlike free invertase, the immobilized form could be reused several times and it is more stable against environmental factors like temperature fluctuations, pH variations, and enzymatic degradation (Hakkoymaz & Mazi, 2020). Fructose and glucose are primary sources of energy for plant metabolism, playing crucial roles in cellular respiration, osmotic regulation, and biosynthesis of essential biomolecules. These sugars also influence root exudation, which can enhance nutrient uptake and promote beneficial microbial activity in the rhizosphere. The immobilized form of invertase on SCN Can enhance sugar availability by the hydrolysis of sucrose into fructose and glucose which provide a controlled and sustained release of these sugars in agricultural environments (Sjölin et al., 2024).

Immobilized invertase also had an impact on beneficial soil microorganisms since many soil microbes utilize fructose and glucose as carbon sources, promoting the growth of plant growth-promoting bacteria (PGPB) and mycorrhizal fungi, which enhance nutrient uptake (e.g., nitrogen and phosphorus absorption). This microbial stimulation can improve soil health, increase organic matter decomposition, and enhance root development. (Kumar, 2016).

Invertase play vital roles during seed germination by breaking down stored carbohydrates into simpler sugars, providing energy for the developing seedling (Wang et al., 2024). Developing simple, cost-effective seed coating technologies has been shown to enhance seedling establishment. For instance, a study focused on paddy seeds found that locally available materials used in seed coatings improved seedling growth, suggesting that incorporating beneficial additives could further enhance outcomes (Fenangad & Orge, 2015). SCN-immobilized invertase could be incorporated into seed coatings or soil treatments to provide a continuous supply of sugars that boost early seedling establishment.

5. Conclusion

This study demonstrated that starch-copper nanocomposites (SCN) provide an effective and sustainable platform for enzyme immobilization, enhancing invertase stability and reusability. Maximum immobilization were observed at pH 9 and temperature 30 °C, the enzyme immobilized under these condition exhibited optimal activity at pH 5 and 35 °C, while free invertase performed best at pH 4 and 40 °C. After ten reaction cycles, the immobilized enzyme retained 49% of its activity, confirming its potential for repeated use. These findings highlight the efficacy of starch-based nanocomposites in stabilizing enzymes, offering a cost-effective and eco-friendly solution for agricultural and biotechnological applications. The ability to reuse immobilized enzymes could improve enzyme-based processes in food industries, biofuel production, and waste management, reducing costs and minimizing environmental impact. Future research could explore the application of SCN-based enzyme immobilization in soil health improvement, bioremediation of agricultural pollutants, and enzymatic enhancement of plant growth which could open new avenues for sustainable agricultural practices.

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Authors' declaration and contribution

Study concept and design: M.C. Analysis and interpretation of data: M.C., S.D. Drafting of the manuscript: M.C. Study supervision: S.D. All authors have read and agreed to publish version of the manuscript.

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