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RESEARCH ARTICLE

ASSESSMENT OF CHITOSAN FROM SELECTED EDIBLE MUSHROOMS FOR ENZYME IMMOBILIZATION

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ARTICLE DETAILS

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ABSTRACT

Assessment of chitosan from some edible mushrooms for enzyme immobilization was carried out using standard methods. The study was aimed to determine functional properties/quality of chitosan from some wild edible Nigerian mushrooms for industrial applications. The results revealed that the V_{max} and K_m of both free and immobilize cellulases were in the range of $1.25 x 10^{-1}$ - $9.25 x 10^{-1}$ / $3.90 x 10^{-1}$- $6.01 x 10^{-1}$mg/ml/sec and $4.67 x 10^{-2}$- $9.25 x 10^{-1}$ / $6.11 x 10^{-1}$ -$8.70 x 10^{-1}$ respectively. Thermostability and optimum pH for the activities of free and immobilize cellulases were 50°C / pH 6 and 40°C / pH 7 respectively; indicating that the immobilized enzyme on different chitosan beads have good thermostability and pH optimum. Storage stability analysis showed a half-life ranging from 3.11 - 6.62 days for free and 8.00 - 232.00 days for immobilize cellulases. The residual activity of immobilized cellulase on standard chitosan and mushroom base chitosan beads achieved after storage for 8, 193, 81, 21, 125 and 232 days were 52.38, 71.79, 65.93, 71.22, 66.45, and 66.81% respectively. The residual activity and half life values were higher in cellulase immobilized on mushroom beads than that of standard chitosan beads. This research suggests that mushroom chitosan has a great potential as a support and carrier for enzyme immobilization.

KEYWORDS

Chitosan, Enzyme, Mushroom, Quality, Immobilization, Industrial

1. BACKGROUND OF THE STUDY

Polymer-based organic and inorganic nanoparticles gain excellent properties from both components as a result of synergy. The properties are not limited to the following; biodegradability, biocompatibility, renewability, enhanced mechanical property and non-toxicity. Hence, these nanocomposite materials are of considerable attention (Gen et al., 2020, Ma et al., 2017, Wu et al., 2017). Chitosan as a cationic bioactive amino polysaccharide is fundamentally composed of β-1,4-D-glucosamine (GlcNAc) linked to N-acetyl-D-glucosamine residues. This polymer is of great economic value due to its multitalented biological activities and chemical applications as an antimicrobial, antitumor, immuno-potentiator and wound healing agent and in slow drugs release, water purification, dietary fiber, packaging films, coatings, tissue engineering and enzyme immobilization among other uses (Gu et al., 2018, Morin-Crini et al., 2019; Nworie, 2018).

Chitosan is a well-known component of fungal cell walls that is formed by the chemical or spontaneous deacetylation of chitin, an insoluble linear GlcNAc polymer. Commercially, chitosan is gotten through chemical deacetylation of crustacean chitin under strong alkali treatment (He et al., 2018). Crustacean chitosan is contradictory in its physicochemical possessions due to inconsistency in raw materials, the harshness of the isolation and conversion processes, the caustic effects of the chemicals used in the isolation process, and variability in the levels of deacetylation and protein contamination (Liu et al., 2012; Fadhil, and Mous, 2021).

In order to obtain chitosan of a more consistent quality, filamentous fungi have been considered an attractive source for industrial applications because their specific products can be manufactured under standardized conditions (Fai et al., 2011; Fadhil, and Mous, 2021). This is achieved by careful manipulation of the growth parameters such as pH, and composition during the fermentation. These manipulations result in a varying molecular weight and degree of deacetylation of the chitosan, which influenced various properties, the application, and the biological response of the polymer (Fai et al., 2011). The use of fungi has demonstrated great advantages like independence from seasonal factors, ease of large-scale production with possible simultaneous extraction of chitin and chitosan. Also, its extraction process is simple and cheap with absence of protein contamination in the co-polymers obtained from fungi biomass, especially those proteins that could cause allergic reactions in individuals with shellfish allergies (Alsaggaf et al., 2017; Alona et al., 2019).

Due to the facts that enzymes are versatile, highly effective eco-friendly catalysts that operate under mild conditions and which experience operational challenges like generation of toxic by-products, temperature and pH variation, immobilization of enzyme will be an alternative solution, allowing enzyme recovery which is an essential prerequisite for its reuse (El-shishtawy et al., 2021). It also provides stability to enzymes against changing conditions in a reaction media because enzymes are responsive to reaction changes and are thus denatured in adverse conditions (Noreen et al., 2016; Mohamed et al., 2017; Lin et al., 2015). Therefore, immobilization provides multiple advantages over free enzymes under identical reaction conditions, enhancing catalytic efficiency and enzyme

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recycling (Aldhahri et al., 2021; Almulaiky et al., 2019). In summary, chitosan's biocompatibility, abundant functional groups, pH-responsive behavior, and other unique properties make it an important material for enzyme immobilization. Its application in various industries underscores its significance in enhancing enzyme stability, reusability, and overall performance in diverse biotechnological processes. This current study is on the assessment of chitosan from selected edible mushrooms for enzyme immobilization.

2. Materials and Methods

2.1 Collection of Mushroom Samples

Five different edible wild Nigerian mushrooms *Lactarus deliciousus (Milk mushroom), Cantharelles cibarius, Laccaria laccata, Hericium erinaceus* and *Pleurotus tueragium* were collected from different areas within the base of a thick forest located in Ugu–Uleri in Biladebia Ntezi in Ishielu Local Government Area of Ebonyi State, Nigeria and were identified by their spore prints and by comparing their morphological, anatomical and physiological characteristics with the standard description in the Department of Applied Biology Ebonyi State University, Abakaliki, Nigeria.

2.2 Preparation of mushroom samples

The mushrooms were uprooted, destalked, washed and air-dried under room temperature for 2-6 days while turning the mushroom to avoid fungal growth. The mushrooms were later milled to obtain the mushroom meals (MRMS) using mortar and pestle and stored in an ovum at 110^{0}C in a container for analysis.

2.3 Chitosan extraction

Chitosan extraction was carried out by a modified method of (Rane and Hoover, 1993; Crestini et al., 1996). The mushrooms powder was suspended with 1 M NaOH solution (1:30 w/v) and autoclaved at 126°C for 15 min. Alkali-insoluble fractions (AIF) were collected after centrifugation at 12 000 X 9.8m/sec^2 for 15 min, washed with distilled water to neutrality. The residues were further extracted using 2% acetic acid (1:40 w/v) at 95°C for 8 h. The extracted slurry was centrifuged at 12 000 9.8m/sec^2 for 15 min. The pH of the supernatant fluids was adjusted to 10 with 2 ml of 1M NaOH, the solution centrifuged at 12 000 x 9.8m/sec^2 for 15 min and the precipitated chitosan was washed with distilled water, 95% ethanol (1:0 w/v) and acetone (1:20 w/v), respectively and dried at 60°C to a constant weight.

2.4 Formation of chitosan beads by ionic gelation method

Chitosan beads were prepared according to the method by (Avadi et al., 2010; Garg et al., 2019). The chitosan powder (3g) was first dissolved in 1.0% acetic acid. The solution of the chitosan powder was then introduced into a 20ml syringe and extruded through the syringe needle into a solution of 1.5% sodium tripolyphosphate under constant stirring. The spherical beads was then picked randomly and measured using vernier caliper to get its diameter (4-5mm in diameter) that were formed as a result of the ionic linkage of the sodium tripolyphosphate. The chitosan beads formed were harvested by filtration and washed with distilled water until neutrality and were stored in distilled water at 4°C until needed.

2.5 Assays for the activity of immobilized cellulase on the mushroom chitosan beads

To 0.9 ml of assay buffer (0.05M acetate buffer, pH 7.0) was added, 0.1ml of appropriately diluted cellulases (20-fold dilution in same buffer) and incubated at 60°C in 1ml of 0.2% carboxymethyl cellulose. After 30 minutes, the reaction was stopped by reducing the temperature to 10°C. An aliquot (1ml) of the reaction mixture was transferred to a 50ml beaker and 1ml of DNS reagent was added with constant swirling in a water bath at a varying temperature between 30 - 80°C to allow the colour to develop and the reaction was stopped after 30 minutes and allowed to cool. The developed colour was measured at 540 nm with UV/VIS spectrophotometer. A blank was run without the cellulase and the CMC. The cellulase unit (U) in this present work was defined as the amount of cellulase required to liberate 1μmol of glucose in 1min under the reaction conditions (0.2% carboxymethyl cellulose, 0.05M acetate buffer, pH 7 at 60°C. The experiments were conducted in triplicate (Abdel-Azizi et al., 2014).

2.6 Immobilization of cellulase enzyme on chitosan beads

The principle of immobilization used here is physical adsorption. Cellulase often binds to the carrier beads by physical interaction such as hydrogen bonding, hydrophobic interaction and vander waal's forces (Abdel-Azizi et al., 2014). Carrier mostly used for physical adsorption is chitosan beads because of its strong adsorptivity due to the fact that, it is a polysaccharide material and which gives room for interaction (hydrogen bonding) between the cellulases amino acid and the hydroxyl group of the polysaccharides. The immobilization was performed by physical adsorption on the chitosan beads as described by (Egwim et al., 2012). The mushroom chitosan beads were immersed in the concentration of cellulase solution with a given enzyme/beads ratio (2ml/gbeads). The mixture was gently stirred for 10 minutes and then placed in a refrigerator at 4°C for 24 hours. The supernatant was removed and the beads washed three times with distilled water. The immobilized cellulase was recovered from the used solution and stored at 4°C for reuse.

2.7 Assay for the activity of free and immobilized cellulase

The cellulase enzyme activities were determined in both free and immobilized enzymes, according to the method described by (Abdel-Azizi et al., 2014). Thereafter, the free and immobilized cellulase was characterized for activity, optimum temperature, substrate concentration, pH, kinetic parameters and storage stability.

2.8 Determination of the kinetic parameters (K_m and V_{max}) of free and immobilize enzymes

To determine the kinetic parameters of the partially purified cellulase, the substrate concentration was varied (0.2-1.0) for both free and immobilized cellulase at optimum temperature, 50°C and citrate buffer pH 7.4. The rate of reaction was measured as earlier described. The value of K_m and V_{max} were determined by using Line weaver-Burke double plots.

2.9 Determination of thermostability of free and immobilized cellulase

To determine the optimal temperature at which the immobilized cellulase can withstand thermal stress, free and immobilized cellulase were suspended in citrate buffer (0.10M, pH 7) and incubated at different temperatures (30 - 80°C) for 30 minutes before the activity was measured.

2.10 Determination of the optimum pH of free and immobilized enzymes

To determine the optimum pH for the free and immobilized cellulase, the enzyme activities were assayed in pH ranging from 3 - 8 at a constant temperature (50°C) and 0.2M CMC for 30 minutes.

2.11 Determination of storage stability of free and immobilize cellulase

To determine the storage stability of free and immobilized cellulase, the free and immobilized cellulase was kept at the temperature of 4°C for one week and its activity was monitored. However, the reusability of the immobilized cellulase was determined by using it repeatedly at 50°C, 2% CMC and 0.1M citrate buffer (pH 7.4).

2.12 Statistical Analysis

The results were presented as a mean ± standard deviation. The statistical analysis shows a significant difference for some parameters at $p<0.05$.

3. Results

The results of immobilization of cellulases on different mushroom chitosan beads are shown in table 1 and figures 1-6

Table 1: The values of K_m and V_{max} of free and immobilize cellulase on standard chitosan beads and different mushroom samples

Sample	K_m of free (mg/ml/sec)	K_m of immobilized (mg/ml/sec)	V_{max} of free (mg/ml/sec)	V_{max} of immobilized (mg/ml/sec)
Standard Chitosan	9.25×10^{-1}	0.56×10^{-1}	7.63×10^{-1}	5.03×10^{-1}
L deliciousus	5.83×10^{-1}	0.93×10^{-1}	8.70×10^{-1}	3.90×10^{-1}
L. laccata	5.83×10^{-1}	0.93×10^{-1}	8.70×10^{-1}	3.90×10^{-1}
C. cibarius	0.59×10^{-1}	0.44×10^{-1}	6.11×10^{-1}	4.32×10^{-1}
H. erinaecius	0.47×10^{-1}	0.13×10^{-1}	7.55×10^{-1}	6.01×10^{-1}
P. tuberagium	0.91×10^{-1}	0.50×10^{-1}	7.23×10^{-1}	5.03×10^{-1}

1/[S] (Reciprocal of substrate concentration),

$1/V_o$ (Reciprocal of activity of free and immobilize enzymes in mg/sec)

3.1 Immobilization of Cellulases on Standard Chitosan Beads

The K_m and V_{max} values of immobilized cellulases on standard chitosan beads and free cellulases were calculated using the Line weaver-Burk plot as shown in the Figure 1 below. The free cellulase had higher values for both K_m (0.069mg/ml) and V_{max} (0.763mg/ml/s) than the immobilized enzyme K_m (0.053mg/ml) and V_{max} (0.503mg/ml/s).

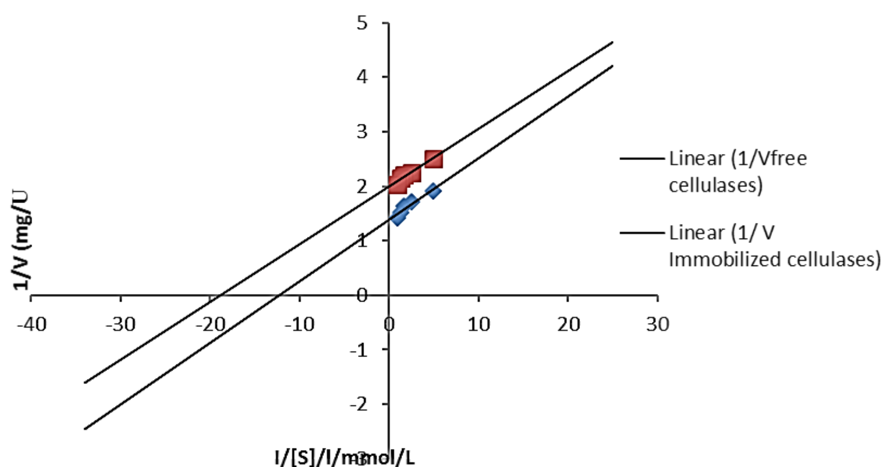

Figure 1: Lineweaver-Burk plots of free and immobilized cellulase on standard chitosan beads.

3.2 Immobilization of Cellulase on *Lactarius deliciousus* Chitosan Beads

Figure 2 shows the Lineweaver-Burk plots of free and immobilized cellulase on chitosan beads from *Lactarius deliciousus.* The V_{max} of immobilized cellulase on *Lactarius deliciousus* chitosan beads (0.390mg/ml/s) was lower than that of free cellulase chitosan (0.870mg/ml/s). The K_m of immobilized cellulase on *Lactarius deliciousus* chitosan beads (0.093mg/ml) was consequently observed to be lower than free cellulase with 0.583mg/ml).

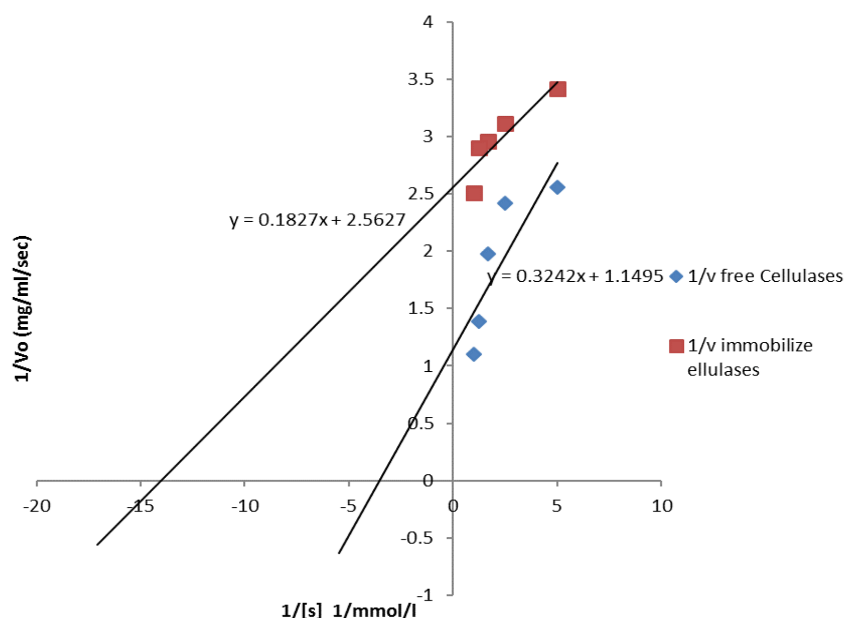

Figure 2: Lineweaver-burk plots of free and immobilized cellulase on chitosan beads from *Lactarius deliciousus*

3.3 Immobilization of Cellulase on *Laccaria laccata* Chitosan Beads

Figure 3 shows the Lineweaver-Burk plots of Free and Immobilized Cellulase on chitosan beads from *L. laccata.* The V_{max} of immobilized cellulase on *L. laccata* chitosan beads, 0.390mg/ml/s was lower than that of free cellulase chitosan (0.870mg/ml/s). The K_m of immobilized cellulase on *L. laccata* chitosan beads was 0.093mg/ml which is lower than free cellulase with 0.583mg/ml).

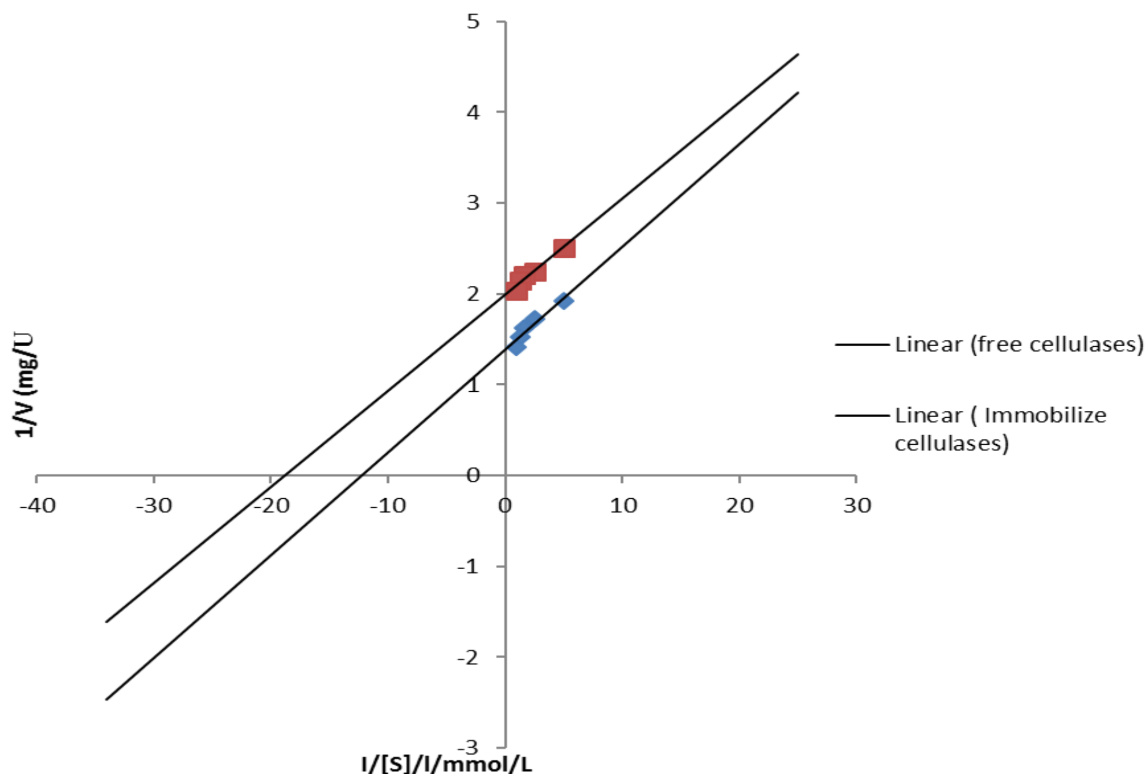

Figure 3: Lineweaver-burk plots of free and immobilized cellulase on chitosan beads from *Laccaria laccata*

3.4 Immobilization of cellulase on C. cibarius chitosan beads

Figure 4 shows the Lineweaver-Burk plots of Free and Immobilized Cellulase on Chitosan beads from *C. cibarius.* The V_{max} of immobilized cellulase on *C. cibarius* chitosan beads (0.432 mg/ml/sec) was lower than that obtained free cellulase enzyme (0.611mg/ml/s). The K_m of immobilized cellulase on *C. cibarius* chitosan beads (0.044mg/ml) was observed to be lower free cellulase of 0.059 mg/ml

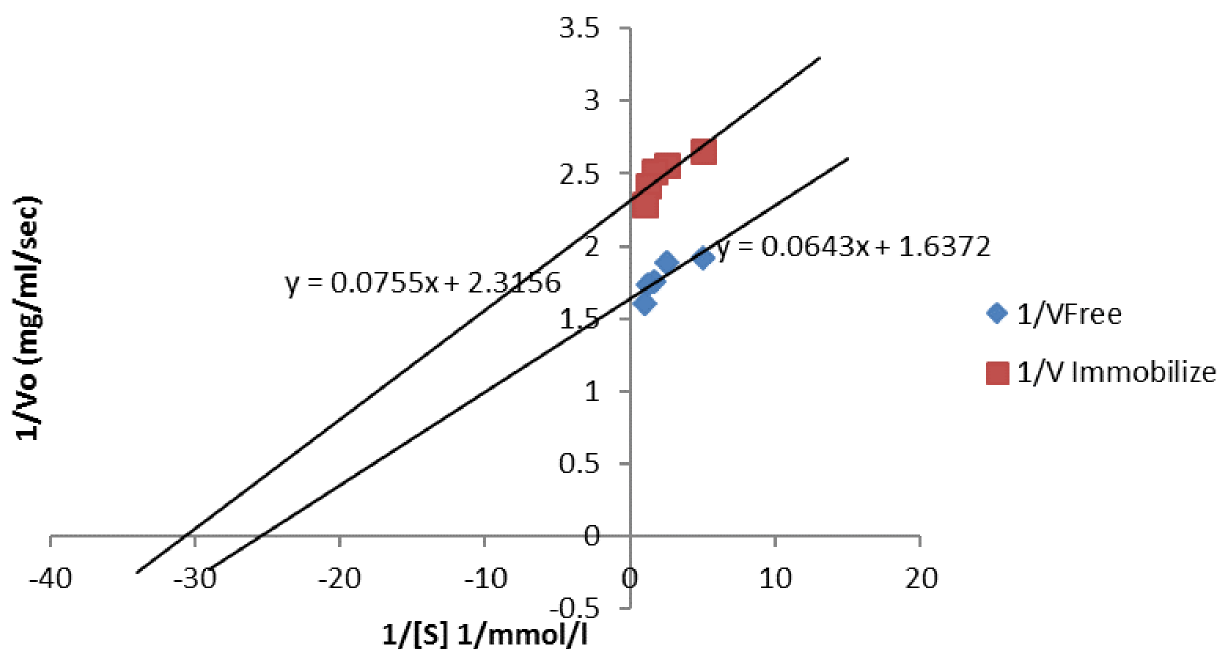

Figure 4: Lineweaver-burk plots of free and immobilized cellulase on chitosan beads from *Cantharelle cibarius*

3.5 Immobilization of Cellulase on Hericum erinacius Chitosan Beads

Figure 5 shows the Lineweaver-Burk plots of free and immobilized cellulase on chitosan beads from *H. erinacius.* The V_{max} of immobilized cellulase on *H. erinacius* chitosan beads (0.601mg/ml/s) was lower than that obtained from free cellulase (0.755mg/ml/s). The K_m of immobilized cellulase on *H. erinacius* chitosan beads (0.013mg/ml) was lower than of free cellulase of 0.047mg/ml.

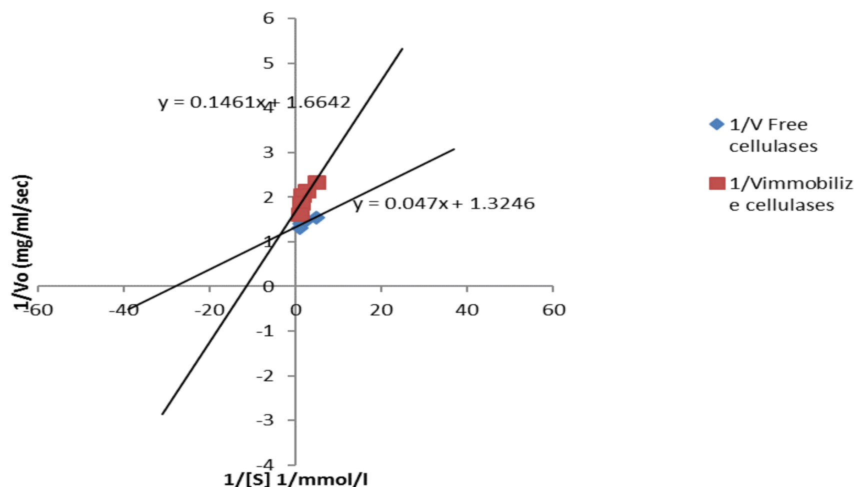

Figure 5: Lineweaver-burk plots ofs free and immobilized cellulase on chitosan Beads from *Hericum erinacius*

3.6 Immobilization of cellulase on *P. tuberagium* Chitosan Beads

Figure 6 shows the Lineweaver-Burk plots of free and immobilized cellulases on chitosan beads from *P. tuberagium.* The V_{max} of immobilized cellulase on *P. tuberagium* chitosan beads (0.503mg/ml/s) was lower to that obtained free cellulase of 0.723mg/ml/s. The K_m of immobilized cellulase on *P. tuberagium* chitosan beads (0.050mg/ml) was lower to that obtained free cellulase with 0.091mg/ml.

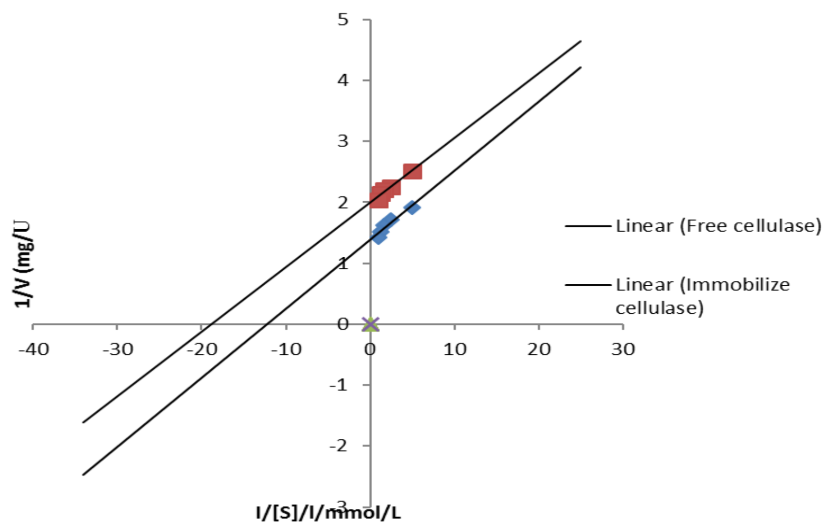

Figure 6: Lineweaver-burk plots of free and immobilized cellulase on chitosan beads from *p. tuberagium*

3.7 Effect of thermostability on the activity free and immobilized cellulase on standard and different mushroom chitosan

The effect of on the activity of free and immobilized cellulase on standard and different mushroom chitosans is as shown in Figure 7-12. The highest enzyme activity observed in immobilized cellulase was 9.34 x10^{-4}mg/ml/s while free cellulase of *H. erinaecius* chitosan had an activity of 5.14 x10^{-5}mg/ml/s at optimum temperature of 60°C (Figure 11). Enzyme immobilized on both *L. deliciousus* had the least activity of 1.21 x10^{-5}mg/ml/s at optimum temperature of 50°c respectively (Figure 8). The highest activity of immobilized cellulase on standard chitosan was 3.21x10^{-4} mg/ml/sec at an optimum temperature of 30°c whereas the free cellulase was 5.08 x10^{-4} mg/ml/sec at an optimum temperature of 50°c (Figure 7). The trend of optimization continues even in the variations of mushroom chitosan as compared with the standard chitosan.

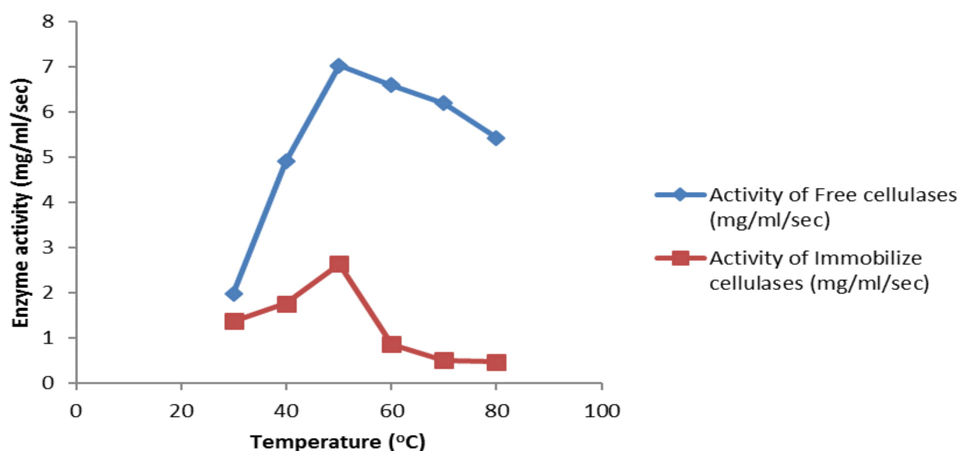

Figure 7: Thermostability of free and immobilized celluase on standard chitosan at different temperatures

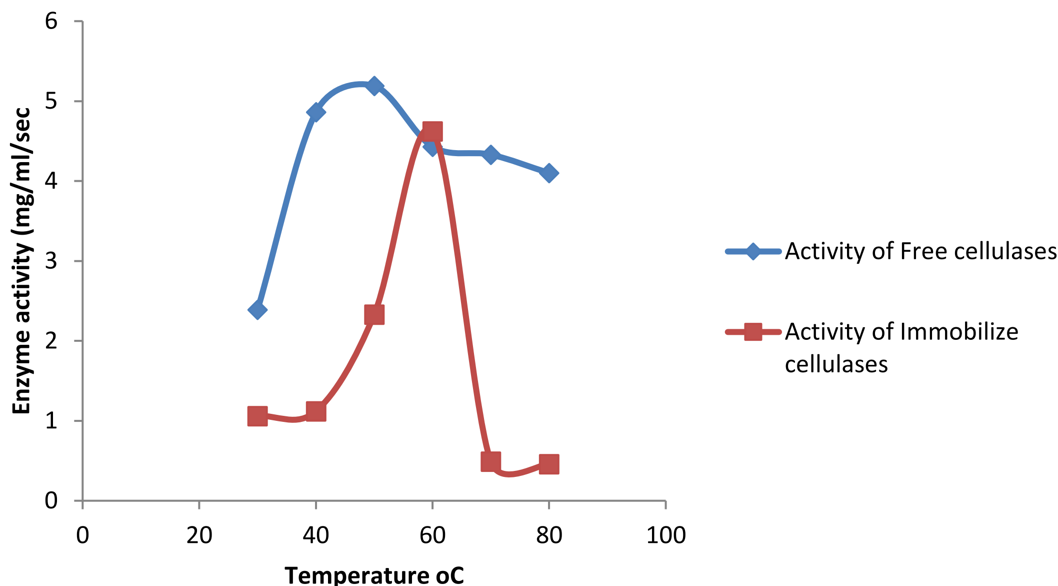

Figure 8: Thermostability of free and immobilized celluase on mushroom chitosan L. *delicousus* at different temperatures

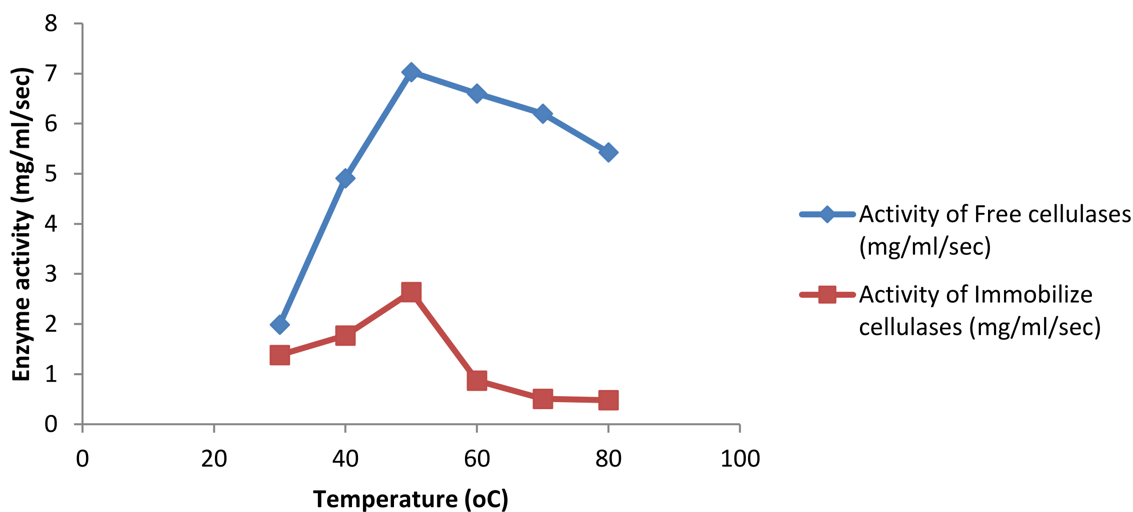

Figure 9: Thermostability of free and immobilized celluase on mushroom chitosan from *L. laccata* at different temperatures

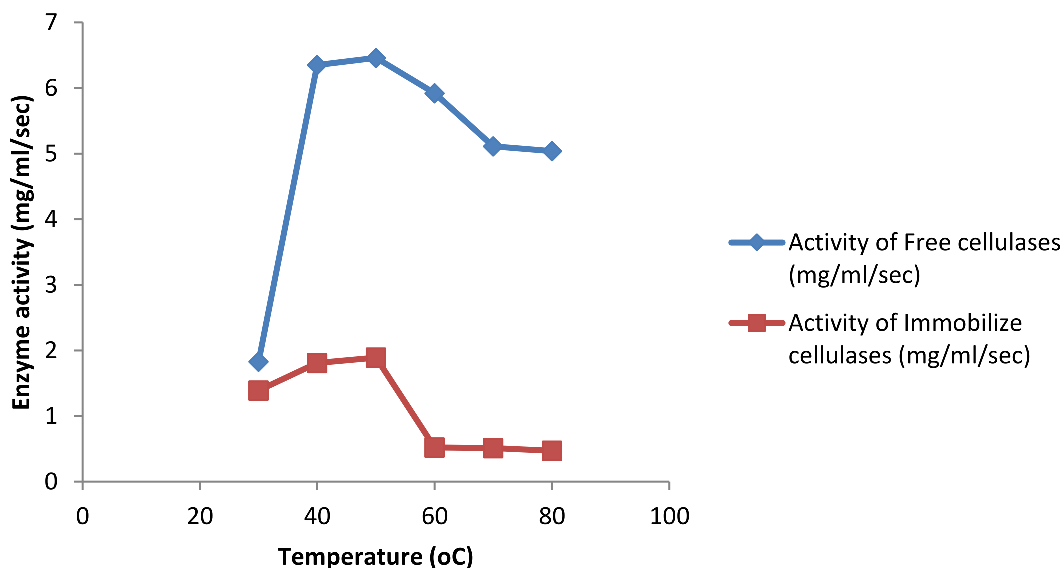

Figure 10: Thermostability of free and immobilized celluase on mushroom chitosan from *C. cibarius* at different temperatures

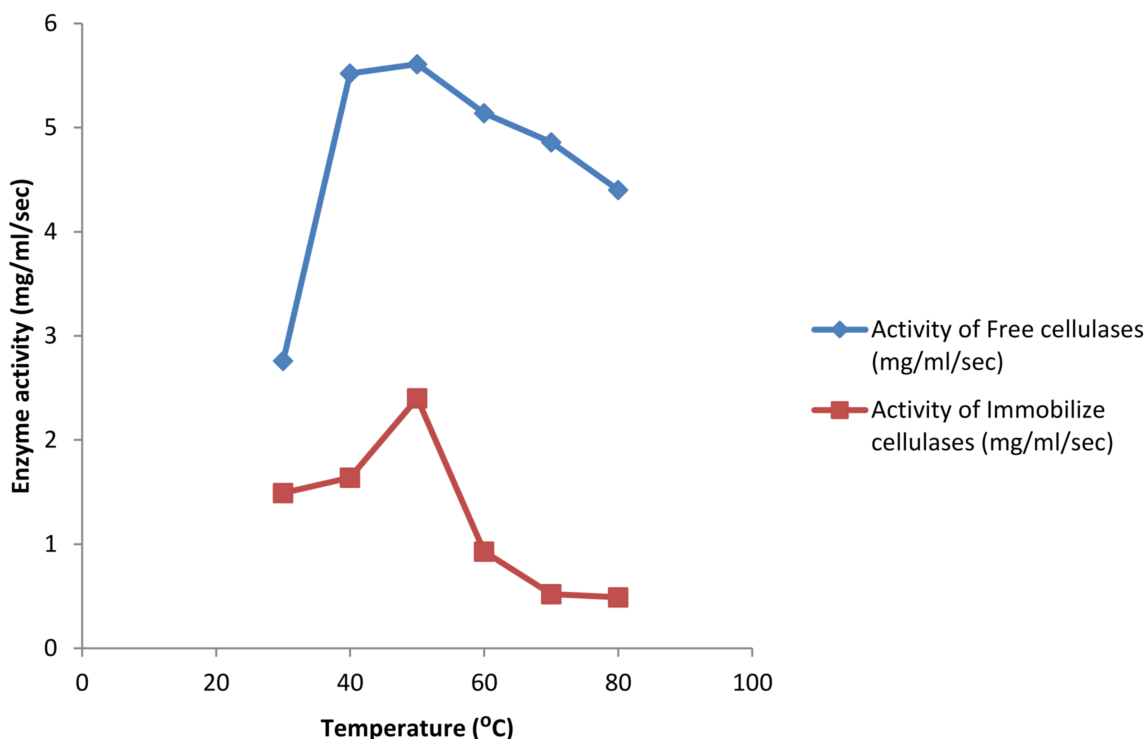

Figure 11: Thermostability of free and immobilized celluase on mushroom chitosan from *H. erinaecius* at different temperature

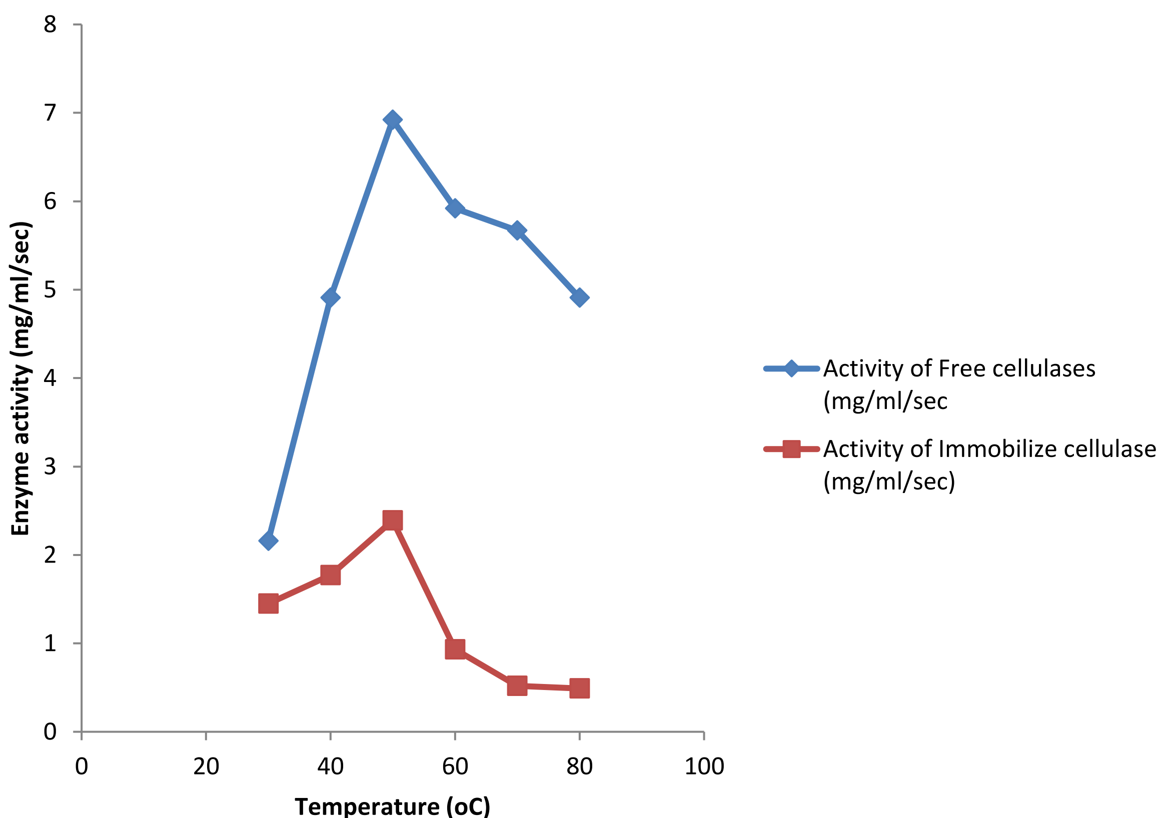

Figure 12: Thermostability of free and immobilized celluase on mushroom chitosan from *P. tuberagium* at different temperatures

3.8 Effect of pH on the activity free and immobilized celluase on standard and differrent mushroom chitosan are shown in figures 13 - 14

The effect of pH on the activity of free and immobilized celluase on standard and differrent mushroom chitosans is as shown in Figures 13 and 14. The highest enzyme activity observed in free cellulase was 9.03 x 10^{-5}mg/ml/s while immobilized cellulases of *H. erinaecius* chitosan had an activity of 6.97 x 10^{-5}mg/ml/s at optimum pH of 7 (Figure 14). Enzyme immobilized on both *L. deliciousus* chitosan had the least activity of 3.30 x10^{-5} mg/ml/s at pH of 5 (Figure 13). The highest activity of immobilized cellulase on standard chitosan was 4.83x10^{-5} mg/ml/s at an optimum pH of 6 whereas the free cellulase was 7.96x10^{-5} mg/ml/s at a pH of 7 (Figure 14).

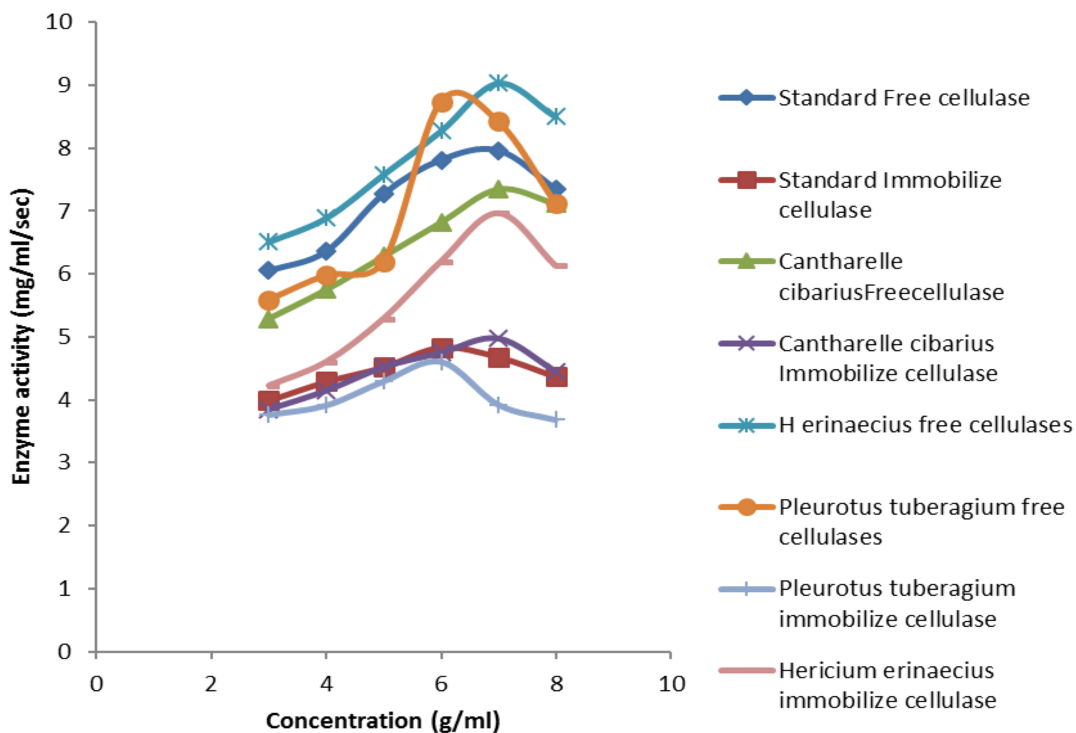

Figure 13: Optimum pH of free and immobilized cellulase on standard and different mushroom chitosan at different concentration

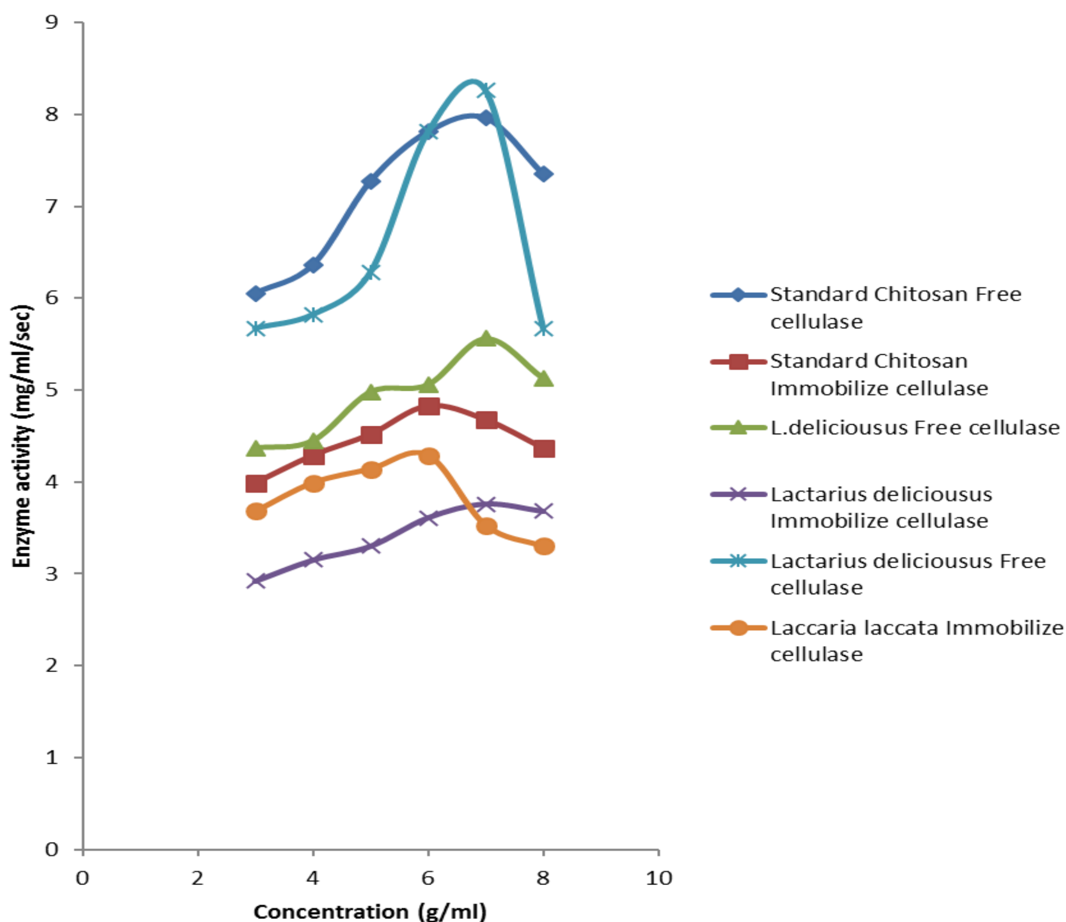

Figure 14: Optimum pH of free and immobilized celluase on standard and different mushroom chitosan at different concentration

3.9 Residual activity of immobilized cellulase on standard chitosan and mushroom chitosan beads

The residual activity of immobilized cellulase on standard chitosan and mushroom base chitosan beads achieved after storage for 8 days, 193 days, 81 days, 21 days, 125 days and 232 days were 52.38%, 71.79%, 65.93%, 71.22%, 66.45%, and 66.81%. The result showed that the residual activity and half life values were higher in cellulase immobilized on mushroom beads than those on standard chitosan beads. Cellulase immobilized on *L. deliciousus* chitosan beads had a higher residual activity while the *P. tuberagium* had the highest half life.

Table 2: Residual of free and immobilize cellulase after one week				
Sample chitosan	Initial Activity (mg/ml/sec)	Final Activity (mg/ml/sec)	Residual activity **(%)**	Half life **(days)**
Standard chitosan	3.99 x10^{-5}	1.90 x10^{-5}	52.38	8.00
L. deliciousus	4.29 x10^{-5}	1.21 x10^{-5}	71.79	193.00
L. laccata	4.52 x10^{-5}	1.54 x10^{-5}	65.93	81.00
C. cibarius	4.83 x10^{-5}	1.39 x10^{-5}	71.22	21.00
H. erinaecius	4.68 x10^{-5}	1.57 x10^{-5}	66.45	125.00
P. tuberagium	4.37 x10^{-5}	1.45 x10^{-5}	66.81	232.00

4. Discussion

Cellulase (EC: 3.2.1.4) is an enzyme that naturally catalyses the breakdown of cellulose. In this study, the cellulases immobilized on mushroom chitosan beads have the properties that are affected by the operating conditions of the immobilization processes; the immobilization of cellulase on mushroom chitosan beads was performed at the optimum conditions. Figure 1-6 shows the Lineweaver-Burk plots for free and immobilize cellulase on the mushroom chitosan beads in catalyzing the breakdown of cellulose. The K_m value for free cellulase from the standard chitosan beads is calculated to be 9.25 x 10^{-1} mg/ml and the V_{max} value of free cellulase on chitosan beads 7.63 x 10^{-1} mg/ml/sec. The corresponding $K_m$and V_{max} for the immobilized cellulase was 5.60 x 10^{-1}mg/ml and 3.03 x 10^{-1} mg/ml/sec respectively. The calculated K_m and V_{max} value of the corresponding cellulase from the different chitosan beads were also calculated to compare with the standard chitosan beads.

In *L. deliciousus* the K_m values for free cellulase was 8.70 x 10^{-1}mg/ml and the V_{max} for free cellulase was 5.83 x 10^{-1} mg/ml/sec and the corresponding K_m and V_{max} value for the free and immobilize cellulase was 9.27 x 10^{-1} mg/ml and 3.90 x 10^{-1} mg/ml/sec. The *L. laccata* K_m and V_{max} value for both free and immobilized cellulase was calculated to be 8.70 x 10^{-1} mg/ml for free cellulase/5.83 x 10^{-1} mg/ml/sec and 9.27 x 10^{-1} mg/ml/ 3.90 x 10^{-1}mg/ml/sec. The free and immobilize cellulase on *C. cibarius* beads were also calculated to be 6.11 x 10^{-1} mg/ml/0.59 x 10^{-1}mg/ml/sec and 4.41 x 10^{-1} mg/ml/0.42 x 10^{-1} mg/ml/sec. In *H. erinaecius*, the free and immobilize cellulase on their chitosan beads was 7.55 x 10^{-1} mg/ml/0.47 x 10^{-1} mg/ml/sec and 6.01 x 10 mg/ml/0.13 x 10^{-1}mg/ml/sec. The *Km and* V_{max} of free and immobilize cellulase for *P. tuberagium* was 9.13 x10^{-1} mg/ml/7.23 x 10^{-1} mg/ml/sec and 5.60 x 10^{-1} mg/ml/5.03 x 10^{-1} mg/ml/sec.

These observations could be attributed to the facts that once the cellulase was immobilized, its affinity toward the substrate decreased due to the steric hindrance of the active sites by the support, the loss of the cellulase flexibility necessary for substrate binding, and the possible change of the cellulase structure resulting from the multi-point covalent attachment with the support. Accordingly, K_m of cellulases immobilized on the chitosan on the different chitosan beads became higher than the free cellulase and V_{max} became lower, demonstrating that the affinity of immobilized cellulase toward the substrate decreased, which is a typical result for the transfer of free enzymes to immobilized state. A group researchers reported the Vmax of free catalase decreased at 40°C by 33,000 -26300, 24,500μmol (min mg protein)-1 and Km increased by 12.5-25 and 20Mm (Kaushal et al., 2018). also, reported that immobilized catalase after 10 consecutive uses, it maintained about 51% of its initial activity and that it maintained about 65% of its initial activity at 25°C with about twofold decreased in Km which showed that affinity to the substrate was improved after immobilization (Sel et al., 2020).

Thermostability of immobilized cellulase on standard chitosan and different mushroom chitosan beads were 50°C while optimum temperature of free cellulase was 45°C. Similiarly, the same optimization was recorded for *L. deliciousus* which showed its optimum temperature at 60°C for immobilized cellulase and that of free at 40°C. *L. laccata* show its optimum at 50°C for immobilize and 45 for free cellulase. The same trend occurs for *C. cibarius* were its optimum temperature for immobilized cellulase was 50°C and the free cellulase was 35°C. *H erinaecius* chitosan beads recorded its optimum temperature at 50°C for immobilize cellulase and 35°C for free cellulase. The same applies to *P. tuberagium* that records 50°C for optimum temperature of immobilized cellulase and 45°C for free cellulase. This result is supported by the findings of who reported that the optimum temperature of immobilized lipase increased from 35°C for free enzyme to 40°C (Egwim et al., 2012). The result is also supported by who depicted that optimum temperature of both free and immobilized phytase was found at 60°C (Onen et al., 2015). The increased in temperature stability of the immobilized enzyme has suggested that immobilized enzymes has a higher resistance to changes in temperature than free enzyme and showing stability and non sensitivity of the immobilize cellulase. The stability of the enzyme up to 70°C could make it less susceptible to thermal inactivation during industrial processes.

The optimum pH of immobilized cellulase was found to be at 6 for standard chitosan beads and 7 for *l. deliciousus,* 6 for *L. laccata*, and 7 for *C. cibarius*, 7 for *H. erinaecius* and 6 for *P. tuberagium*. Optimum pH of immobilized cellulase was shifted to acidic range from pH 8 which was the optimum pH for free enzyme. The acidic shift in the optimum pH was in agreement with the general observation that the positively charged supports displace pH-activity curve of the enzyme attached to them towards lower pH values (Egwim et al., 2012). The stability of pH in acidic region could be an advantage in fruit processing such as citrus fruits as a result of their acidic nature. Also, the report is in consonance with result of this study (Sel et al., 2020).

The residual activity of immobilized cellulase after 7 days ranged from 52.38-71.79%. The lowest residual activity was recorded for commercial chitosan while the highest residual activity was recorded for *L. deliciousus* while the half ranged from 8 - 232 days. Thus, it would take 8-232 days for immobilize cellulase to attain half of its activity (half-life). These results are similar to the findings of who reported 96% residual activity of immobilized lipase after storage for 90 days (Egwim et al., 2012). Furthermore, the storage stability observed was higher than the previous study of who reported that half life of immobilized pectinase was 30 days under 4°C condition (Li et al., 2002). The higher half-life of immobilized cellulase has wide industrial applications since enzymes could be reused thereby reducing the cost of industrial process. This difference could be as a result of the effect of support use for immobilization on enzyme storage or the nature of the enzyme itself. The half-life indicated possible reusability of the immobilized enzyme while residual activity showed the potency of the immobilized enzymes after each cycle. The storage stability of immobilized enzymes could be due to intramolecular linkage conferred by the carriers (chitosan) and depends on the length of such linkage (Torcilin et al., 1979). It could also be due to the restriction of the enzyme within the carrier.

5. Conclusion

For industrial applications, cellulase immobilized on chitosan beeds can be reused over a long period of time and can withstand harsh industrial conditions (high temperatures and acidic medium). Therefore, study concludes that chitosan from mushroom have high quality and excellent residual activity with half life after 7days with quite resistance to temperatures and Ph. And that it could be use for industrial enzyme immobilization.

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Disclosure Statement

The authors declare that there is no conflict of interest.

Authors Contributions

The authors declare that all data were generated in-house and that no paper mill was used. *This work was carried out in collaboration of all authors.* UAJ and EC conceived and designed the research. UAJ and EC conducted experiments as well as contributed new reagents or analytical tools. UAJ analyzed data. UOH wrote *the protocol, and the first draft of the manuscript*. All authors read and approved the *final* manuscript and all data were generated in-house and that no paper mill was used.

Data Availability Statement

All data generated or analyzed during this study are included in this submitted article. The raw data shall be made available upon request to the corresponding author.

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