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Partial Purification of Cellulase Enzyme from *Fusarioum oxysporium* by Using Egg White Matrix

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Authors' contributions

This work was carried out in collaboration between both authors. Author JS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MK managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: Protein matrix possesses a capacity to bind organic and inorganic matter. Egg white matrix as an adsorbent was utilized for partial purification of cellulase enzyme in detailed batch study.
Methodology: Effect of pH, matrix size, enzyme concentration, contact time and temperature on the binding of cellulase on egg white matrix was studied. Desorption studies was carried with variable pH, sodium chloride and ammonium sulphate system to desorbs the cellulase for the egg white matrix.
Results: Equilibrium isotherms for the adsorption of the cellulase enzyme on egg white matrix were well fitted to Freundlich and Langmuir isotherm models. The adsorption process has been found endothermic in nature and thermodynamic parameters, Gibb's free energy ($\Delta G°$), change in enthalpy ($\Delta H°$) and change in entropy ($\Delta S°$) have been calculated as -13276.21, 2025.47 and 698.64 (KJ mol^{-1}) respectively. Thermodynamic studies revealed that the adsorption process is spontaneous and endothermic in nature. In desorption studies it was concluded that maximum elution (66.8% was obtained for cellulase enzyme with 0.3M $(NH_4)_2SO_4$ with respect to different pH and sodium chloride solution. Cellulase enzyme obtained after desorption process has maximum purification fold of 4.25 with 60% recovery.
Conclusion: The results from the current research suggest that egg white can be used

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for partial purification of cellulase enzyme with less cost.

Keywords: Adsorption; cellulose; isotherm; egg white matrix.

1. INTRODUCTION

Cellulose, a polysaccharide of β- 1,4-linked glucose residues, is a primary product of photosynthesis in terrestrial environment. It is most abundant renewable bioresource produced in biosphere. The annual yield is approximately 1×10^{12} tons [1]. The biological hydrolysis of cellulose to glucose occurs by the action of cellulase enzymes. It involves three type of reactions; first, breakage of non covalent interactions present in amorphous structure of cellulose, second, hydrolysis of chain ends to break the polymer into smaller sugars and third hydrolysis of disaccharides and tetra saccharides into glucose [2,3]. Cellulases are widely distributed in nature and are produced by fungi, bacteria, plants, protists, and invertebrate animals [4,5]. At present, cellulase and related enzymes are used in food, brewery, wine, animal feed, textile, laundry, pulp & paper industries. [6]. Due to industrial applications, cellulase enzyme purification is essential to increase its specific activity. Purification is process of extraction of single enzyme/protein from cells, tissues, etc. which may contain more than 1000 different proteins and lots of other biomolecules. Most purification methods, involves chromatographic techniques, which use matrixes like DEAE-Sephadex, sepharose, silica gel and BioGel P-60. However, these matrixes are very costly and make purification, a costly process. So research was performed to develop economical egg white matrix (EWM), for purification of cellulase produced form *F.oxysporium.*

2. MATERIALS AND METHODS

2.1 Microorganism and Enzyme Production

Cellulase enzyme was obtained from fungal culture of *F. oxysporum* (MTCC, 1755). Potato Sucrose Broth media was used for growth and fungal culture was kept at agitation speed of 150 rpm at 37ºC. The supernatant containing extracellular cellulase was harvested after 68 h cultivation by centrifugation at 10000g for 20 min and stored at 4ºC for further use.

2.2 Cellulase Assay

The cellulase (CMCase) activity was assayed according to Stewart and Leatherwood [7], where appropriately enzyme solution (0.5 ml) was mixed to 0.5 ml CMC (0.5% CMC in phosphate buffer 0.2M, pH 6.0) and incubated at 40ºC for 30 min. In the assay, release of reducing sugars from was determined by Miller [8] method. One international unit (IU) of enzyme activity was defined as the amount of enzyme that catalyzed the liberation of reducing sugar equivalent to 1.0 µM glucose min^{-1} under assay conditions.

2.3 Determination of Protein Concentration

The protein concentration of the crude and purified enzyme fractions was determined by the method of Lowry et al. [9] using bovine serum albumin (BSA) as standard.

2.4 Preparation of EWM

Hen eggs were boiled and white portion of eggs was separated from yellow one and washed with distilled water to remove dirt and other materials, dried at 120-140ºC for overnight and powdered it. The powered material was sieved to particle sizes (200 and 300 µm) and washed thoroughly with ethanol (20% v/v) till leaching of protein stops. The material was then dried at 120-140ºC and cooled to room temperature (22 to 25ºC). Finally the EWM was ready to be used for enzyme purification.

2.5 Adsorption Process Parameters

The adsorption investigations of cellulase on egg white matrix were carried out in batches at different conditions of pH (4, 5 and 6), concentration (1, 2 and 3%), time (10-90 minu) and temperature (20, 27 and 37ºC) to ensure the tendency of enzyme adsorption process. In each experiment 100 ml of enzyme solution of specific concentration (10 IU/ml) was mixed with defined quantity of adsorbent and mixed for different time intervals (min) for saturation. The supernatant was harvested by centrifugation (6000 rpm for 10 min 4ºC), to enumerate enzyme and protein content by standard procedure.

2.6 Adsorption Kinetic

Typically, the mathematical correlation, which has an important role towards the modelling analysis and applicable practice of the adsorption systems are depicted by graphically expression of adsorbate on solid-phase vs. its residual concentration in liquid [10]. Adsorption equilibrium with dynamic balance with the interface concentration is established when an adsorbate has been contacted with the adsorbent for sufficient time [11,12]. Kinetic investigations are carried out to measure the rates of adsorption under various experimental conditions; enzyme concentration, pH, temperature and time on the rates of reaction to attain of equilibrium during the adsorption process by Langmuir [13] and Freundlich [14] adsorption isotherm. In different measuring flasks, 100 ml of enzyme solution (10-15 IU/ml) with definite pH and adsorbent conc. was taken at different temperatures of 20, 27 and 37ºC with periodic shaking. Enzyme and protein adsorbed on EWM was determined by standard procedure. The amount of equilibrium adsorption, q_e (mg/g) was calculated by following equation (1)

$$\mathrm{qe} = \frac{(Co - Ce)V}{W} \quad (1)$$

(where $C0$ and C_e (mg/L) are the liquid-phase concentrations of cellulase enzyme at initial and equilibrium, respectively. V is the volume of the solution and W (g) is the mass of dry sorbent used. Empirical constant (n) and Kf (IU of enzyme/ g of adsorbent) was determined from slope and intercept respectively from the Freundlich plot log q_e vs log q_c. Dimensionless constant, separation factor r was calculated by following equation:

$$r = \frac{1}{1+\mathrm{KaCo}}$$

where, K_a is Langmuir constant and C_o is initial concentration. The value of r was less than one which showed that the adsorption process was favourable. The values of r indicates the nature of the isotherm, if the conditions are r>1, r=1, r<1 and r= 0, the adsorption process is

unfavourable, linear, favourable and irreversible respectively. The thermodynamic data were evaluated from Langmuir isotherms using following equations:

$$\Delta G^{o} = - RT \ln K$$

$$\Delta H^{o} = R \frac{T_2 T_1}{T_2 - T_1} \ln \frac{K_2}{K_1}$$

$$\Delta S^{o} = \frac{\Delta H^{o} - \Delta G^{o}}{T}$$

Where K, K_1 and K_2 are the equilibrium constants obtained from the slopes of adsorption isotherms

3. RESULTS AND DISCUSSION

3.1 Adsorption Studies

Mesh size of EWM 200 µm was more effective for the adsorption of enzyme as compare to 300 µm (Fig.1a.), as the adsorption process is a surface phenomenon, the enzyme binding efficiency of the matrix with size 200 µm registered high efficiency due to larger surface area.

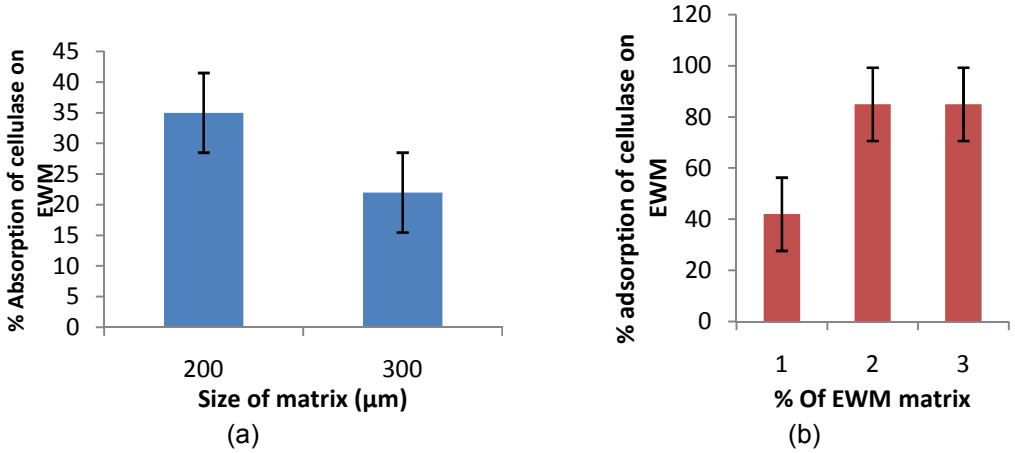
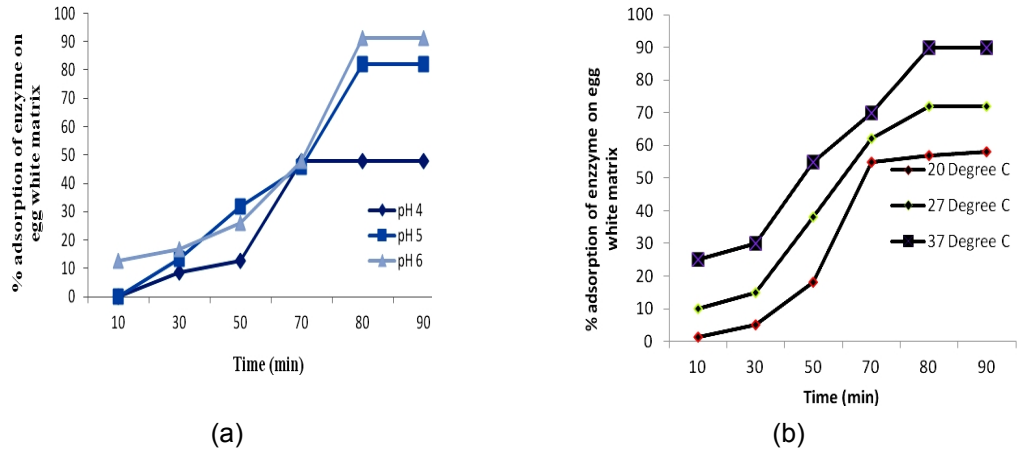


Fig. 1. Effect of (a) size and (b) % of EWM on the adsorption of cellulase enzyme on EWM

Different % of EWM (1, 2 and 3%) was mixed with 100 ml enzyme solution (10 IU/ml phosphate buffer of pH 6) and % enzyme binding was determined. EWM matrix concentration 2 gm/100 ml of enzyme solution (10 IU/ml) was optimum for maximum adsorption of the enzyme cellulase. As concentration of matrix was increased from 1-2 % (Fig. 1b.) % enzyme absorption increases and equilibrium is attained, but higher to this concentration of matrix adsorption remains constant. The adsorption strength of cellulase enzyme on EWM at varying pH (4, 5 and 6.), (Fig. 2a.) illustrate that pH of solution effected the adsorption capacity and it was maximum (91.33%) at pH 6. Increase in pH increases negative charge which lead to a formation of separate protein aggregates [15]. This increases protein - protein interaction. The adsorption of cellulase enzyme (10 IU/ml) was also recorded in the different time intervals (10,30,50,70,80 and 90 min) at a pH 6.0 and temperatures 20, 27 and 37ºC (Fig. 2b.).



(a) (b)

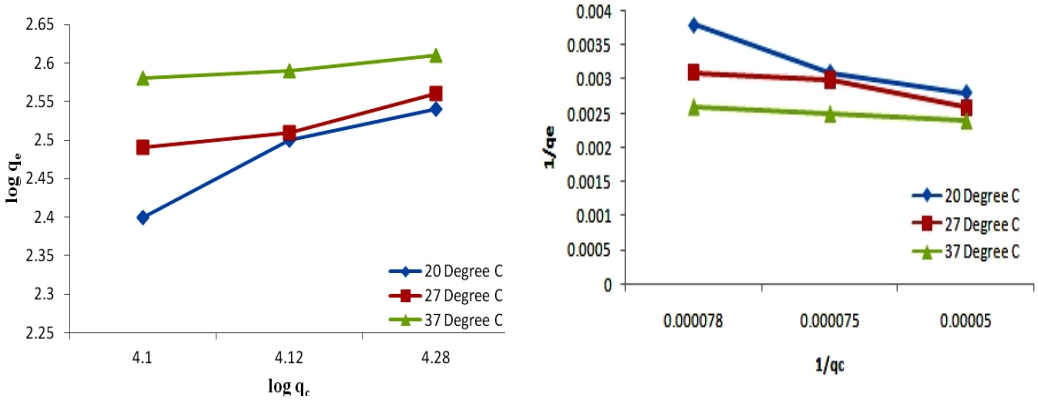
Fig. 2. Effect of (a) pH and (b) temperature on % adsorption of cellulase enzyme on EWM

Results specify that the adsorption of cellulase enzyme increases with increase in temperature, indicating endothermic nature of process. It is also observed that at concentration 10 IU/ml of cellulase adsorption rate is fast but with rise in concentration (above 15 IU/ml) the percentage adsorption gradually remains constant.

3.2 Langmuir and Freundlich Adsorption Isotherms for Cellulase Binding on EWM

The binding profile of cellulase on EWM at different temperature and enzyme concentration was determined by experimentation to obtain Langmuir (1916)[13] and Freundlich (1906)[14] adsorption isotherms. In both the cases linear plots were obtained, which reveal the applicability of these isotherms on the ongoing adsorption process.

Freundlich and Langmuir plots (Fig.3(a) and (b)) respectively for the adsorption of cellulase enzyme on egg white was applied to calculate different Freundlich and Langmuir constants, K_f (1.36, 1.46 and 1.58 IU/mg of matrix) and K_a (233.33, 33.33 and 33.33) at different temperature 20, 27 and 37ºC. This reveals the endothermic nature of the ongoing process. Evaluated thermodynamic parameters, change in free energy (ΔG^o), change in enthalpy (ΔH^o) and change in entropy (ΔS^o) (Table 1) establish the feasibility of adsorption process. Further, the decrease in the values of ΔG^o with the increasing temperature indicates the spontaneity of the process at higher temperatures. The endothermic nature was also confirmed from the positive values of enthalpy change (ΔH^o), while good affinity of cellulase enzyme towards the adsorbent materials is revealed by the positive value of ΔS^o.



(a) (b)

Fig. 3. (a) Freundlich and (b) Langmuir adsorption isotherm for cellulase on EWM at different temperature

Table 1. Thermodynamic parameters for the adsorption of cellulase on EWM

Thermodynamic parameters	Values
1. ΔG^{o} (KJ mol^{-1}) at	
20 ^{o}C	-13276.21
27 ^{o}C	-13593.39
37 ^{o}C	-14046.50
2. ΔH^{o} (KJ mol^{-1})	2025.47
3. ΔS^{o} (JK^{-1} mol^{-1})	698.64

3.3 Thermodynamic Equilibrium Constant (Kºc)

Thermodynamic equilibrium constant for adsorption of cellulase enzyme on EWM was calculated as following-

$$K^{o}_{C} = C_a / C_e$$

Here C_a was concentration of enzyme on adsorbent at equilibrium and C_e is the concentration of enzyme in solution at equilibrium. Thermodynamic equilibrium constants for cellulase enzyme on EWM at different temperature were calculated. It was observed that Kºc was 0.05, 0.09 and 0.185 at temperature 20, 27 and 37ºC. Thermodynamic equilibrium constants for cellulase enzyme on egg white matrix at different pH were also calculated and it was 0.05, 0.09 and 0.2 at pH 4, 5 and 6 respectively. Higher Kºc reveal the higher adsorption of enzyme concentration on EWM at equilibrium and less concentration left in solution at 37ºC.

3.4 Desorption of Cellulase from EWM

For economical adsorption process, it is necessary to regenerate adsorbent, therefore, desorption test on cellulase bound EWM were carried out with buffer solution (pH 4, 5 and 7) sodium chloride & ammonium sulphate. It was concluded that maximum elution (66.8% was obtained for cellulase enzyme with 0.3M $(NH_4)_2SO_4$ (Table 2). It has given maximum purification fold of 4.25 with 60% recovery. The reversibility of adsorption depends on binding bond such as ionic or covalent bonding or weak binding forces such as Van der

Waals' forces or a dipole–dipole interaction formed between the adsorbent surface and the cellulase enzyme. Therefore, different surface characteristics would help to explain the reversibility of adsorption

Table 2. Effect of Elution system on % elution of cellulase enzyme from EWM

Elution system	Value of elution system	Elution (%) of enzyme from matrix
pH	5.5	20
	6.5	15
	7.0	15
NaCl (M)	0.1	7.5
	0.2	10.5
	0.3	16.5
	0.4	16.7
	0.5	14.0
$(NH_4)_2SO_4$ (M)	0.1	40
	0.2	44
	0.3	66.8

4. CONCLUSION

The results predicted from the current research suggest that EWM can be used to adsorb cellulase enzyme for partial purification. The operational parameters i.e. size of matrix, (200m) pH (6), temperature (37ºC), contact time (80 min) and enzyme concentration (15 IU/ml) was effective for maximum adsorption (91.33%) of enzyme on egg white matrix. The adsorption data was satisfactorily explained by Freundlich and Langmuir isotherm. 4.25 purification fold and 60% recovery was obtained after desorption of enzyme from EWM with ammonium sulphate. Results of Freundlich and Langmuir isotherm explain the binding capacity of EWM for cellulase enzyme. Thus, this cheap biological matrix can be explored at large scale for enzyme purification. This will reduce purification cost of process.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Percival ZYH, Himmel ME, Mielenz JR. Outlook for cellulose improvement: screening and selection strategies. Biotechnol Advances. 2006;24:452-481.
2. Mosier NS, Hall P, Ladisch CM, Ladisch MR. Reaction kinetics, molecular action and mechanisms of cellulolytic proteins. Adv Biochem Eng Biotechnol. 1999;65:23-40.
3. Wilson DB, Irwin DC. Genetics and properties of cellulases. Adv Biochem Eng Biotechnol. 1999;65:1-21.
4. Watanabe H, Tokuda G. Animal cellulases. Cell Mol Life Sci. 2001;58:1167-1178.
5. Davison A, Blaxter M. Ancient origin of glycosyl hydrolase family 9 cellulase genes. Mol Bio Evol. 2005;22:1273-1284.
6. Bhat MK. Cellulase and related enzymes in biotechnology. Biotechnol Advances. 2000;18(5):355- 83.

7. Stewart B, Leatherwood J. Derepressed synthesis of cellulase by *Cellulomonas*. J. Bacteriol.1976;128:609–61
8. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959;31:426–8.
9. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951;193:265-275.
10. Ncibi MC. Applicability of some statistical tools to predict optimum adsorption isotherm after linear and non-linear regression analysis. J. Hazard. Mater.2008;153:207–212.
11. Ghiaci, AM, Abbaspur, R, Kia F, Seyedeyn A. Equilibrium isotherm studies for the sorption of benzene, toluene, and phenol onto organo-zeolites and as-synthesized MCM-41, Sep. Purif. Technol. 2004;40:217–229.
12. Kumar KV, Sivanesan S. Sorption isotherm for safranin onto rice husk: comparison of linear and non-linear methods. Dyes Pigments. 2007;72:130–133
13. Langmuir I. The constitution and fundamental properties of solids and liquids. J. Am. Chem. Soc. 1916;38(11):2221–2295.
14. Freundlich, HMF. Over the adsorption in solution, J. Phys. Chem. 1906;57:385–471.
15. Vasbinde AJ, Kruif CG. Casein–whey protein interactions in heated milk: the influence of pH. International Dairy J. 2003;13(8):669–677.

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