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Immobilization kinetics of lipase on mesoporous material (Santa Barbara Amorphous-15)

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Abstract : The present study focuses on the immobilization kinetic of *Aspergillus niger* lipase on SBA-15 (Santa Barbara Amorphous-15) obtained from water glass. Freundlich and Langmuir isotherms were used to describe equilibrium nature of immobilization. Lipase immobilization was dependent on the pH of solution and the lipase uptake was greater at pH 6-7. The maximum adsorption capacity for SBA-15 calculated from the Langmuir equations reaches to 35.6 mg g^{-1}. The kinetics of adsorption was examined using pseudo first-order and pseudo second-order. The adsorption of lipase on SBA-15 followed pseudo second-order kinetics.
Keywords : SBA-15, Immobilization, Lipase, Isotherms.

1. Introduction

Mesoporous materials has various applications such as catalysis, drug delivery, chromatography etc., The mesoporous silica material serves as a potential host for enzyme immobilization due to their high specific surface and high thermal stability[1,2]. Immobilization of enzyme to the mesoporous material facilitate them to be used for several cycles of operations. Immobilization reduces the enzyme cost and reduces the cost of separation of pure enzyme from the processes. The objective of the current study is to investigate the kinetic and equilibrium data of immobilization studies were processed to identify with the immobilization mechanism of the lipase on SBA-15.

2. Materials and Methods

The water glass obtained from Kiran Global Chems Limited, Tamilnadu was used as the silica precursor. Hydrochloric acid (98%, Merck) were obtained from Merck Chemicals Pvt. Ltd. Plutonic P123 (99%, Aldrich) purchased from Sigma Aldrich.

2.1 Synthesis of SBA-15

Pluronics P123 (PEO_{20} PPO_{70} PEO_{20}) was used as a template for the synthesis of SBA-15. 5g of Pluronics was dissolved in 190 g 2N HCl. 15 mL of silica source (sodium silicate) was added. The silica deposited over the micelle was aged in Teflon coated stainless steel container at 80ºC for 16 hr. the precipitated white solids was washed with 500 ml of deionised water and room dried for 24 hr. The template was removed by calcinations at 500ºC for 5 hr[3].

2.2 Lipase immobilization and Lipase activity

0.05 mg of SBA-15 was added to pH 7 phosphate buffer solution containing 0.5 mg/ml lipase and kept for 12 h at 30°C. The immobilized matrix was separated and the percentage immobilization and specific enzyme activity were determined. The enzyme concentration was measured by Lowry method[4]. The enzyme entrapped on support was calculated by following equation (Eq.1).

$$C_E = C_0 - C_t \quad (1)$$

Where:

Then, the enzyme loading percentage can be calculated by equation (Eq.2)

$$\%immobiliza\ tion = \frac{C_E}{C_0} \times 100\% \quad (2)$$

C_E = the concentration of the immobilized enzyme (g/mL)

where C_o is the initial concentration of lipase in the buffer solution (mg/L);

C_t is the concentration remaining in solution at time't' (mg/L);

The specify enzyme activity of the lipase immobilized on mesoporous material was determined by Olive oil emulsion method[5].

2.3 Isoelectric point and PZC measurements

Percentage immobilization of lipase on SBA-15 explained by zero point charge (pH_{zpc}) of SBA-15 and iso electric point of lipase (pI) [6]. The lipase molecule has a positive charge at a pH below the pI and it is negatively charged at a pH above the pI.

0.1 g of SBA-15 was added to 50 ml of 0.1 M NaCl solution. The mixture was left to equilibrate for 24 h. The final pH of the solution was noted. The same procedure was repeated for different initial solutions of varying pH. The difference in solution pH ($\Delta pH = pH_i - pH_f$) was measured and plotted against initial pH. The pH at which ΔpH becomes zero is called pH_{pzc}.

3. Characterization

The external morphology of the mesoporous material was analyzed by Scanning Electron Microscope

4. Results and Discussion

4.1 Characterization

SEM image of SBA-15 (Fig 1) shows that the particles consist of rod like morphology with diameter and length of 0.3 and 0.7 μm approximately[7].

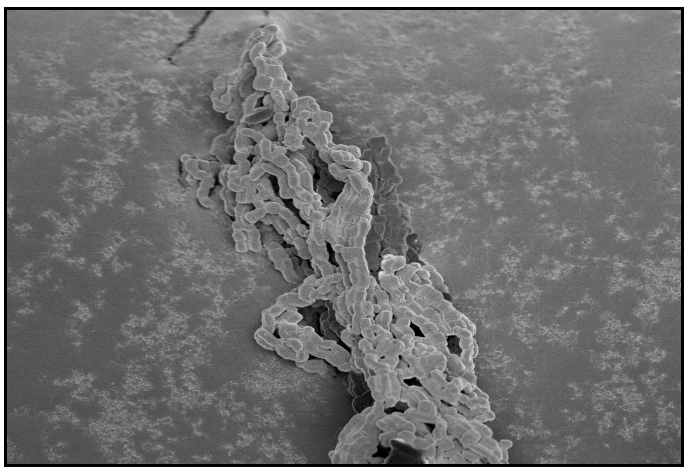

Figure 1: SEM image of SBA-15.

4.2 Effect of pH

Effect of pH on immobilization of lipase on SBA-15 was studied by conducting batch experiments by varying solution pH between 3 and 9. pH of the buffer solution was altered between pH 3 and pH 6 using dilute phosphoric acid and between pH 7 and pH 9 using potassium hydroxide. After adjusting the solution pH to the desired value, 0.1 g of lipase was added to the buffer and the mixture was placed in horizontal shaker at 100 oscillations/min at 35 ºC for 24 h [8].

Fig. 2 shows the effect of pH on the percentage immobilization and specific enzyme activity is shown in the. Adsorption capacity increases from pH 2 to pH 7 and decreases further. Therefore, further experiments were conducted at solution pH 7 for buffer solution.

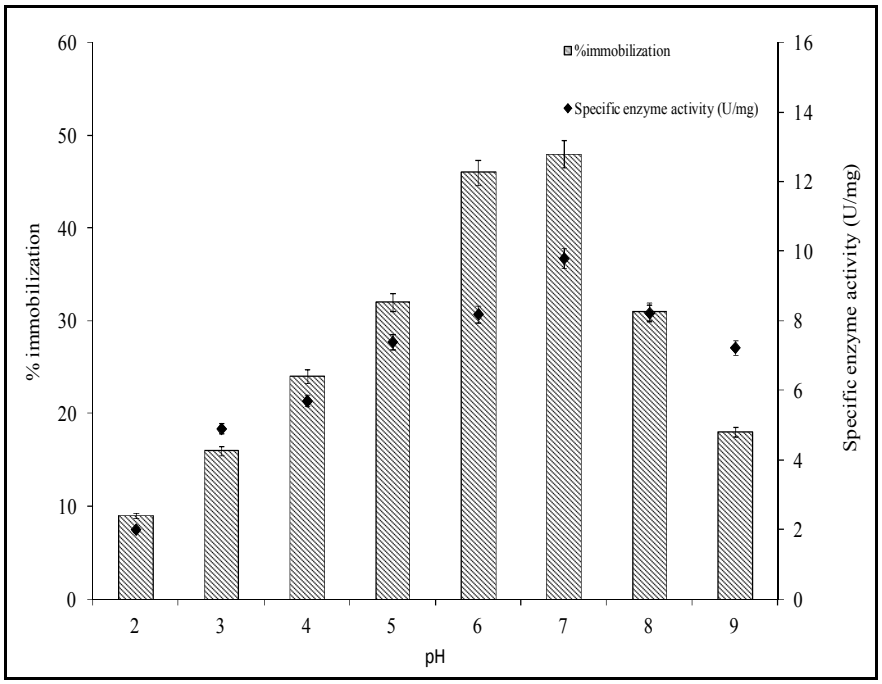

Figure 2: The effect of pH on the percentage immobilization and specific enzyme activity of lipase on SBA-15.

This experiment was repeated for SBA-15 to determine iso-electric point of lipase molecule. From the Fig. 4 the zero point charge (pH_{zpc}=3.7) of SBA-15 and iso-electric point of lipase (pI≈ 6.8) was obtained. A good interaction was observed between the enzyme and silica at a pH between the isoelectric point (pI) of enzyme and the zero point charge of silica (pzc). At a pH below pI (6.8) of lipase, the protein became positively charged. If the pH was above the isoelectric point of silica surface, the surface becomes negatively charged [9].

The coulombic repulsive force between the molecules can be minimised if the pH of the buffer solution is maintained near iso-electric point [11]. Reduction in the repulsion forces enhances percentage immobilization by bringing the lipase molecules closer to each other. When the pH of the buffer solution is maintained above the pI of lipase the molecules will become negatively charged and the repulsion between SBA-15 and lipase does not favour adsorption. Thus the pH of the buffer solution is maintained above pI of lipase [12].

pH of the buffer solution has a major role in varying the electrostatic charge of polar group in the lipase. pH of the solution alters the charge of the polar group which is responsible for the enzyme activity. pH memory state of lipase is the charge retained by the lipase during the hydrolysis is the same charge maintained in the buffer solution. The effect of pH on activity of lipase is shown in the Figure 6. The highest enzymatic activity was observed near the isoelectric point (pI 6.8)**.** Increase in pH beyond optimum caused a rapid decrease in the enzyme activity.

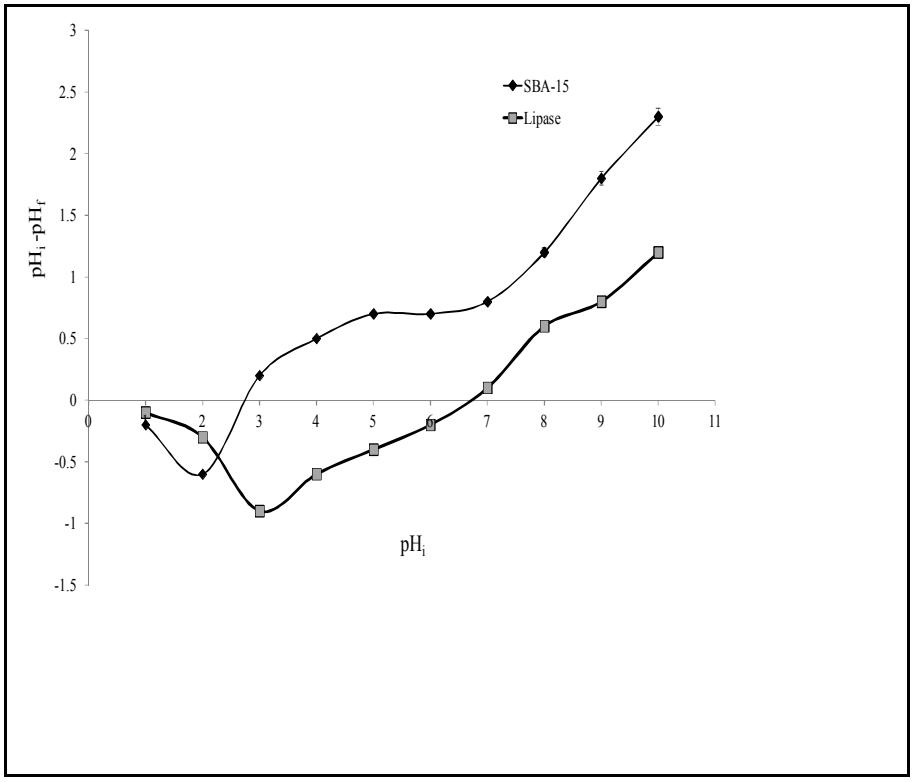

Figure 3 1. Isoelectric point of lipase (pI) and Zero point charge (zpc) of SBA-15

4.3 Adsorption isotherm

The concentration of lipase was varied from 0.2 to 4.5 mg/L with SBA-15 concentration of 0.05 g/mL. Equilibrium data were analyzed for both Langmuir and Freundlich isotherms[13]. The non-linear regression analysis was used to obtain the equilibrium parameters. Results are illustrated in Table 1.

Sorption isotherms are presented in Figure 5. This particular equilibrium data fit Langmuir isotherm well and does not fit Freundlich isotherm equation[14].

Table 1: The equilibrium parameters were obtained by non-linear regression analysis.

Langmuir isotherm			**Freundlich isotherm**		
K_L (dm^3/mg)	1.3845		K_F (mg/g)(dm^3/ mg)$^{1/n}$	14.37	
q_m (mg/g)	35.587		N	1.348	
R^2	98.14		R^2	95.52	

Numerous models describe the kinetics of Adsorption. The pseudo first-order model of Lagergren and pseudo second-order kinetic models had been utilized widely to describe the kinetics of adsorption.

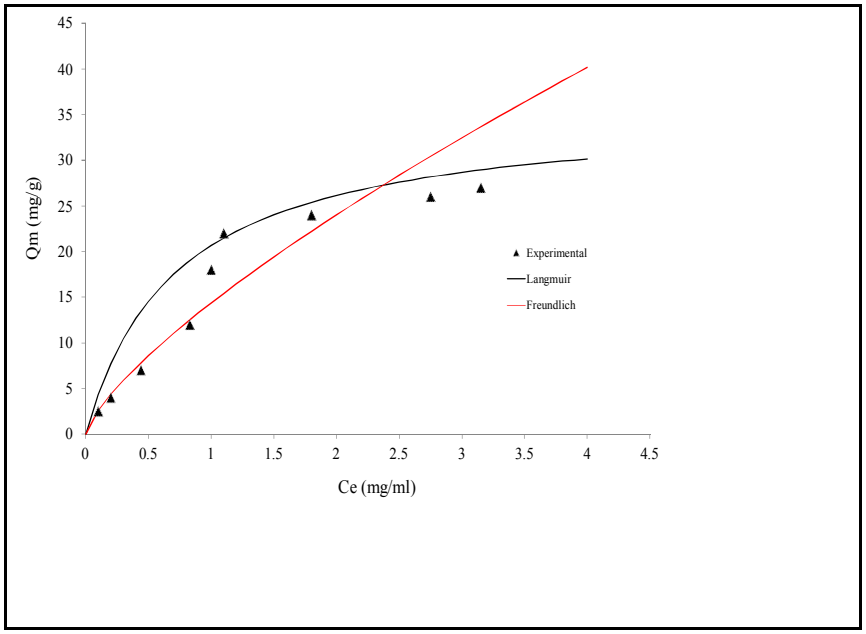

Figure 5: Equilibrium data isotherm curves.

The Lagergren Pseudo first order was found to non- linear in nature the rate constant found from the slopes of best-fit points (correlation coefficient R = 0.9154) is found to be $K_{ad} = 0.2317\ h^{-1}$.

Figure 6 shows that immobilization of lipase on SBA-15 is chemisorption controlling[13]. The second-order rate constant was found to be 0.0378.

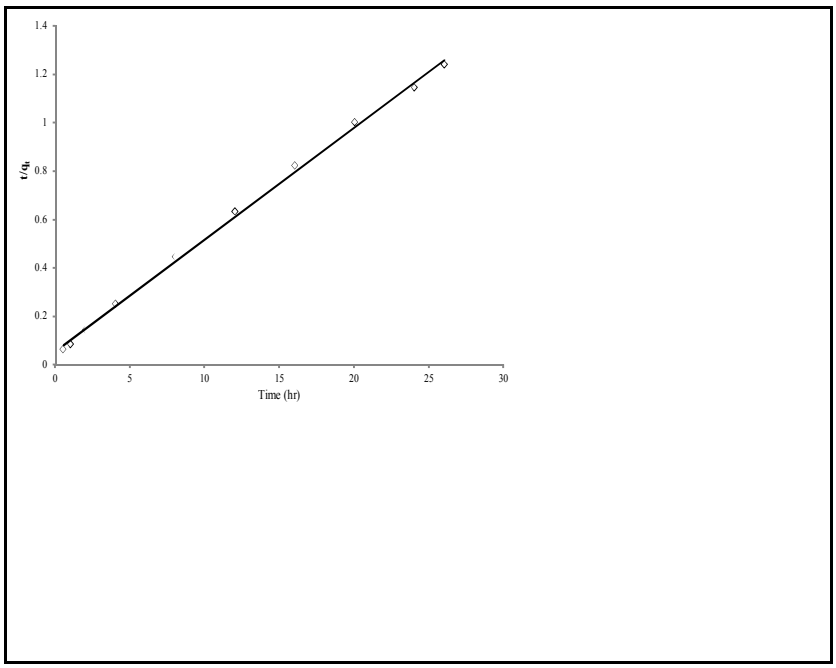

Figure 6: Pseudo second order plots of adsorption of lipase on SBA-15.

5. Conclusion

The present work immobilization of lipase on SBA-15 was investigated. The lipase immobilization capacity of the SBA-15 was quantitatively evaluated by Langmuir isotherm model. The kinetics of the process was predicted by pseudo second order model.

6. References

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