Jurnal Teknologi

Full Paper

α-CHYMOTRYPSIN IMMOBILIZED ENZYME: PHYSICAL, ACTIVITY & STABILITY PROPERTIES

Mohd Hakimi Nazir, Norzita Ngadi*

Department of Chemical Engineering, Faculty of Chemical Engineering Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia

Article history
Received
6 November 2014
Received in revised form
18 March 2015
Accepted
1 August 2015

*Corresponding author
norzita@cheme.utm.my

Abstract

In this study, a-chymotrypsin enzyme was used as a substrate while micropore Y-zeolite, which is HY, USY and NaY as a support. The purpose of this study was to compare the physical properties of zeolite and immobilization of enzyme with zeolite. The characteristics such as BET surface area, isotherm adsorption, BJH adsorption, pore size, t-plot and pore volume have been studied. Furthermore, a comparison has been conducted between immobilized and mobile enzyme for their ability to adsorb hydrolysate at λ=410 nm. The stability of the immobilized enzyme was also determined by varying the parameters of phosphate and tris-chloride buffer and loading of sample solution. Based on the result obtained, HY zeolite has the best physical properties compared to USY and NaY zeolite. Besides that, immobilized enzyme gave higher hydrolysate adsorption activity than the free enzyme. Stability results showed that pH of phosphate and tris-chloride buffer and amount of sample solution play an important role in obtaining the stable immobilized enzyme.

Keywords: Y zeolite, immobilized, enzyme activity, adsorption, *a*-chymotrypsin

Abstrak

Dalam kajian ini, enzim a-kimotripsin telah digunakan sebagai substrat sementara liang mikro Y-zeolite, iaitu HY, USY dan NaY sebagai penyokong. Tujuan kajian ini adalah untuk membandingkan ciri-ciri fizikal zeolite dan juga penyekat gerakan enzim dengan zeolit. Ciri-ciri fizikal seperti BET luas permukaan, isotem penjerapan, penjerapan BJH, saiz liang, t-plot dan isipadu liang dikaji. Tambahan itu, pembandingan telah dijalankan antara enzim dan mobil enzim untuk kemampuan menjerap hydrolisat pada λ=410 nm. Kestabilan penyekat gerakan enzim telah ditentukan dengan mengubah parameter buffer phosphate dan tris-klorida dan dos sampel. Daripada keputusan yang diperolehi, HY zeolit mempunyai ciri-ciri fizikal yang lebih baik berbanding USY dan NaY zeolit. Selain itu, penyekat gerakan enzim memberikan penjerapan hydrolisat yang tinggi berbanding mobil enzim. Keputusan kestabilan menunjukkan bahawa pH buffer phosphate dan tris-klorida dan dos sampel memainkan peranan penting untuk mendapatkan penyekatan gerakan enzim yang stabil.

Kata kunci: Y zeolite, bergerak, aktiviti enzim, penjerapan, *a*-chymotrypsin

© 2015 Penerbit UTM Press. All rights reserved

1.0 INTRODUCTION

There are growing interest in the use of biocatalyst in organic media, where hydrolytic enzymes can catalyze various synthetic reactions such as esterification, transesterification, peptides synthesize, etc. [1]. However, enzymatic catalysis in organic media has disadvantage that enzymes are often inactivated by the organic solvent. One of the main purposes of biotechnology is obtaining a stable biocatalyst in organic solvent system. It is because organic solvent molecules tend to displace water from the hydration shell of proteins and this destruction is one of the main reasons of the denaturation of proteins by organic solvents. Possibilities of enzyme stabilization might be obtained from the modification and immobilization of enzyme.

The support used for immobilizing enzyme should possess mechanical strength, microbial resistances, thermostability, chemical durability, chemical functionality, low cost, hydrophylicity, regenerability and a high capacity of enzyme. The various

immobilization protocols used with enzymes have been extensively studied. Immobilization of enzyme involves four different methods: adsorption of enzyme on a carrier, entrapment of the enzyme within an insoluble gel matrix, containment of enzyme within porous hollow fibers or microcapsules, binding of the enzyme to dry mycelia and ion exchange between the enzyme and support [2]. All the methods have their strength and weakness. However, immobilization of enzyme through physical method is still the most favorable and commonly used because it is the easiest to perform and least expensive. In this method, the forces between a support and the enzymes include hydrogen bonding, Van der Waals forces and hydrophobic interactions.

There are varieties of commercially available supports such as polymer resin, zeolites and hydrotalcites that can be used for the immobilization of enzyme. In this study, zeolite from family Y was used as a support. Zeolites have attracted much attention in recent years due to their unique structural characteristic and resistant to biodegradation. Furthermore, it possess novel properties such as high surface areas, hydrophobic or hydrophilic behavior and electrostatic interactions as well as it can be readily prepared with cavities ranging from micropore (<20 Å) to mesopore (20-500 Å) according to the control of preparation technology [3].

In this study, three different zeolites from family Y, namely zeolite HY, USY and NaY were used as a support to immobilize a-chymotrypsin enzyme using adsorption method. The physical properties of zeolites before and after immobilization process were investigated. The activity and stability of immobilized enzyme on adsorption of hydrolysate were also investigated and compared to the free enzyme.

2.0 EXPERIMENTAL

2.1 Preparation for the Analysis of Physical Properties of Catalysis in organic Zeolite

The analysis of the physical properties of zeolite were carried out using micrometritis ASAP 2010. The analysis was carried out at temperature of 77.35 K, vacuum pressure, 400 μmHg and degass temperature at ambient temperature [4].

2.2 Preparation of Immobilized *a-Chymotrypsin* on Different of Zeolite

First, a-Chymotrypsin was dissolved in phosphate buffer (4ml, pH 7.95, 50 Mm), then zeolite (200mg) was added and the solution was stirred at room temperature for 1 hour. After that, the pH value of the mixture was measured by pH meter and the suspension was lyophilized overnight to obtain the zeolite immobilized a-Chymotrypsin [3].

2.3 Preparation for the Measurement of Activity of Free and Immobilized Enzyme

The activity of enzyme was measured by N-Glutaril-L-phenylalanine-P-nitroanilida (GPNA) [5]. The substrate solutions were prepared by dissolving 20 mg GPNA into 1 ml ethanol and then evaporated [6]. Subsequently, this solution was diluted into 50 ml by using 0.05M buffer tris-chlorida solution with pH value at 7.6 and contains 0.02 M calcium chloride. Finally, the solution was stabilized for several minutes.

In order to measure the enzyme activity, the substrate solution were pipetted into a closed cylinder and then were put into a water bath at temperature of 25 ± 0.05 °C. Then the solution containing enzyme and zeolite was injected to the cylinder and were mixed for 30 minutes. Before the reaction was stopped, 1 ml of 30% acetic acid was added. Meanwhile for pure enzyme activity, the procedure was done as above except no zeolite was added.

After 30 minutes, the solution was injected into cuvette and the analysis was done using IR Spectroscopy. The activity of the enzyme was measured based on the adsorption of hydrolysate at wavelength 410 nm [7].

3.0 RESULTS AND DISCUSSION

3.1 Physical Properties of Pure Zeolite Y and Combination of Zeolite Y with *a*-Chymotrypsin

Tables 1 and 2 show the physical properties of zeolite before and after immobilization, respectively. According to Table 1, HY zeolite had better physical properties compared to zeolite USY and NaY since it has high BET value and Langmuir surface area. Furthermore, it has more microporous structure, pore volume and microporous adsorption diameter pores, which make HY zeolite more prominent to be used as catalyst in adsorption process.

Table 1 Zeolite physical properties before immobilization

Physical properties of Zeolites	Zeolites HY	Zeolites USY	Zeolites NaY
BET surface area (m^2/g)	602.1663	534.6643	440.4233
Langmuir surface area (m^2/g)	794.3522	705.5929	581.1638
Micro porous area (m^2/g)	557.9317	478.1031	405.2235
Adsorption of BJH surface area(m^2/g)	26.5247	49.4990	17.5269
Desorption of BJH surface area (m^2/g)	36.3282	60.0144	17.2682
Total pore volume(cm^3/g)	0.3134	0.2975	0.2150
Adsorption of BJH volume (cm^3/g)	0.0675	0.0913	0.0299
Desorption of BJH volume (cm^3/g)	0.0726	0.0962	0.0288
Micro porous volume (cm^3/g)	0.2600	0.2228	0.1888
Average pore diameter for BJH adsorption(cm^3/g)	80.9356	71.7260	85.1912
Average pore diameter for BJH desorption((cm^3/g)	67.1898	61.3918	88.6419

Table 2 Zeolite physical properties after immobilization

Physical properties of Zeolites	Zeolites HY	Zeolites USY	Zeolites NaY
BET surface area (m^2/g)	441.5481	355.9790	287.2837
Langmuir surface area (m^2/g)	582.9665	470.5617	379.5596
Micro porous area (m^2/g)	405.6789	317.8117	262.2482
Adsorption of BJH surface area(m^2/g)	22.7498	38.1673	12.6572
Desorption of BJH surface area (m^2/g)	29.6332	30.2892	11.0905
Total pore volume(cm^3/g)	0.2225	0.1917	0.1422
Adsorption of BJH volume (cm^3/g)	0.0460	0.0543	0.0270
Desorption of BJH volume (cm^3/g)	0.0498	0.0559	0.0246
Micro porous volume (cm^3/g)	0.1892	0.1483	0.1223
Average pore diameter for BJH adsorption(cm^3/g)	80.9356	71.7260	85.1912
Average pore diameter for BJH desorption((cm^3/g)	67.1898	61.3918	88.6419

From the data, it was found that the immobilization process causes a decrease in zeolite physical properties. As shown in Table 2, the BET surface area of zeolite HY before immobilized was recorded to be 602.1663 m^2/g. When the zeolite was subjected to immobilization process, the BET surface area was decreased to 441.5481 m^2/g. The same trend can also be observed for zeolites USY and zeolite NaY, whereby the BET surface area decreases from 534.6643 to 355.9790 m^2/g and from 440.4233 to 287.2837 m^2/g, respectively. The reduction occurred might be due to the entrapment of enzyme in zeolite matrix.

3.2 Activity of Free and Immobilized Enzyme a-Chymotrypsin: Effect of Temperature

In this study, the activity of enzyme was measured by its ability to adsorb hydrolysate at wavelength, λ of 410 nm. Table 3 shows the hydrolysate adsorption activity between free a-chymotrypsin enzyme and immobilized a-chymotrypsin enzyme with HY, USY and NaY zeolites. It can be observed that the free a-chymotrypsin enzyme gave the lowest adsorption activity, at 0.02215 Å compared to immobilized enzyme as shown in Table 3. Meanwhile, immobilization with HY zeolite gave the highest hydrolysate adsorption activity at 1.7803Å followed by USY and NaY zeolite at 1.1986Å and 1.0965Å, respectively. These results indicate that the immobilization of enzyme with zeolite Y was able to

enhance the enzyme activity in adsorption of hydrolysate.

Table 3 Adsorption Activity of Free and Immobilized Enzyme

Sample	Adsorption Activity (Å)
Free Enzyme	0.02215
Enzyme +Zeolite HY	1.7803
Enzyme + Zeolite NaY	1.0965
Enzyme + zeolite USY	1.1986

3.3 Stability of Immobilized Enzyme *a*-Chymotrypsin with Zeolite HY

The stability of immobilized enzyme was determined by varying the parameters such as pH phosphate buffer, pH tris-chloride buffer and loading of sample solution. The experiment had been carried out using zeolite HY only since this catalyst offered the best physical properties and adsorption activities. Tables 4, 5 and 6 show the adsorption activity of immobilized enzyme with zeolite HY after varying the pH phosphate buffer, pH tris-chloride buffer and loading of sample solution, respectively.

Table 4 Adsorption Activity of Immobilized Enzyme with Different pH Phosphate Buffer

pH value	Adsorption Activity (Å)
5	-0.0887
6	0.0015
7.95	1.7803
8	-0.0934
9	0.0002

Table 5 Adsorption Activity of Immobilized Enzyme with Different pH Tris-Chloride Buffer

pH value	Adsorption Activity (Å)
6	-
7.6	1.7803
8	-0.1967
9	-0.1533

Table 6 Adsorption Activity of Immobilized Enzyme with Different Sample Solution Loading

Sample solution (mL)	Adsorption Activity (Å)
0.2	-0.6233
0.4	-0.3766
0.5	1.7803
0.6	-0.2213
0.8	-0.2003

As seen from Table 4, the adsorption of hydrolysate increased as pH increased, from - 0.0887 Å to almost 1.8 Å at pH 5 to 7.95, respectively. However, the increasing of pH into more alkali resulted in decrement in adsorption activity as shown by pH 8.0 and 9.0. Overall, adsorption of hydrolysate is low at condition where very acidic or alkali [8].

Meanwhile, by varying the pH value in tris-chloride buffer also influence significantly to the adsorption activity as shown in Table 5. Increasing the pH into 8 and 9 for example, has reduced significantly the adsorption activity of the sample. There are no data on pH 6, because the graph obtained gives very different in trend and it is suggested that immobilization process did not suitable using tris-chloride buffer in acidic condition.

Amount of sample being loaded also obviously affected the adsorption activity. Reducing the sample solution from 0.5 ml to 0.4 ml and 0.2 ml resulted in reduction of hydrolysate adsorption activity as shown in Table 6. Addition of sample solution to 0.6 ml and 0.8 ml also reduced the adsorption activity.

From the results, it is showed that the pH of phosphate and tris-chloride buffer and amount of sample solution play an important roles in obtaining the stable immobilized enzyme.

3.0 CONCLUSION

It can be concluded that HY zeolite has the best physical properties compared to either USY or NaY zeolite to immobilize the *a*-chymotrypsin enzyme. Moreover, immobilization of *a*-chymotrypsin with HY zeolite gave the highest enzyme activity in adsorption of hydrolysate. In overall, immobilization of *a*-chymotrypsin with zeolite Y gave better adsorption activity compared to free enzyme. The stability of immobilized enzyme was affected by the pH of phosphate and tris-chloride buffer and amount of buffer solution.

Acknowledgement

The authors would like to extend their sincere gratitude to the Ministry of Higher Education Malaysia (MOHE) for the financial supports received under University Grant (Vote no. Q.J130000.2544.08H05) and Fundamental Research Grant Scheme (Vote no. R.J130000.7844.4F489).

References

[1] Erlanger, B. F., Cooper, A. G. and Bendich, A. J. 1964. *Protein Extraction Using Reverse Micelles: System Parameters and Mass Transfer Studies*. Ph.D Thesis, University of London.

[2] Dumitriu, E., Francesco, S., Joel, P. and Ioana, F. 2003. Preparation and Properties of Lipase Immobilized on MCM-36 Support. *Journal of Molecular Catalysis B: Enzymatic*. 22: 119-133.

[3] He, F., Zhuo, R. X., Liu, L. J. and Jin, D. B. 2002. Immobilized Lipase on Porous Silica Beads: Preparation and Application for Enzymatic Ring-Opening Polymerization of Cyclic Phosphate. *Reactive and Functional Polymers*. 1: 81-85.

[4] Flank, H. W. and Jr, W. E. T. 1998. Perspectives In Molecular Sieve Science, Washington, DC. *American Chemical Society*. 1: 542-554.

[5] Sara, M., Pum, D., Messner, P. and Sleytr, B. U. 1993. *Immobilized Macromolecules: Application Potentials*. London: Springer-Verlag. 161-166.

[6] Freeman, S. K., Lee, S. S., Kiserow, J. D. and Megrawn, B. L. 1998. Increased Chymotrypsin activity in AOT/ Bile Salt Reversed Micelles. *Journal of Colloid and Interface Science*. 207: 334-348.

[7] Guo, W. X., Xuan, W. L., Gui, L.T. and Yun, H. Y. 2000. Enzymatic Peptide synthesis In Organic solvent with Different Zeolite as Immobilization Matrixes. *Tetrahedron*. 56: 3517-3522.

[8] Murakami, Y., Hoshi, R. and Hirata, A. 2003. Characterization of Polymer Enzyme Complex as a Novel Biocatalyst for Nonaqueous Enzymology. *Journal of Molecular Catalysis B: Enzymatic*. 22: 79-88.