Synthesis of Aldehyde-Silica Nanoparticle for Matrix Immobilization of Endo-\(\beta\)-1,4-D-xylanase

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Abstract
Synthesis of silica nanoparticles has been conducted by sol gel method using Tetraethylorthosilicate (TEOS) as precursor. Silica nanoparticle was prepared by varying the molar ratio of NH\(_3\) and TEOS i.e. 0.03, 0.06 and 0.12. Mixture of TEOS, ethanol, distilled water and NH\(_3\) as catalyst was stirred at temperature of 50 ± 2º C for 5 hours to produce sol. The sol was allowed to form gel for 48 hours. Gel of silica was kept at temperature of 70 ºC to evaporate the solvent resulting in white powder of silica followed by calcination at 400ºC for 8 hours. Characterization by means SEM revealed that the silica particle size was governed by the molar ratio of NH\(_3\) and TEOS. Higher NH\(_3\) concentration gave a bigger particle size i.e. 88.48, 271.31 and 473.52 nm respectively. Modification of the silica surface with glutaraldehyde was aimed to make the silica as immobilization matrix of endo-\(\beta\)-1,4-D-xylanase. Percentage of immobilized protein enzyme on silica 88.48, 271.31 and 473.52 nm were 30.49\%, 15.05\%, and 10.56\% respectively.

Keywords: xylanase, immobilization, aldehyde-silica nanoparticle

1. Introduction
Silica nanoparticle/silica in nano scale (10\(^{-9}\) m) recently has more industrial application. It has good stability and chemical inertness.[1] Nanomaterial such as silica nanoparticle as a raw material results in product with different properties and quality improvement as well.[2] One of silica nanoparticle utilization is as a matrix in enzyme immobilization process. Silica nanoparticle is a good matrix since it has large surface area and pore volume, uniform pore size, easiness for chemical modification, reusability and environment friendliness.[3]

Silica nanoparticle is usually synthesized by sol-gel, reverse micro emulsion ad solid state. Sol-gel is widely employed in producing pure silica as it can control the particle size, size distribution and morphology by systematic controlling the reaction parameters.[4] Sol-gel is a method of solid preparation at low temperature by involving transition of a system of microscopic particles dispersed in a liquid (sol) to a microscopic material containing liquid (gel) and result in hard material like glass as evaporated. Material obtained by this method is amorphous with not uniform pores. It is usually involving hydrolysis and condensation process.[5]

Enzyme endo-1,4 xylanase is an enzyme with ability to hydrolyze xylan producing oligosaccharide such as XOS which beneficial in health. The use of this enzyme in free form is not efficient as it cannot be reused. Hence it is observed to immobilized endo-\(\beta\) -1,4-D-xylanase onto silica nanoparticle. It is initiated by synthesis silica nanoparticle followed by enzyme immobilization and determine efficiency of immobilized enzyme protein.

2. Experimental Method
Synthesis of silica nanoparticle by sol-gel technique using Tetraethylorthosilicate (TEOS) as precursor is adopted from previous work.[6] [7] Mole ratio of H\(_2\)O : TEOS : EtOH is constant i.e. 4:1:8, with a variation in the amount of ammonia to control the particle size, the mole ratios of NH\(_3\)/TEOS are 0.03 ; 0.06 and 0.12. TEOS and ethanol 90% was mixed using a magnetic stirrer and dropwise of solution containing ammonia and water was added periodically. It was conducted for 5 hours at temperature of...
50 ± 2 °C to produce sol. The mixture was left at room temperature to form a gel. The gel was evaporated at 70 °C leaving a fine white powder and it was calcined at 400 °C for 8 h.

Immobilization was conducted through a covalent bond. The bond was formed after a modification on the functional groups of silica using glutaraldehyde. This modification was adopted from previous work by washing the silica in water for twice and separated by centrifugation. Silica nanoparticle was mixed with 3 mL of glutaraldehyde and separated through a centrifugation (150 rpm) for 4 hours at room temperature.[8] [9] Silica nanoparticle was then washed three times using 30 mL of water to remove unreacted glutaraldehyde. Immobilization of endo-β -1,4-D-xylanase is through mixing the modified silica and enzyme in a shaker (150 rpm) at temperature of 25 °C for 36 h. The free enzyme was washed using 3 mL of water for three times.

The protein content of the enzyme solution before and after immobilization in washing buffer solution were determined by the Bradford method using bovine serum albumin as a standard protein [10]

The percentage of immobilization yield (YI) enzyme was calculated using formula of %YI= . Where P_i is total protein in endo-β-1,4-D-xylanase, P_w is total protein left in washed solution, and P_s is total protein in supernatant after immobilization.

In addition, several analyses were conducted to support the present study, such as a Scanning Electron Microscope and a Fourier Transform Infra Red.

3. Results and Discussion

Figure 1 is SEM images of silica synthesized via sol-gel using TEOS as silicon precursor and ammonia as catalyst. Particle size of silica varies as a function of mole ratio of NH_3 : TEOS. The average of particle size of silica are 88.48, 271.31 and 473.52 nm respectively as mole ratio of NH_3 : TEOS of 0.03, 0.06 and 0.12.

Previous work has synthesized silica nanoparticle through sol-gel by varying mole of NH_3 catalyst with mole ratio NH_3 : TEOS 0.11 : 0.28 resulting in silica with particle size of 55 nm, whereas mole ratio of NH_3 0.3 : TEOS 0.28 gave silica with particle size of 130 nm[11]. Another study have immobilized xylitol dehydrogenase through covalent bond onto silica with particle size of 15, 30, 80 and 3000 nm and reported that highest immobilization was shown by silica with particle size of 80 nm[12]. Immobilization of endo-β -1,4-D-xylanase onto silica nanoparticle was first confirmed by FTIR analysis. For this purpose, FTIR spectra of unmodified silica nanoparticle, glutaraldehyde-modified 20 nm silica nanoparticle, glutaraldehyde-modified 88.48 nm silica nanoparticle, glutaraldehyde-modified 271.31 nm silica nanoparticle, and glutaraldehyde-modified 473.52 nm silica nanoparticle were measured and compared.

Figure 1. Particle size of silica of (a) mole ratio NH_3:TEOS 0,03, (b) mole ratio NH_3 :TEOS 0,06, (c) mole ratio NH_3:TEOS 0,12

Figure 2 (control) shows peaks unmodified silica nanoparticle at 800.49 cm^-1 are assigned to the Si-O symmetric stretching vibration, 958.65 cm^-1 are assigned to the Si-OH asymmetric stretching vibration, and peaks at 1091.75 cm^-1 are assigned to the Si-O asymmetric stretching vibration.[13]

Modification of functionalized silica nanoparticle was treating the nanoparticles with glutaraldehyde. During the reaction, one of the aldehyde groups of glutaraldehyde conjugates to the silica nanoparticle while another aldehyde groups conjugates to the endo-β -1,4-D-xylanase through the amino group. Modification of functionalized silica nanoparticle was confirmed by FTIR analysis. FTIR analysis of modified silica nanoparticle revealed the presence of aldehyde groups on silica nanoparticle shown in
Figure 2 (A,B,C,D). There is two new peaks at 1710.92 – 1718.63 cm\(^{-1}\) are assigned to C=O bond of aldehyde group and peaks at 2928.04 – 2974.33 cm\(^{-1}\) are assigned to stretching vibration of C-H bond of glutaraldehyde. [14] [15]

Protein content of free endo-\(\beta\)-1,4-D-xylanase is 2.65 mg whereas the immobilized ones are 0.808, 0.399 and 0.280 mg in silica with particle size of 88.47, 271.31 and 473.52 nm respectively. In respect to the particle size of silica percentage yield of enzyme immobilization are 30.49, 15.05 and 10.56 %. It shows that silica with smaller particle size increases the amount of protein immobilized onto the silica.

Figure 2. FTIR spectra of control unmodified silica nanoparticle, (A) glutaraldehyde-modified 88.48 nm silica nanoparticle, (B) glutaraldehyde-modified 271.31 nm silica nanoparticle, (C) glutaraldehyde-modified 473.52 nm silica nanoparticle, and (D) glutaraldehyde-modified 20 nm silica nanoparticle

4. Conclusion
Silica nanoparticle synthesized through sol-gel with mole ratio NH\(_3\) : TEOS of 0.03; 0.06 and 0.12 result in silica with particle size of 88.48, 271.31 and 473.31 nm respectively. Silica-aldehyde was obtained on modification of silica surface with glutaraldehyde. Immobilization of endo-\(\beta\)-1,4-D-xylanase onto nanoparticle silica-aldehyde gave percentage of immobilized endo-\(\beta\)-1,4-D-xylanase protein of 30.49, 15.05 and 10.56 % in respect to the particle size of silica of 88.48; 271.31 and 473.52 nm.

References
[1] Fernandez, B. R. 2012. Sintesis Nanopartikel SiO2 Menggunakan Metoda Sol-gel Dan Aplikasinya Terhadap Aktifitas Sitotoksik Sel Dalam Review Jurnal Nanoteknologi. Review Jurnal Nanoteknologi. Padang: Jurusan Kimia, Program Pascasarjana Universitas Andalas.
[2] Siswanto, M. Hamzah, Mahendra, and Fausiah. 2012. Perekayasaan Nanosilika Berbahan Baku Silika Lokal Sebagai Filler Kompon Karet Rubber Air Bag Peluncur Kapal Dari Galangan. Prosding INSINAS 2012. Jakarta: Badan Pengkajian dan Penerapan Teknologi.
[3] Zhang. Y. W., Tiwari. M. K, Jeya. M, and Lee. J. K. 2011. Covalent immobilization of
recombinant Rhizobium etli CFN42 xylitol dehydrogenase onto modified silica nanoparticles. App Microbiol Biotechnol. 90 2 499-507.

[4] Rahman, I. A., and V. Padavettan. 2012. Synthesis of Silica Nanoparticles by Sol- Gel: Size-Dependent Properties, Surface Modification, And Applications In Silica-Polymer Nanocomposites In Review. Journal of Nanomaterials. 12 1 1-15.

[5] Wijaya, K. 2010. Nanomaterial Berlapis Dan Berp ori: Sintesis, Karakterisasi Dan Perannya Sebagai Material Multi Fungsi. Yogyakarta: UGM

[6] Brach, D., V.N. Dougnac, H. Palza, and R. Quijada. 2012. Functionalization of Silica Nanoparticles for Polypropylene Nanocomposite Applications. Journal of Nanomaterials. 20 1477- 1482.

[7] Stoeber, W. Fink, A. 1968. Controlled Growth of Monodisperse Silica Spheres in the Micron Size Range. Journal of Colloid and Interface Science. 26 1 69-68.

[8] Singh, R., K., Zhang. Y. W, N. Nguye, M. Jeya, and J. K. Lee. 2010. Covalent immobilization of β-1,4-glucosidase from Agaricus arvensis onto Functionalized Silicon Oxide Nanoparticles. Appl Microbiol Biotechnol. 89 2 337-344.

[9] Dhiman, S., Jagtap. S. S, Jeya, M. Haw, J. R, Kang. Y. C, and Lee. J. K. 2011. Immobilization of Pholiota adiposa xilanase onto SiO2 nanoparticles and its application for production of xilooligosaccharides. Biotechnology. 34 7 1307-1313.

[10] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin reagent. J. Biol. Chem 1951; 31:426–428.

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