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The 12th Congress of Indonesian Society for Biochemistry and Molecular Biology in Conjunction With The 2nd International Conference “Collaboration Seminar of Chemistry and Industry (CoSCI)” and AnMicro Workshop

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Preface

Second International Conference "Collaboration Seminar of Chemistry and Industry (CoSCI 2018) in conjunction with 23rd Indonesian Society for Biochemistry and Molecular Biology (ISBMB) seminar was taken place at Universitas Airlangga, Surabaya-Indonesia on October 11-12, 2018. The event presented a theme" Recent Development of Omics Technology For Human Prosperity".

"Omics is a general term for a broad discipline of science and engineering for analyzing the interactions of biological information objects in organism. The main focus is on: 1) mapping information objects such as genes, proteins, and ligands; 2) finding interaction relationships among the objects; 3) engineering the networks and objects to understand and manipulate the regulatory mechanisms; and 4) integrating various omes and omics subfields.

The event was held to facilitate for the scientists, scholars, engineers and students from universities, research institutes and industries to present ongoing research activities, especially in Biochemistry, Biology, Chemistry, Medicine and other in related fileds. It was also to encourage future collaboration among all participants.

The conference of CoSCI and ISBMB created proceedings from the papers that were submitted by participants, after they were reviewed by committee members and international reviewers. This volume intends to provide readers with the recent advances in the Omics Technology such as Chemistry, Biochemistry and Molecular Biology, and Medicine field.

We would like to thank to all authors who contributed to the proceedings and also to the organizing committee, reviewers, speakers, sponshor, and all the conference participants for their supporting in the conference of CoSCI 2018 and 23rd Seminar of ISBMB.

Dr. Purkan, M.Si
Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia, Nov. 10, 2018

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Peer review statement

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Peer review statement

All papers published in this volume of *IOP Conference Series: Earth and Environmental Science* have been peer reviewed through processes administered by the proceedings Editors. Reviews were conducted by expert referees to the professional and scientific standards expected of a proceedings journal published by IOP Publishing.

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Synthesis of Aldehyde-Silica Nanoparticle for Matrix Immobilization of Endo-β-1,4-D-xylanase

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Synthesis of Aldehyde-Silica Nanoparticle for Matrix Immobilization of Endo-β-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 °C 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-β-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-β -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_2O : 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 ºC to produce sol. The mixture was left at room temperature to form a gel. The gel was evaporated at 70 ºC leaving a fine white powder and it was calcined at 400 ºC 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 ºC 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.

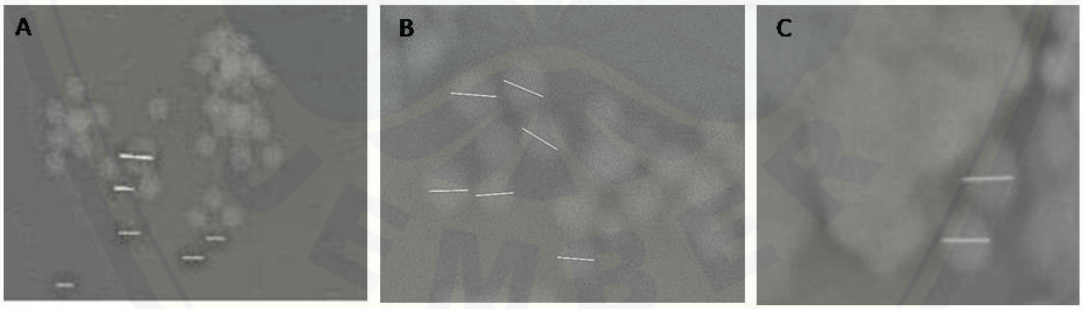

Figure 1. Particle size of silica of (**a)** mole ratio NH_3:TEOS 0,03, (**b)** mole ratio NH_3 :TEOS 0,06, (**c**) mole ratio NH_3:TEOS 0,12

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 asymetric stretching vibration, and peaks at 1091.75 cm^{-1} are assigned to the Si-O asymetric 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-β -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.

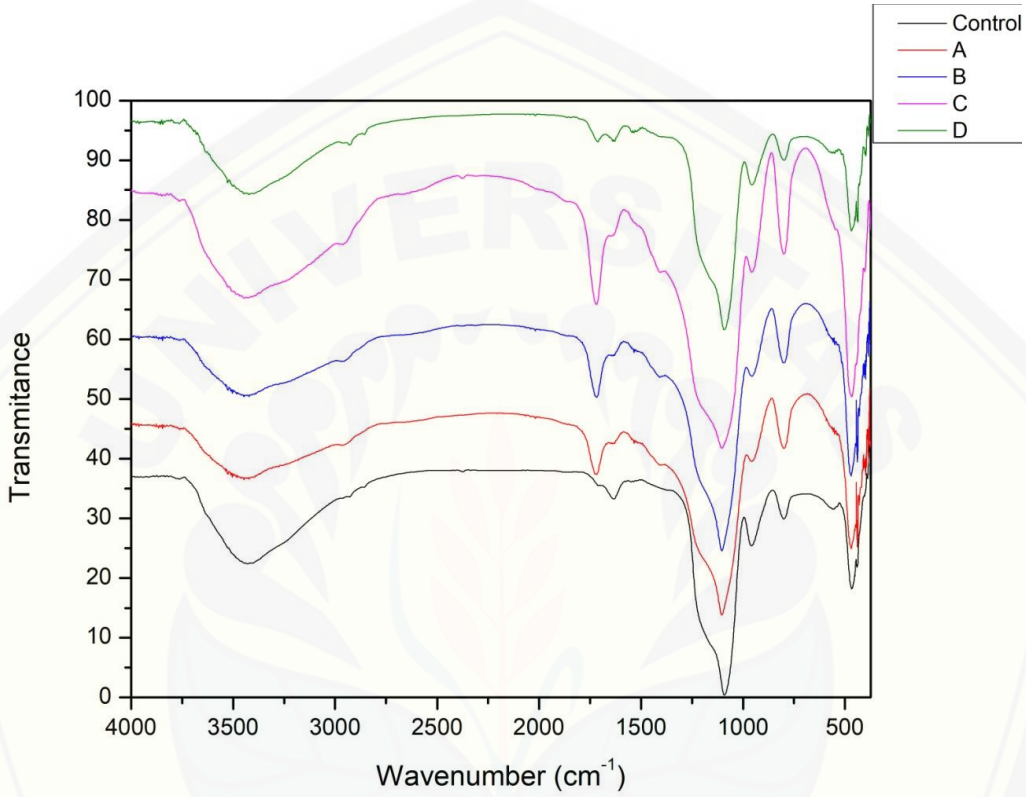

Figure 2. FTIR spectra of **control** unmodified silica nanoparticle, (**A)** glutaraldehyde-modified 88.48 nm silica nanoparticle, (**B)** glutaraldehyde-modified 271.31 nm silica nanoparticle, (**C**) glutaraldehyde-modified 473.52 nm silica nanoparticle, and (**D)** glutaraldehyde-modified 20 nm silica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

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-β -1,4-D-xylanase onto nanoparticle silica-aldehyde gave percentage of immobilized endo-β -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.

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