Effect of Agitation Speed for Enzymatic Hydrolysis of Tapioca Slurry Using Encapsulated Enzymes in an Enzyme Bioreactor

Siti Noraida Abd Rahim, Alawi Sulaiman, Ku Halim Ku Hamid, Nurul Aini Edama, and Azhari Samsu Baharuddin

***Abstract*—Agitation speed is an important factor in the industrial application for the mixing efficiency in order to increase productivity. In this study, the effect of agitation speed was investigated in a stirred bioreactor for hydrolysis of tapioca slurry into glucose by using encapsulated enzymes within calcium alginate-clay (alg-clay) beads. The hydrolysis process was tested at various agitation speeds from 40 to 200 rpm. The stability of alg-clay beads was examined in term of morphology of beads, enzyme leaching behavior and enzymes activity. The morphology of the alg-clay beads was investigated by Field Emission Scanning Electron Microscopy (FESEM). It was found that vigorous speed ruptured the beads structure. The result also showed that by increasing the agitation speed, the leakage of enzymes also increased. Therefore, it can be concluded that there was a strong correlation between the speed of agitation and the beads morphology. The optimum agitation speed which gave the highest amount of enzymes activity was 120 rpm.**

***Index Terms*—Stirred bioreactor, agitation speed, alginate-clay beads, enzymes, tapioca slurry.**

I. Introduction

An enzyme bioreactor is a device used for biochemical reactions that are performed by the catalysis of enzyme to generate the expected products under mild conditions. The enzyme bioreactor is widely applied for food processing, industrial biotransformations, pharmaceutical processing, biosensors, and so on [1]. On the basis of enzyme bioreactor, it can be categorized as free enzyme and immobilized enzyme. The use of immobilized enzyme has attracted many attentions due to its reusability, easy separation from the reaction media and possible in high thermal stability compared to free enzyme [2]. The immobilized enzyme bioreactor can be designed as stirred, packed, fluidized and membrane reactor that can be operated either in batch or continuous system [3]. Among these bioreactor, the stirred bioreactor is a most applied in bench scale and industrial scale application because it is versatile and easy to operate [4].

The performance of hydrolysis process that is carried out in the immobilized stirred bioreactor is influenced by many factors such as size of particle, concentration of reaction media and geometrical and operational characteristics of the vessel [3]. However, the mixing efficiency and its distribution into the bioreactor is the most important parameter in order to obtain higher productivity. Purwanto *et al.* [5] had mentioned that the lower and excessive speed will reduce the productivity of hydrolysis process because the enzyme is sensitive to mechanical shear stress. Thus, the mixing efficiency should be considered in the optimization process.

For this reasons, the aim of this work was to investigate the effect of agitation speed for enzymatic hydrolysis of tapioca slurry into glucose using encapsulated enzymes in the stirred bioreactor. The morphology of encapsulated enzymes, enzymes leaching behavior and activity of enzymes were determined.

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II. Materials and Methods

A. Materials

Alpha-amylase from *Bacillus subtilis*, glucoamylase from *Rhizopus niveus Lyophilized* and cellulase from *Aspergillus niger* were supplied from MP Biomedicals, United States. All other chemicals reagent used were analytical grade.

B. Tapioca Slurry Preparation

Cassava root was washed free of dirt, hand peeled and sliced to small pieces. Then, the chips were dried in oven at 65 ^{o}C for 24 hr and then ground into powder. For the hydrolysis process, 1 % (w/v) of tapioca slurry was prepared by boiling tapioca powder using citrate phosphate buffer (pH 5.0) to gelatinize the starch.

C. Calcium Alginate-Clay (Alg-Clay) Beads Preparation

Approximately 2.5 g of kaolinite clay was dissolved in 100 mL of citrate phosphate buffer solution (pH 5.0) and stirred for 1 hr at room temperature. The kaolinite clay used in this study was prepared as described in the previous study [6]. After that, 2.5 g of alginate powder was added to the clay solution and stirred for 4 hr. Next, 2.5 mL of glycerol was added to the alg-clay solution. For encapsulation of enzymes, 1 mL of each enzyme solution (1 mg solid/mL of

alpha-amylase, glucoamylase and cellulase) was mixed with 12 mL of alg-clay solution. The mixture solution was stirred thoroughly to ensure complete mixing and dropped into 0.2 M $CaCl_2$ solution by using syringe. After 3 hr of hardening, the beads were collected by filtration and then washed with buffer solution several times to remove any unbound enzymes [7], [8].

D. Bioreactor System

A stirred bioreactor was used to hydrolyze tapioca slurry into glucose catalyzed by encapsulated enzymes (see Fig. 1). The cylindrical glass of 10 cm inner diameter and 27 cm height was used in this study. The bioreactor was also set up with water jacket and propeller. The water jacket will control the reaction temperature by circulating the water from the water bath tank. Meanwhile, the propeller plays a role as agitation system to improve mixing efficiency and it was connected to an electrical motor.

For hydrolysis process, 27 g of beads was added into bioreactor that consisting 1000 mL of tapioca slurry. The reaction was conducted at 50 ^{o}C under various agitation speed (40, 80, 120, 160 and 200 rpm) for 28 hrs. The aliquots were taken at 4 hrs interval times and heated in boiling water for 10 min to stop the reaction. The enzymes activity and protein content was analyzed.

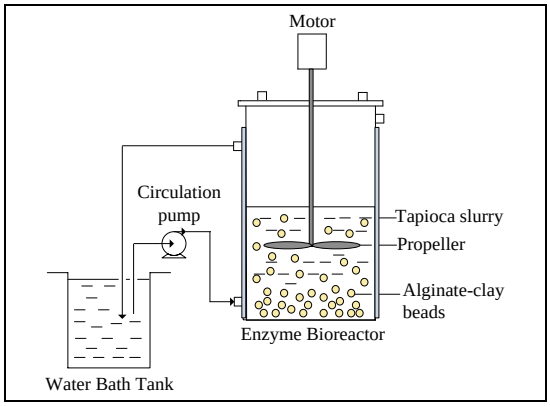

Fig. 1. Schematic diagram of stirred bioreactor.

E. Bead Morphology

The surface morphology of alg-clay beads before and after reaction was studied using Field Emission Scanning Electron Microscopy (FESEM) (ZEISS Supra 40VP, USA). Before being observed by FESEM, the beads were mounted on stubs using double sided adhesive tape and sputter coated with gold using a sputter coater.

F. Enzymes Leaching Behavior

The amount of enzymes (protein content) leached into bulk solution was determined by Lowry's procedure modified by Hartree [9] using bovine serum albumin (BSA) as a standard.

G. Enzymes Activity Assay

The activity of enzymes was measured by using spectrophotometer at 540 nm with 3,5-dinitrosalicylic acid (DNS) as an indicator [10]. Theoretically one unit of alpha-amylase activity is defined as the quantity of enzyme that releases 1 mg of reducing sugar per minute at pH 6.6 and 30 ^{o}C. One unit of glucoamylase is expressed as the amount of enzyme releasing 10 mg of reducing sugar (glucose) per minute at pH 4.5 and 40 ^{o}C. One unit of cellulase activity is liberated as the amount of enzyme that produces 1 μmole of reducing sugar (glucose) at pH 5 and 37 ^{o}C in 1 min.

III. Results and Discussion

A. Beads Morphology

The morphology of alg-clay beads before and after hydrolysis process was investigated by using FESEM. The FESEM micrographs of the beads were taken at various agitation speeds after 28 hrs of hydrolysis process in a stirred bioreactor (Fig. 2). As shown in Fig. 2 (a), the surface of beads before conversion process had a rough surface and flat. After hydrolysis process, the external surface of the beads that were agitated at 40 rpm had the same external surface morphology with the beads before the process (Fig. 2 (b)). This can be explained due to minimal collision between the beads. However, by increasing the agitation speed higher than 40 rpm, the external structure of the beads began to crack and rupture. From Fig. 2 (c), the beads start to crack at 80 rpm while the surface of the beads at 120 and 160 rpm (Fig. 2 (d and e)) had a deeper and more severe crack. The vigorous agitation speed (200 rpm) caused the rupture of the beads as can be clearly observe in Fig. 2 (f). This can be explained due to higher shear stress and shear forces acted on the beads surfaces as a result of collisions.

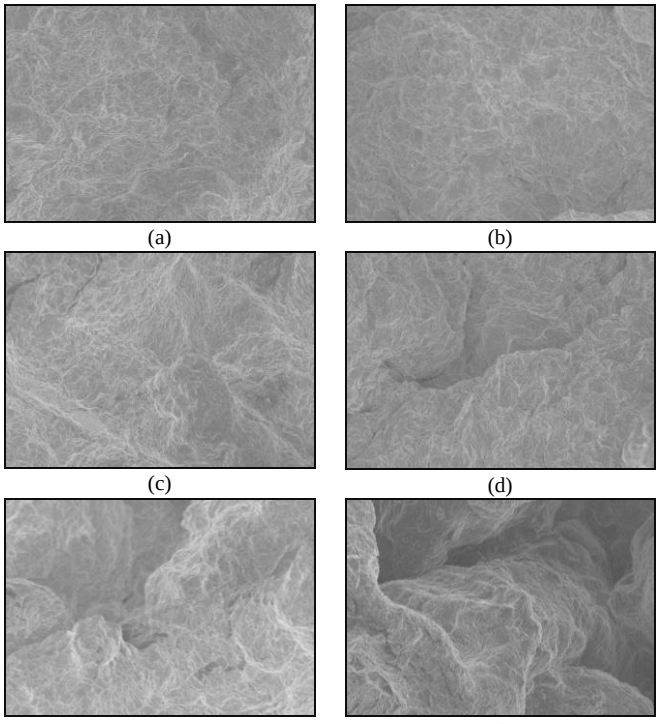

Fig. 2. FESEM micrographs of alg-clay beads at 1000x magnification. (a) before hydrolysis; (b) 40 rpm; (c) 80 rpm; (d) 120 rpm; (e) 160 rpm; (f) 200 rpm.

B. Beads Morphology

The enzymes leaching from alg-clay beads are illustrated in Fig. 3. The result showed that at lower agitation speed which is 40 rpm, the beads had a smaller leakage of enzymes. Based on the morphology of the beads at 40 rpm (Fig. 2 (b)), there

was no crack observed on the beads external surface. Even that so, the leakage of enzymes still occurred at low agitation speed. According to Kulkarni *et al*. [11], the leakage of enzymes may be due to the swelling of the alg-clay beads. The swelling behavior was occurred when the beads tend to absorb water in order to fill the void regions of the polymer network within the beads that remain dehydrated until they reach the equilibrium state. This phenomenon will cause the losing of polymer network at the presence of osmotic pressure as well as weakened mechanical strength and lastly tend to the leakage of enzymes [12]. This explanation also supported by Li *et al*. [13] where the serious swelling of the beads may reduce the enzyme loading efficiency, mechanical strength and recycling stability.

As can also be seen in Fig. 3, the high agitation speed in a stirred bioreactor result in high shear stress causing the enzymes from the beads will leach out easily. In this study, it was found that the higher leakage of enzymes was observed at speed of 200 rpm. Compared with the morphology from Fig. 2 (f), the surface of the beads seem ruptured was in agreement with result of enzymes leaching behavior. And then, it was proved that the higher leakage of enzymes from the beads due to high shear stress that produce from the higher collision between the stirrer and beads.

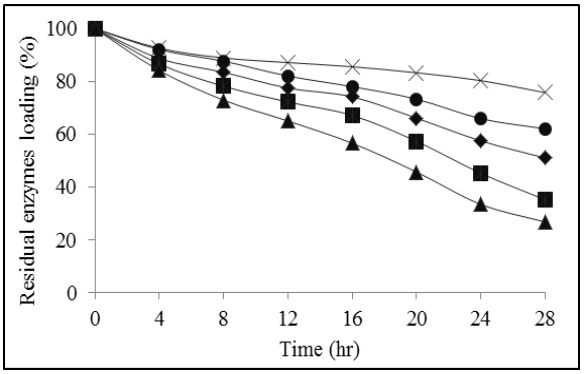

Fig. 3. Residual of enzymes loading in the beads at various speeds. (×) 40 rpm; (●) 80 rpm; (◆) 120 rpm; (■) 160 rpm; (▲) 200 rpm.

C. Activity of Encapsulated Enzymes

In theory, for the case of encapsulated enzymes, the reactant (in this case starch) needs to diffuse from the bulk liquid to the external surface of the beads and from there into the active sites of the enzymes. The external mass transfer and internal diffusion limitations can therefore be minimized by carrying out the reaction at an optimum agitation speed. In this study, the effect of agitation speed on the tapioca slurry hydrolysis process was studied in the range of 40 to 200 rpm (Fig. 4). It was found that the activity of encapsulated enzymes increased with the increase of agitation speeds from 40 to 120 rpm and then slightly decreased from 120 to 200 rpm. Thus, the optimum agitation speed could be concluded at 120 rpm. The minimum mass transfer was noticed to occur at lower agitation speed (40 rpm). This can be explained because at this agitation speed the external mass transfer rates between the bulk phase of the reaction mixture and the surface of enzymes decreased. Furthermore, the activity of enzymes at higher speed was also lower due to perturbation of protein structure by the shear effect during hydrolysis process and then, it will inactivate the catalytic power of enzymes [14], [15].

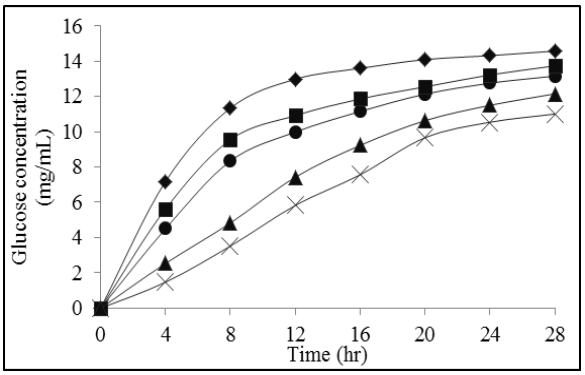

Fig. 4. Activity of encapsulated enzymes at various speeds. (×) 40 rpm; (●) 80 rpm; (◆) 120 rpm; (■) 160 rpm; (▲) 200 rpm.

IV. Conclusion

As a conclusion, the encapsulated enzymes onto alg-clay beads were successfully studied using a stirred bioreactor at various agitation speeds. For the enzymes leaching study, the leakage of enzymes from beads was increased with the increase in agitation speed. The result also showed that the leaching of enzyme from the beads was consistent with the damage of external surface morphology. Besides, the maximum activity of encapsulated enzymes was found at 120 rpm. Thus, the mechanical stress gave greater impact on the encapsulated enzymes and should be considered in the optimization process.

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