Immobilization of *Candida rugosa* lipase by adsorption-crosslinking onto corn husk

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**Abstract.** Corn husk is one of the agricultural waste that has not been used optimally. Corn husk waste allows to be used as immobilized support for biocatalyst because it is easy to obtain, available abundant, renewable and easy to decompose. This research was conducted in two phases, namely the adsorption of enzyme immobilization on the support, followed by crosslinking between the enzyme and support through the addition of glutaraldehyde. The optimum conditions for cross-linked adsorption immobilization using support of corn husk were achieved at concentrations of 0.75 mg / ml at 4 hour reaction time. The biggest unit activity value is obtained at 2.37 U / g support through 0.5% glutaraldehyde addition.

1. Introduction

Corn crop (*Zea mays ssp. mays*) is grain crops that have the largest production capacity every year compared to other grain crops [1]. The composition of the corn crop morphology consists of roots, stems, leaves, flowers and fruit. The fruit consists of a corn cob, seeds and leaves wrapping (skin). Corn husk is a part of the plant that protects the corn kernels which is light green when young, and dries up when the old tree. Corn husk as agricultural waste is generally used conventionally as a source of raw materials for crafts and fodder, while on an industrial scale, among others, have been used as raw material for bioethanol industry [2] and the paper industry [3].

The main composition of the corn husk are composed of cellulose and lignin [3]. Corn husk can be used as a raw material for enzyme immobilization support because its availability is abundant, are renewable and biodegradable [4]. Some criteria of a good support is inert, physically strong, stable, can be regenerated, and able to increase and decrease the activity of enzymes inhibitors [5].

Utilization of agricultural waste biomass as a biocatalyst immobilization support has been carried out which utilizes coconut fibber to immobilize α-amylase [6,7]. Through method of adsorption-crosslinking, an agricultural waste such as of corn cobs, banana stem, rice husk, coconut fibers, and stems salak were reported as the support in immobilization lipase from acinotebacter [8]. Utilization of corn husk was studied to be used as a support on proteinase and amylase enzyme immobilization by adsorption [4]. In 2013, continue research on core utilization of corn stalks as support for the immobilization amylase, lipase and proteinase enzymes [9].

Enzyme immobilization process is highly dependent on the type of support and immobilization method used [10]. Adsorption method is a method of enzyme immobilization as it is the most simple, easy and economical. A disadvantage of adsorption immobilization method is the weak interaction between the enzyme and the support that results in low activity and stability [11]. The use of glutaraldehyde as a cross linker is one way to improve the interaction between the enzyme and support. This process begins with enzyme adsorption on its support, followed by cross-linking between enzymes with the addition of glutaraldehyde. Immobilization by adsorption-cross-linking has been made using 0.01% glutaraldehyde as a cross-linker activating agent however it was not successful [6,8]. In this research, effect of different concentration of glutaraldehyde was investigated.
The use of support materials from waste biomass in the immobilization process is largely determined by the preparation of the biomass. The main purpose of the pretreatment is to increase the surface area, decrystallize cellulose, hemicellulose and eliminate lignin [12][13]. Pretreatment of the support of corn husk is important in physics and chemistry to facilitate the process of immobilization.

The purpose of this research is to investigate the abilities of corn husk as the support for lipase obtain from commercial *Candida rugosa*.

2. Material
Commercial *C. rugosa* lipase was purchased from Sigma- Aldrich (Singapore). Agricultural waste biomass corn husk was obtained from people’s a plantation in Ciomas-Bogor, West Java. NaCl, HCl and other chemicals standard uses analytic of merck.

3. Methods

3.1. Pretreatment of the corn husk
A modified pre-treatment method using NaCl and HCl from previous studies was used [8]. Corn husk was cut to a length of 10 - 20mm, washed with clean water, then soaked in 0.8% (w/v) NaCl solution for 30 minutes. It was subsequently rinsed with distilled water 3 times. It was put into the autoclave for sterilization and dried at 65°C for 24 hours. Corn husk was then crushed and screened to obtain a particle size of 0.1 - 0.5 mm. As much as 10 grams of corn husk that has been filtered and then was soaked in 2N HCl solution, and triple washing with 0.1 M phosphate buffer (pH 7.0). Final was obtained by drying at 60 °C for 24 hours.

3.2. Immobilization adsorption-crosslinking
The process of immobilization was done using adsorption-crosslinking method [8, 12]. One gram support was suspended in 100 ml solutions (0.5 mg/ml) prepared in a phosphate buffer 20 mm (pH 7.5), stirred (350 rpm) for two hours at room temperature, then 0.5% glutaraldehyde was added. Variations crosslinking reaction time used were 2, 4 and 6 hours. Support that has been immobilized separated from the filtration. Lipase activity was tested in the supernatant.

3.3. Measurement of hydrolytic activity for *Candida rugosa* lipase
The activity of lipase was done by a reaction call hydrolysis [14]. The substrate was prepared by mixing 50 ml of olive oil with 150 ml of polyvinyl alcohol solution (2%, w/v) to obtain emulsion. The reaction mixture consists of phosphate buffer 5.0 ml (0.025 M, pH 7) and 5 ml of the emulsion above and a certain amount of free *C. rugosa* lipase was incubated for 15 min at 37 °C in a vessel. The reaction was terminated by adding 15 ml of 95 wt.% ethanol. The liberated fatty acid was titrated with 0.05M sodium hydroxide solution in the presence of phenolphthalein indicator. In addition, a blank experiment for comparison without adding *C. rugosa* lipase was also carried out. One unit (U) of CRL activity was defined as the amount of CRL which produced 1 mol of free fatty acids per min under the assay conditions.

4. Result and discussion

4.1. Effect of pretreatment on corn husk
Biomass used in this study is a waste of agricultural produce. Require a special pretreatment to clean the debris from the source of the waste attached to biomass, as well as to facilitate the absorption of lipase *C. rugosa* at the time of immobilization. Pretreatment early done in physics by the process of grinding to get smaller size of particles (0.1 and 0.5 mm). This process aims to get the surface area of particles greater. The corn husk were soaked in NaCl for 30 minutes. This stage serves as a impurities solvent still attached to agricultural waste biomass when washing. Further pretreatment using HCl solution. The use of solution HCl at the time of treatment is too destructive to structures lignocellulose
that is a component authors biomass. That accessibility of adsorption enzyme when immobilization increased. Treatment using hydrochloric acid (HCl) serves to damage the lignocellulosic structure followed by the removal of hemicellulose thereby increasing the porosity and the ability of biomass digestion enzymatically. [12]. Initial treatment of lignocellulose with acid at ambient temperature is done to improve anaerobic digestibility [15].

The result on the influence of the surface structure before and after treatment using scanning electron microscopy (SEM) can be seen in figure 1 and 2.

Figure 1 was the SEM test results before pretreatment. Looks larger pore structure, contrary to the Figure 2, which look having a smaller pore structure. Effect of HCl addition at the time of treatment could have damaged the rice straw structure thus enables increased accessibility of the enzyme to the support at the time of immobilization (Figure 1). The opposite occurs before the pretreatment, the larger pore size so that the enzymes are adsorbed more easily dislodged (Figure 2).

4.2. Influence of unit activity on reaction time and concentration of Candida rugosa lipase

_Candida rugosa_ activity for 2 hour reaction time is shown in Figure 3. Activity lipase was the results of immobilization adsorption was seen to rise fit with the increase in time reaction. After the crosslinking process through the addition of glutaraldehyde precisely causes a decrease in the value of _C._ _rugosa_ lipase activity for all lipase concentration variations (Figure 3). This is probably caused by the reaction conditions that are inconsistent with glutaraldehyde concentration so that the crosslinking process is not been successful [11]. The biggest activity value is the optimum condition because it shows the number of enzymes adsorbed on the support of corn husk is increasing [8]
For reactions which lasted 4 hours, the addition of glutaraldehyde led to an increase in value of the activity of 1.2 and 2.37 U / g support respectively at lipase concentrations of 0.5 and 0.75 mg / ml (figure 4). When 6 hours reaction there was increased activity at smaller cockroach concentrations of 0.25 and 0.5 mg / ml with an activity value of 1.77 and 1.56 U / g support (figure 5).

5. Conclusions
The optimum activity for adsorption-crosslinking immobilization using the corn skin support for 2.4 and 6 hours was obtained with an activity value of 1.42; 2.37 and 1.77 U / g. This result was obtained from lipase concentration of 0.5 mg / ml for reaction time of 2 and 6 hours. whereas for 4 hours is obtained from lipase concentrations of 0.75 mg / ml.

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