Preparation and Thermal Protease Stability of Biopesticide to Control the Pomacea Canaliculata’s Eggs

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Abstract—The snail of Pomacea canaliculata attacks young leaves and stem of paddy, thus causing great losses to farmers in rice-producing countries. In general, farmers prefer to use chemical methods to eradicate the snail because of efficacy reason. However, since the chemical methods might not environmental friendly, it is necessary to find an alternative bio-based effective method for controlling the snail. In the present paper, we are reporting the preparation of biopesticide for disrupting the snail’s eggs hatching process based on the action of immobilized protease. The immobilization was meant for maintaining enzyme stability and activity rather than for recycling purposes. Hence, it is preferable to use a simple immobilization method and cheaper but larger surface area of support material. For this purpose, protease was immobilized through adsorption onto mesoporous activated carbon of rice husk and formulated to form biopesticide. It was found that the optimum temperature for immobilized protease was at 30°C. The enzyme was stable at 20°C to 60°C with average activity of 0.034 U/ml and 0.033 U/ml. The high thermal stability of the immobilized protease promises its application in the tropical countries as a potent biopesticide for Pomacea canaliculata’s eggs.

Index Terms—activated carbon, immobilization, protease, thermal stability

I. INTRODUCTION

Pomacea canaliculata is a freshwater snail that could easily be found in water irrigation and in paddy fields particularly in Asian countries. The invasion of the snails in rice field has caused substantial damage to leaves and stems of paddy, thus causing great loss to rice growers. This snail has a very high reproductivity with high hatching rate. Therefore, it is preferable to disrupt the eggs instead of the snail itself. Various methods have been introduced to control the growth of Pomacea canaliculata, but most of them are chemically based, which might not compatible to the environment [1]. On the other hand, biological treatment towards the snails is not very effective because the hatching rate is very high where the juveniles outnumber the matured snails. Therefore, it is preferable to disrupt the hatching process of the eggs rather than the matured snails. There are some applications of several microbes to control the snails’ eggs [2], [3], [4] with limited applicability. For example, Paecilomyces lilacinus will require 7 days to disrupt the snails’ eggs effectively [2]. Considering this issue, it might be possible to use enzyme instead of microbes for this purpose. Protease might be a good potential in controlling the Pomacea canaliculata’s eggs as they would hydrolyzed the protein of the eggs to terminate the embryo’s growth. The efficacy of enzyme-based biopesticide was largely determined by the activity of enzyme. The free enzyme however, might not be stable and easily denatured. Through appropriate immobilization technique, most of the drawbacks associated with the free enzyme could be minimized.

This paper deals with preparation of the support materials for protease immobilization to be used as a biopesticide for disrupting the growth of the snails’ eggs. There are several techniques to carry enzyme immobilization namely covalent binding, adsorption or encapsulation/entrapment [5]. Among these, direct adsorption onto solid support is the simplest [6]. This technique depends on the interaction of van der Waals force between the enzyme and the support, therefore, conserved most of the enzyme active site.

For the support material, it is preferable to use activated carbon obtained from local waste. Rice husk might be a good candidate for the support material because it is abundantly available and high carbon (42.2%) content [7].

To improve the applicability, it is important to ensure that the enzyme is active at wide range of temperature. This will guarantee that the biopesticide could be useful in tropical countries. Therefore, the paper is concerned with the preparation of the support material as well as thermal activity of immobilized protease for developing biopesticide to disrupt the hatching process of the eggs.

II. MATERIALS AND METHODOLOGY

A. Materials

Protease of Aspergillus oryzae was purchased from Sigma Aldrich (Kuala Lumpur, Malaysia). Rice husk was collected from rice milling factory in Kedah, Malaysia. Pomacea canaliculata’s eggs were collected from paddy

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field in Perlis, Malaysia. All other reagents were of analytical grade.

B. Activated Carbon Preparation

Rice husk was washed and oven dried at 110°C before sieving at 500µm. Activated carbon was prepared as described before [8] with a slight modification. Briefly, precarbonization of rice husk was carried out at 400°C in an inert condition for 4 hrs followed by chemical activation with NaOH at 750°C for 2 hrs to increase its surface area. After activation, the activated carbon was washed thoroughly with distilled water to remove any residue left before drying and grinding.

C. Characterization of Rice Husk Activated Carbon

The surface area and pore size were determined by N₂ adsorption-desorption isotherms. The N₂ adsorption-desorption isotherms of rice husk activated carbon was measured using Quantachrome Corp., Nova-1000 gas sorption analyzer. The rice husk activated carbon was degassed for overnight at 150°C. The specific surface area was measured using Brunner-Emmett-Teller (BET) isotherm equation. The morphology of rice husk activated carbon before and after immobilization was examined under Scanning Electron Microscope (Jeol JSM-5610LV, Japan).

D. Free Protease Effect on the Pomacea Canaliculata’s Eggs

Free protease of Aspergillus oryzae was diluted with phosphate buffer of pH 7 and was applied to the Pomacea canaliculata’s eggs for 24 hr.

E. Protease Immobilization

Sample of 0.05 g of activated carbon was introduced to 1 ml of protease 25U/ml dissolved in phosphate buffer at pH 7. The immobilization was carried out at 20°C, agitated at 160rpm for 4 hrs. Protease immobilization was investigated with protease assay.

F. Protease Assay

Protease was assayed by spectrophotometrically according to [9] with a slight modification. Casein (0.6%) was used as the substrate. 0.02g of immobilized protease was mixed with 300µl of casien before being incubated for 30 min at different temperature (20°C to 60°C) in water bath. The reaction was stopped by the addition of 600µl of trichloroacetic acid (10%) for 15 min before being centrifuged to separate the precipitate. The filtrate was measured at 275 nm. One unit of protease activity was defined as the amount of enzyme required to liberate 1 µg of tyrosine per minute.

G. Thermal Stability

The thermal stability of immobilized protease was studied by incubating the immobilized protease with casein as the substrate at various temperatures (20°C-60°C) for 30 min.

III. RESULTS AND DISCUSSION

A. Characterization of Rice Husk Activated Carbon

The surface area, pore volume and pore size of rice husk activated carbon was determined using Brunauer-Emmett-Teller (BET) as listed in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Rice husk activated carbon</th>
<th>Non-activated rice husk [9]</th>
<th>Improvement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface area (m²/g)</td>
<td>1646.62</td>
<td>36.44</td>
<td>97.79</td>
</tr>
<tr>
<td>Pore size (nm)</td>
<td>2.54</td>
<td>4.26</td>
<td>65.48</td>
</tr>
<tr>
<td>Pore volume (cm³/g)</td>
<td>1.04</td>
<td>0.03</td>
<td>97.12</td>
</tr>
</tbody>
</table>

The surface area, pore size and pore volume of rice husk activated carbon clearly showed good adsorption capacity as compared to non-activated rice husk according to [10] (Table 1). The improvement is due to the lignin, cellulose and hemicellulose being hydrated, polymerized and solubilized certain volatile components to create pores [11]. It was reported that during the carbonization step, the sample diameter was gradually increased and developed the micropores followed by mesopores during the activation step with NaOH [12]. It was found that utilization of NaOH would create larger pore diameter of activated carbon than those with KOH activation [13]. The pore size in Table 1 indicated that the rice husk activated carbon was in mesopores range of 2-50 nm. The high surface area of rice husk activated carbon developed through the process would serve as a good support for amine functionalization and protease immobilization.

The surface morphology of the activated carbon before and after protease immobilization was shown in Fig. 1 (a) and (b). Fig. 1 (a) clearly shows the porous structure of rice husk upon activation by NaOH. Fig. 1 (b) shows the protease was adsorbed well within the channel of the support.

Figure 1. Surface morphology of rice husk activated carbon before immobilization (a) and after protease immobilization (b)

B. Thermal Stability

Before attempting to measure the thermal activity of immobilized protease, it is necessary to evaluate the efficacy of free protease to disrupt the hatching process.

The effect of free protease on the Pomacea canaliculata’s eggs is shown in Fig. 2. After 24 hrs of incubation with protease, the protease solution was withdrawn and the hatching possibility was monitored for 12 days. After 12 days, the untreated of Pomacea canaliculata’s eggs hatched (Fig. 2a), while the treated of
Pomacea canaliculata’s eggs did not hatched. It is believed that it is due to the protease action towards the protein in the embryo of the egg (Fig. 2b). Therefore, it shows that protease has the potential to be used as a potent biopesticide of Pomacea canaliculata’s eggs.

The thermal stability of immobilized protease was investigated in range of 20°C-60°C. Based on Fig. 3, it is found that the optimum temperature for immobilized protease is at 30°C with enzyme activity of 0.034 U/ml. The improvement of thermal stability was highly dependent on the immobilization method and the nature of the support [14].

IV. CONCLUSION

Activated carbon prepared from rice husk showed high surface area giving high adsorption capacity; thus, could be a good support for enzyme immobilization. The immobilized protease on the rice husk activated carbon has successfully increased the thermal stability up to 60°C. Through immobilization technique, the immobilized protease was robust and stable to disrupt the hatching process of the eggs. Due to that reason, it has high potential to be used for controlling the Pomacea canaliculata’s eggs.

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REFERENCES


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