Enzyme Bioencapsulation Using Magnetic Fe_3o_4-Chitosan

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INTRODUCTION

Application of enzymes can be achieved more economically and efficiently by immobilization to enhance its activity, selectivity, and operation stability. However, the immobilized derivatives are difficult to separate from the reactive medium, except though the use of high-speed centrifugation. Enzymes immobilized in the presence of Fe_3O_4 nanoparticles have the advantage of being easily and effectively recovered by application of magnetic fields. The aim of this work was to investigate the potential of bioencapsulation method for *C. kikuchii* lipase immobilization using magnetic Fe_3O_4-chitosan microparticles. Two different techniques were evaluated: entrapment by cross-linking with sodium tripolyphosphate (TPP) and spray drying.

MATERIALS AND METHODS

Lipase Production

The *Cercospora kikuchii* lipase production was carried out according to Costa-Silva et al. (2011) and used for immobilization process.

Preparation of magnetic chitosan and enzyme immobilization

Fe_3O_4 nanoparticles were prepared by coprecipitaion method with a ferrous complex in presence of NH_4OH (Xie et al. 2009). Firstly, $FeCl_2.4H_2O$ and $FeCl_3.6H_2O$ [Fe^{2+}:Fe^{3+}=1:2] were dissolved in about 100 ml dionized water at a final concentration of 0.3 M iron ions. Next, this iron solution source was added drop-wise into NH_4OH under strong agitation and subsequently the solution was heated at a constant temperature of 80 °C for 30 min and then filtered and washed with distilled water and ethanol. Finally, the resultant precipitates were dried in oven at 102 °C and add to the chitosan acetate solution. The suspension cross-linking technique was used for the preparation of magnetic chitosan microparticles and enzyme immobilization. In this specific procedure, a 4% chitosan solution was prepared using a 5% aqueous acetic acid solution containing Fe_3O_4 dry magnetic nanoparticles (Fe_3O_4 content/chitosan ratio: 1/4) and 20 ml of lipase solution (1.2 g of lipase). The contents were stirred gently to ensure complete homogenization. This solution was then extruded dropwise through a peristaltic pump into a beaker containing 1000 ml of 0.136 mM sodium tripolyphosphate ($Na_5P_3O_{10}$) solution, which was prepared in 0.05 mM Tris–HCl (pH 8.0). The formed beads were recuperated by simple filtration and dried by oven (35 °C) for 24 hours – Figure 1A. The magnetic chitosan acetate solution containing the enzyme was also dried by spray drying. Drying was conducted in a bench-top spray dryer (model SD-05, Lab-Plant, Huddersfield, U.K.), with a concurrent flow regime. The feed flow rate of atomizing air was set at 17.0 L/min at a pressure of 147.1 kPa. The flow rate of the drying air was maintained constant at 60 m^3/h. The drying conditions were performed according to Costa-Silva et al. (2011). The Figure 1B shows the powder produced after spray drying.

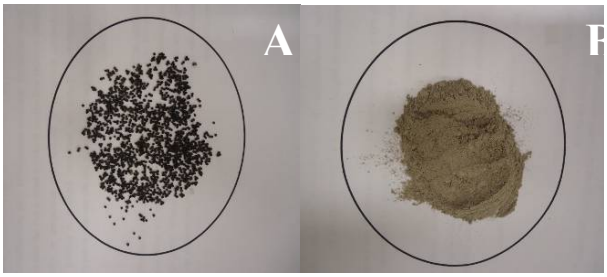

Figure 1. Lipase immobilized onto chitosan microparticles. A) Beads produced after cross-linking using sodium tripolyphosphate B) Powder produced after spray drying

Enzymatic activity

Lipase activity assay was performed using ρ-nitrophenyl palmitate (*p*-NPP) as substrate according to Mayordomo et al. (2000). One unit (U) of lipase activity was the amount of enzyme that released 1 µmol of p-nitrophenol/min under the aforementioned conditions. The activity retention (REA %) was calculated following the equation (1):

$$\text{REA (\%)} = 100 \times \frac{\text{Immobilized enzyme activity } \left(\frac{U}{mg}\right)}{\text{Soluble enzyme activity } \left(\frac{U}{mg}\right)} \quad (1)$$

Residual enzymatic activity determination for immobilized lipase after 5 batch of reaction (five cycles) was carried out. The immobilized derivative was recovered by centrifugation and washed with buffer (sodium phosphate buffer 50 mM, pH 6.5) for the next reuse.

RESULTS AND DISCUSSION

Table 1 shows the effect of immobilization conditions on outlet drying gas temperature (T_{go}), process yield, moisture content, and residual lipase activity (REA), of the immobilized derivatives.

Table 1: Dryer performance and product properties after bioencapsulation process

	Bioencapsulation Method	
	Spray drying	Cross-linking (TTP)
T_{go} (°C)	69.0±0.5	-
T_{in} (°C)	55.0±0.9	-
R_E (%)	53.5±1.4	-
REA (%)	85.9±1.2	75.6±1.6
aw (-)	0.3±0.03	0.2±0.02
Moisture (%)	5.8±0.5	3.8±0.8
Five cycles	25.9±1.1	52.8±1.4

T_{go}: outlet drying gas temperature; T_{in}: bed temperature; R_E: process yield; aw: water activity; REA: residual lipase activity.

The immobilization of lipase onto chitosan acetate powder obtained by spray drying process was more suitable for lipase activity retention than the cross-linking method. However, when it is considered the enzyme activity after five batches the use of sodium tripolyphosphate as a cross-linking agent for the beads production was the best immobilization condition. It was found that the immobilized derivative prepared by cross-linking method retained an activity of about 52.8% using *p*-NPP as substrate, after five reuses. In general, low values of moisture content (and water activity as well) are excellent for product stability. The moisture content in obtained immobilized derivatives varied between 3.8 and 5.8 % *(Table 1)*. The water activity is another factor that affects the enzyme stability. The water activities of immobilized derivatives are in the range 0.2-0.3. These low values are important because the dehydration could provide an acceptable protein shelf life, ease storage and transport and protect the biological activity of these molecules. The yield of immobilized lipase powder production by spray dryer was 53.5 %.

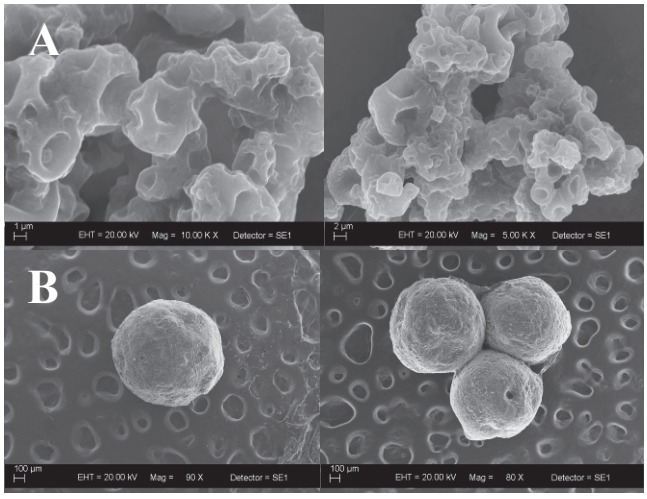

Figure 2: Scanning electron photomicrographs of microparticles: A) Powder produced by spray dryer B) Beads produced by cross-linking.

The outer morphologies of the immobilized derivatives are illustrated in Figure 2. The particles obtained by spray dryer did not show a defined format and the particle surface appeared grooved. For beads obtained by cross-linking method, the particle surface showed a rounded and well-defined shape. Figure 3 shows that the immobilized derivatives dispersed well in water, and aggregated within 10 s when a permanent magnet was nearby. The magnetic property will assist in the process of separation of the enzyme of the reaction medium and open new perspectives for enzymes utilization.

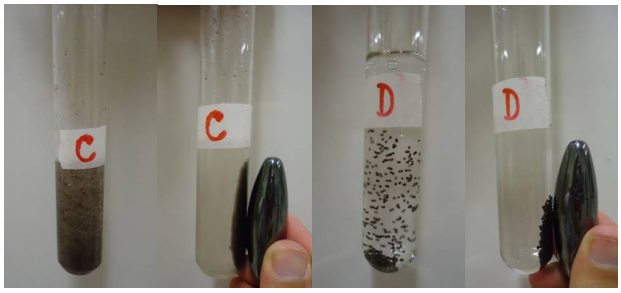

Figure 3: Dispersion and magnetic effect of immobilized derivatives

CONCLUSIONS

This work describes a successful method of magnetic chitosan microparticles production suitable for *Cercospora kikuchii* lipases immobilization. The bioencapsulation of lipase onto these particles showed little loss of enzyme activity, and the stability for the reuse cycles. Due to the good biocompatibility of chitosan, these particles may be used in magnetic-field assisted drug delivery, enzyme or cell immobilization and many other industrial applications.

REFERENCES

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