

Immobilization of hydrolytic enzymes on functionalized ferromagnetic nanoparticles coated with silica

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Abstract

Endoglucanase and β -glucosidase of *Trichoderma reesei*, and also pectinase from *Aspergillus niger*, all commercial enzymes, will be immobilized on spherical nanoparticles having a ferromagnetic core embedded in a functionalized silica. This immobilization technique might enable the target enzymes reuse in hydrolysis of different cellulosic biomass, such as orange bagasse, and make the enzyme hydrolysis less expensive and a greener way for ethanol second generation production.

Key words: Cellulase, pectinase, enzyme immobilization, magnetic nanoparticles.

Introduction

The high cost of hydrolases production can be overcome by developing the cost-effective strategy that allows the recovery and reuse of the enzymes, especially ones that hydrolyse lignocellulosic biomass. Herein, an enzyme immobilization technique which allows an easy magnetic separation of the immobilized enzyme from the reaction mixture aiming enzyme reuse is presented.

Results and Discussion

The ferromagnetic nanoparticles¹ were synthesized by co-precipitation method and 1:2 Fe²⁺/ Fe³⁺ ration. Then, the coating with silica was conducted by the Stöber method using silica and 3-aminopropyltriethoxysilane² (Image 1). The carboxylate function of these particles will be modified by adding succinic anhydride with glutaraldehyde (Image 2) and furthermore by adding humic acid or an aromatic acid functional group. The citrate buffer (pH 4.0) was the optimal condition for *A. niger* free pectinase at 50 °C, corresponding to 0.80 \pm 0.01 U mL⁻¹ activity (Image 3). The endoglucanase and β -glucosidase of *T. reesei* had their activities³ determined as 1.87 \pm 0.05 U mL⁻¹ and 2.32 \pm 0.06 U mL⁻¹ as free enzymes, respectively, at pH 5.0.

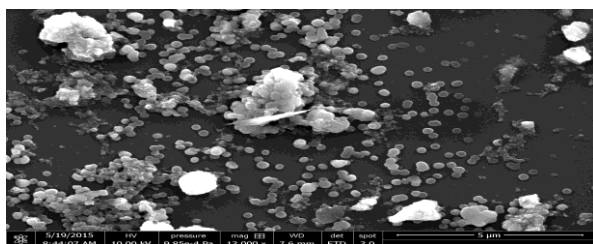


Image 1. Scanning electron micrograph showing the ferromagnetic nanoparticles coated with silica.

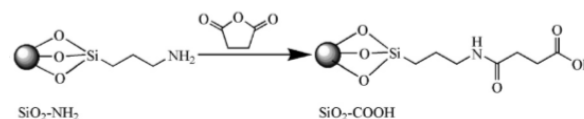


Image 2. Reaction used in silica surface modification just before the enzymes immobilization.

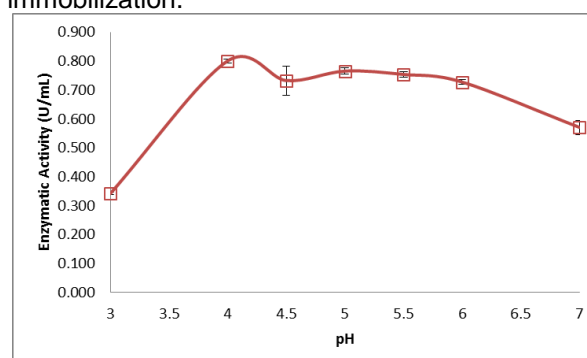


Image 3. Evaluation of pectinase activity (*Aspergillus niger*) under different pH conditions.

Conclusions

The immobilized enzymes can be magnetically recovered and reused until their remnant enzymatic activities continue to be high. Enzymes reuse aims to add in production of fermentable sugars from orange bagasse and similar cellulosic biomass. The hydrolyses yields and stabilities of all target, free and immobilized, enzymes are being determined.

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