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Immobilization of pectinase on silica-based supports: Impacts of particle size and spacer arm on the activity

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ABSTRACT

The pectinase was separately immobilized onto Florisil and nano silica supports through both glutaraldehyde and 3-glyoxypropyltrietoxysilane spacer arms. The effects of spacer arm, particle size of support and ionic liquids on the activities of pectinase preparations were investigated. The immobilization of pectinase onto Florisil and nano silica through 3-glyoxypropyltrietoxysilane spacer arm completely led to inactivation of enzyme; however, 10 and 75% pectinase activity were retained when it was immobilized through glutaraldehyde spacer arm onto Florisil and nano silica, respectively. The pectinase immobilized onto nano silica through glutaraldehyde spacer arm showed 6.3-fold higher catalytic efficiency than that of the pectinase immobilized onto Florisil through same spacer arm. A 2.3-fold increase in thermal stability of pectinase was provided upon immobilization onto nano silica at 35 °C. The effects of IL/buffer mixture and volume ratio of IL/buffer mixture on the catalytic activities of free and immobilized pectinase preparations were also tested. All the pectinase preparations showed highest activity in 10% (v/v) 1-butyl-3-methylimidazolium hexafluorophosphate containing medium and their activities significantly affected from the concentration of 1-butyl-3-methylimidazolium hexafluorophosphate.

1. Introduction

Pectinases catalyze the hydrolysis of α-1-4-glycosidic linkage of polygalacturonic acid [1] and widely used in food and wine industries to clarify fruit and vegetable juices [2]. In spite of their high catalytic properties, free enzymes suffer from their cost, low stability and lack of repeated continuous uses in industrial operations [3,4]. Enzyme immobilization is one of the most popular strategies to enhance enzyme stability, recyclability and to also facilitate product recovery [4,5]. To date, pectinases have been immobilized onto various supports such as silica-coated chitosan [6], oxidized pulp fiber [7], agar–agar [8], and entrapped in polyvinyl alcohol sponge [1] and alginate [9].

The geometric properties of the selected support, such as shape, size, thickness, and length are one of the important factors to obtain high retention of activity upon immobilization [10]. In recent years, using nanomaterials in enzyme immobilization has become interesting research area to enhance activity and stability of enzymes due to their large specific surface area [11–13]. Silica nanoparticles have been increasingly used as enzyme support material due to the their easy synthesis, handling and modification, high thermal and mechanical stability, resistance in organic solvents or acidic conditions [14]. Lei et al. [15] immobilized pectinase covalently onto amino (-NH_2) and carboxylic acid (-COOH) functionalized nanoporous silica and reported that pectinase activity was increased by the immobilization. Seenuvasan et al. [16] immobilized pectinase onto amino functionalized silica-coated magnetic nanoparticles activated with glutaraldehyde and reported that the immobilization led to enhancement stability and activity of pectinase.

Another way for enhancing enzyme activity and stability is medium engineering [17,18]. Ionic liquids (ILs) offer many advantages in biocatalytic applications since their properties depending on the structure of the ions can be easily tunable by changing them [19]. In recent years, reaction media containing IL have been used as an alternative to reaction media containing pure buffer in carbohydrate industry to dissolve carbohydrates which are difficult to be dissolved in buffers and/or to enhance activity and stability of enzymes [20–23].

Although many protocols are available for the covalent immobilization of enzymes, using supports activated with oxirane group and/or the supports containing primary free amino groups

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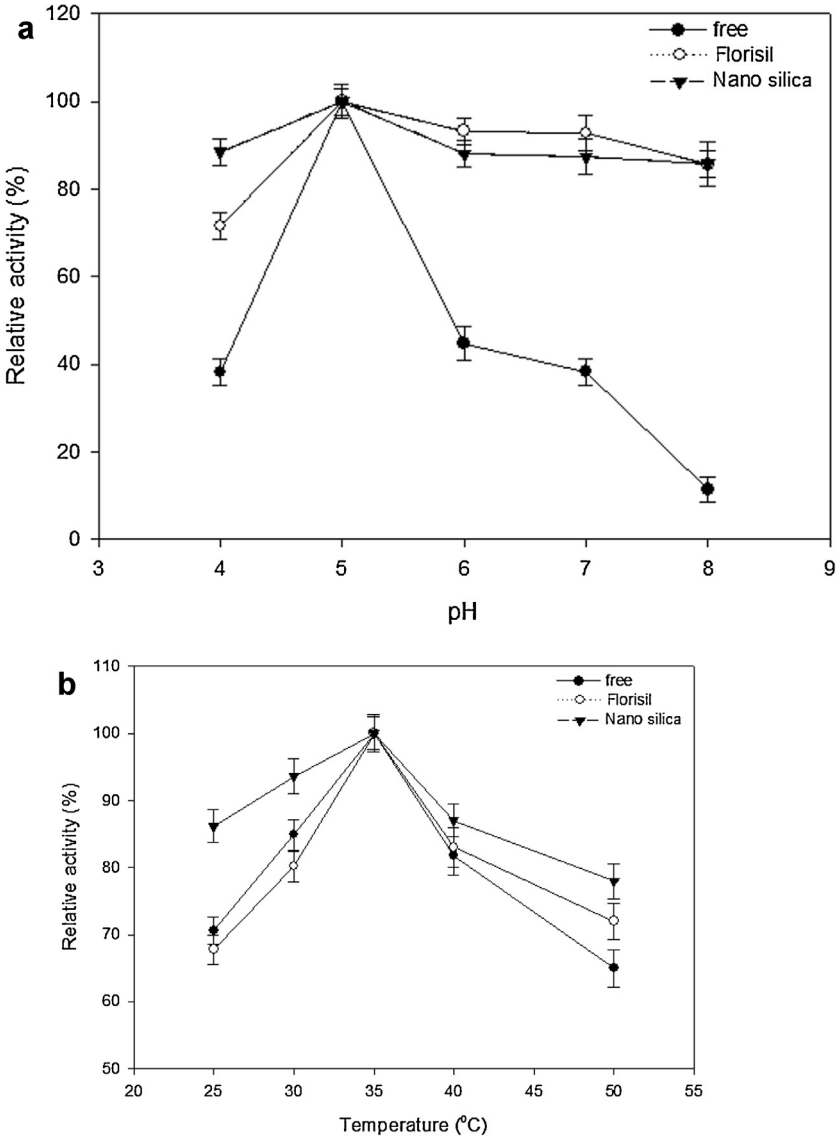

Fig. 1. (a) The effect of pH on the activities of free and immobilized pectinase preparations. The pectinase activity at pH 5.0 was taken as 100% for all the preparations. The experiments were run in triplicate. (b) The effect of temperature on the activities of free and immobilized pectinase preparations. The enzyme activity at 35 °C is taken as 100% for all the preparations. The experiments were run in triplicate.

and then extended with glutaraldehyde are widely applied immobilization protocols. Because, these methods are quite simple and efficient, and allow the improvement of enzyme activity and stability [24–27]. The aim of this study is to develop a highly active and durable immobilized pectinase preparation. Hence, the pectinase was covalently immobilized onto Florisil and nano silica supports through both 3-glyoxypropyltrietoxysilane and glutaraldehyde to generate oxirane and aldehyde reactive groups. The effects of particle size and spacer arm on the catalytic activities of immobilized pectinase preparations were investigated. Furthermore, the effects of IL/buffer mixture and volume ratio of IL/buffer mixture on the catalytic activities of free and immobilized pectinase preparations were tested. The ILs used in this study were 1-butyl-3-methylimidazolium acetate ([Bmim][OAc]), 1-butyl-3-methylimidazolium trifluoromethanesulfonate ([Bmim][TfO]), 1-butyl-3-methylimidazolium tetra fluoroborate ([Bmim][BF_4]), and 1-butyl-3-methylimidazolium hexafluorophosphate ([Bmim][PF_6]).

2. Material and methods

2.1. Material

Pectinase from *Aspergillus aculeatus* (aqueous solution, ≥3800 units/mL), 3,5-dinitrosalicylic acid, potassium sodium tartrate, sodium hydroxide, glutaraldehyde (50% solution, w/w), (3-Aminopropyl) triethoxysilane (3-APTES), (3-Glycidyloxypropyl) trimethoxysilane (3-GPTMS), nano silica (mesoporous, 200 nm particle size, pore size 4 nm) were purchased from Sigma Chemical Co (St. Louis, MO, USA). Florisil (magnesium silicate) (0.150–0.250 μm) and ILs were supplied from Merck (Darmstadt, Germany).

2.2. Modification of supports

2.2.1. Glutaraldehyde spacer arm

Florisil and nano silica supports were separately modified with 3-APTES and glutaraldehyde according to [28]. Briefly, 1 g of the supports were rinsed with 10 mL of HNO_3 solution (5% in water,