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Full Length Article

Polyaniline-coated magnetic diatomite nanoparticles as a matrix for immobilizing enzymes

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ABSTRACT

This work proposes a simple, cost-effective, and efficient preparation of a composite made from magnetic diatomaceous earth (mDE) coated with polyaniline (mDE@PANI). The material was used as a matrix for immobilizing industrial enzymes: invertase, β-galactosidase, and trypsin. The mDE@PANI was characterized by several methods, and the results suggested that the nanoparticles were approximately 12 nm in size, exhibited superparamagnetic behavior, and displayed a good magnetic response and that magnetite comprised the main iron oxide phase. Moreover, several studies were conducted for all immobilized derivatives, including determination of optimal pH and temperature; kinetic parameters; thermal stability and reusability. The obtainment of three novel magnetic biocatalysts with superior performance (in terms of activity and stability) compared to their free counterparts demonstrated the efficacy of the mDE@PANI nanoparticles. In addition, the enzymatic derivatives can be easily recovered from the reactor by using an external magnetic field. Finally, the present methodology allowed the achievement of good mDE@PANI matrix together with three promising magnetic biocatalysts with several potential biotechnological applications.

1. Introduction

Enzymes are important biological macromolecules widely used in industrial applications. Their immobilizations confer advantages, such as improved thermal stability and reusability. The literature is replete with studies about the immobilization of enzymes on different matrices [1,2]. It is well known that the matrix has significant effects on the performance of the catalyst. Preparation of a composite material comprising two or more organic and/or inorganic compounds could be an interesting approach toward developing a matrix for biomolecule immobilization. Among inorganic materials, diatomaceous earth (DE), a lightweight mineral clay composed mainly of amorphous hydrated silica ($SiO_2{\cdot}nH_2O$), has attracted much attention due to many of its properties, such as high cation exchange capacity, large surface area, porous structure, chemical inertness, low thermal conductivity, low cost, and ready availability [3]. DE (natural or modified) has been explored in several biotechnological applications, with the removal of heavy metals [4–11] being the main application reported in the literature. However, more recent studies have described the use of this material as a potential functional drug carrier [12–15] and as a new poultry vaccine adjuvant [16]. Recently, Chen et al. [17] have reported the wide potential applications of functional nanocomposites produced from both magnetite and this type of material (mineral clay). In addition, some researchers have produced electrically conducting diatomite by coating it with polyaniline (PANI), one of the most-studied conducting polymers [18–21]. To the best of our knowledge, little attention has been paid to modified DE as a matrix for biomolecule immobilization [22–26]. PANI is a material highlighted by its direct electron transfer capability with immobilized enzymes [27]. The effects of PANI on catalytic performance of enzymes have been related to the immobilization methods. For adsorptive interactions, repercussions in enzymatic activity were reported as a consequence of the change on the

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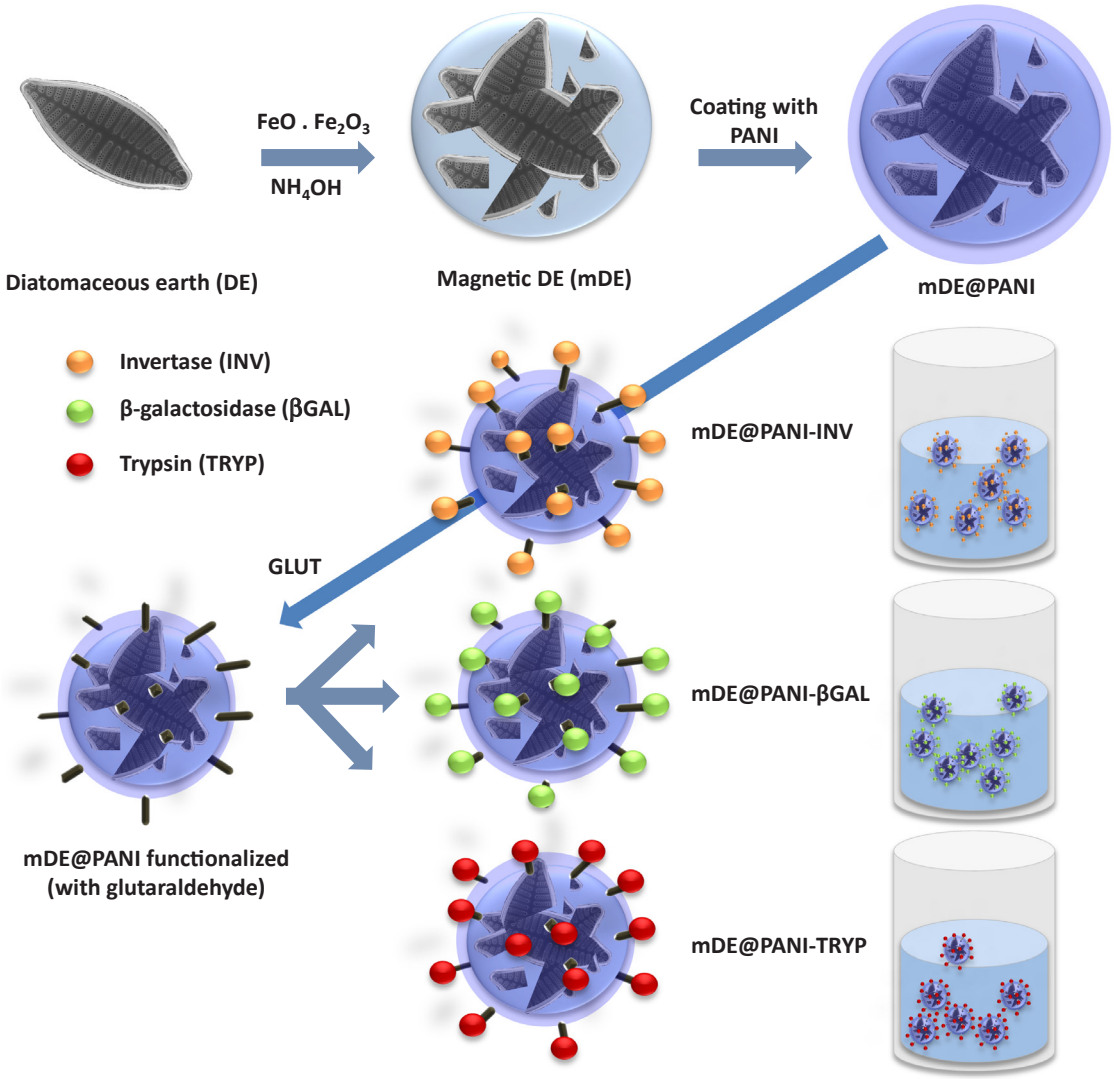

Scheme 1. Chemical strategy applied for the synthetic preparation of mDE@PANI nanoparticles and the three magnetic biocatalysts obtained from them.

enzyme active site after immobilization process [28]. And for covalent immobilization, intermediate oxidation states of PANI implied a greater amount of glutaraldehyde bound which, in turn, allowed better activity retention when compared with the fully oxidized or reduced states of the conducting material [29]. Here, PANI coating on magnetic DE (mDE) added chemical groups ($-NH_2$) on magnetic surface allowing covalent binding of enzymes. Therefore, the mDE coated with PANI (mDE@PANI) followed the chemical strategy (Scheme 1) to covalently immobilize three industrial enzymes: invertase (EC 3.2.1.26), β-galactosidase (EC 3.2.1.23), and trypsin (EC 3.4.21.4). Invertase is mostly used to convert sucrose to invert sugar (glucose and fructose) that is an important product in the food and beverage industries. In our laboratory invertase has been previously immobilized on magnetic DE (mDE) [30,31]. However, the enzyme was covalently immobilized on mDE functionalized by a silane agent (APTES – aminopropyltriethoxysilane). Another enzyme of particular interest is β-galactosidase that hydrolyzes lactose to glucose and galactose in dairy products. This is important β-galactosidase application for the production of free lactose foods aiming people suffering from lactose intolerance. Furthermore, production of galacto-oligosaccharides (non-digestible carbohydrate) from lactose by β-galactosidase immobilized on magnetic materials has been widely explored [32–38]. Trypsin also has many different industrial and biomedical applications including, in particular, as a laboratory reagent for proteomic analysis [39–42].

The major contribution of this work is the employment of the mDE@PANI nanoparticles as an efficient and attractive matrix for covalent immobilization of the enzyme. Several physical–chemical techniques were used to characterize the mDE@PANI as well as some properties of the three novel magnetic biocatalysts were studied: optimal pH and temperature; kinetic parameters; thermal stability and reusability.

2. Materials and methods

2.1. Materials

DE was kindly donated by TAMER Company (Salta, Argentina). Invertase (Baker's yeast), β-galactosidase (*Aspergillus oryzae*), trypsin (porcine pancreas), potassium permanganate, aniline, glutaraldehyde, bovine serum albumin, lactose, N-α-benzoyl-DL-arginine-p-nitroanilide (BApNA), and dimethylsulfoxide (DMSO) were purchased from Sigma Aldrich Chemicals (St. Louis, USA). All other chemicals were of the highest purity available commercially.

2.2. Preparation of mDE@PANI nanoparticles ($mDE@PANI_{nano}$)

The mDE was prepared according to the procedure described by Cabrera et al. [31]. Before the PANI-coating process, mDE was washed

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