Journal of Magnetism and Magnetic Materials 375 (2015) 117–123

Contents lists available at ScienceDirect

Journal of Magnetism and Magnetic Materials

journal homepage: www.elsevier.com/locate/jmmm

Increase in stability of cellulase immobilized on functionalized magnetic nanospheres

Wenjuan Zhang [a], Jianhui Qiu [a,*], Huixia Feng [b], Limin Zang [a], Eiichi Sakai [a]

[a] Department of Machine Intelligence and Systems Engineering, Faculty of Systems Engineering, Akita Prefectural University, Akita 015-0055, Japan
[b] College of Petrochemical Engineering, Lanzhou University of Technology, Lanzhou 730050, China

ARTICLE INFO

Article history:
Received 2 July 2014
Received in revised form
11 September 2014
Available online 5 October 2014

Keywords:
Magnetic nanoparticle
Immobilized cellulase
Biofule
Stability

ABSTRACT

Functionalized magnetic nanospheres were prepared by co-condensation of tetraethylorthosilicate with three different amino-silanes: 3-(2-aminoethylamino propyl)-triethoxysilane (AEAPTES), 3-(2-aminoethylamino propyl)-trimethoxysilane (AEAPTMES) and 3-aminopropyltriethoxysilane (APTES). Then three functionalized magnetic nanospheres were used as supports for immobilization of cellulase. The three functionalized magnetic nanospheres with core–shell morphologies exhibited higher capacity for cellulase immobilization than unfunctionalized magnetic nanospheres. The increasing of surface charge of functionalized magnetic nanospheres leads to an enhancement of the capacity of cellulase immobilization. Particularly, AEAPTMES with methoxy groups was favored to be hydrolyzed and grafted on unfunctionalized magnetic nanospheres than the others. AEAPTMES functionalized magnetic nanospheres with the highest zeta potential (29 mV) exhibited 87% activity recovery and the maximum amount of immobilized cellulase was 112 mg/g support at concentration of initial cellulase of 8 mg/mL. Immobilized cellulase on AEAPTMES functionalized magnetic nanospheres had higher temperature stability and broader pH stability than other immobilized cellulases and free cellulase. In particular, it can be used in about 40 °C, demonstrating the potential of biofuel production using this immobilized cellulase.

1. Introduction

Bioethanol from cellulosic biomass is one of the most promising biofuels to lessen the environmental pollution generated by burning of fossil fuels [1,2]. Conversion of cellulosic materials to ethanol is a complex process. Initially, cellulose is hydrolyzed into reducing sugar by cellulase and reducing sugar is subsequently converted into bioethanol [3,4]. These two steps can be performed in a combined hydrolysis and fermentation, referred to as simultaneous saccharification and fermentation (SSF), which is mainly used for bioethanol production [5,6]. Cellulase is a multicomponent enzyme consisting of three different enzymes (1,4-β-D-glucanases or endoglucanases, exo-1,4-β-D-glucanases or cellobiohydrolases and 1,4-β-D-glucosidases). Enzymes are biocatalysts possessing some excellent properties, such as of high activity, selectivity and specificity [7]. Nevertheless, the cellulase is often easily inactivated and difficult to be separated [8,9]. The optimal temperature for SSF is about 38 °C, which is a compromise between the optimal temperatures for hydrolysis (45–50 °C) and fermentation (30 °C). The efficiency of free cellulase is limited by their deactivation at about 38 °C [10]. Therefore, developing effective strategies for improving enzyme stability is an important target for ethanol production.

Immobilization of enzymes on supports in general helps to improve the enzyme stability by increasing the resilience of enzymes to variation of pH and temperature [11–14]. Immobilization of cellulase has been achieved using a diverse range of methodologies (adsorption on nanostructured materials and covalent binding of cellulase on nanomaterials) [15]. Covalent binding normally leads to the partial inactivation due to conformational restrictions caused by covalent binding of amino acid residues to support. Adsorption has the highest commercial potential, due to its relatively low cost, simplicity, and high retention of catalytic activity [16].

Magnetic particles are important material for a wide range of application, such as bioseparation [17], hyperthermia [18–20], and biomolecules immobilization. Fe_3O_4 nanoparticles have received extensive attention in enzyme immobilization to improve enzyme activity, loading and stability. Fe_3O_4 nanoparticles with low toxicity, biocompatibility, and easy synthesis are more suitable supports for enzyme as compared to others [21,22]. Surface modification using organic functional groups has been found to be useful for the immobilization and adsorption of enzymes to the surface of

* Corresponding author. Fax: +81 184 272134,
E-mail address: qiu@akita-pu.ac.jp (J. Qiu).

http://dx.doi.org/10.1016/j.jmmm.2014.09.067
0304-8853/

the silica material [23,24]. Magnetite nanoparticles coated with silica and modified by organic-silanes, biocompatible and with hydrophilic properties, are promising approach in nanobiotechnology. The huge diversity of alkoxysilanes allows for different types of nanoparticle surface functionalization, i.e., introducing charges on the system that can prevent the aggregation of the particles in liquids and improve their biocompatibility [25,26]. Surface modification by silanes is a complex process [27,28]: First, the silane monomers are hydrolyzed liberating alcohol and yielding reactive silanol groups. Second, during the hydrolysis process, the concomitant condensation of silanols (aging) also takes place. Finally, silanes are adsorbed and chemically grafted on magnetic silica nanospheres surfaces. However, few papers have reported on the functionalized magnetic silica nanospheres is used as supports for immobilization of cellulase through the interface-containing amino groups. Silane structure is correlated to silane grafting on the surface of functionalized magnetic silica nanospheres and immobilization of cellulase. So the control of grafted silane becomes an important step in this system.

In the present work, functionalized magnetic nanospheres were prepared by hydrolysis of amino-silane and grafting of amino-silane on the surface of unfunctionalized magnetic nanospheres, and then immobilization of cellulase on functionalized magnetic nanospheres was conducted by electrostatic adsorption. (Scheme 1). The influence of silane structure in alkoxysilanes containing the amino groups on modification of unfunctionalized magnetic nanospheres surface was studied by transmission electron microscopy (TEM), zeta potential analysis, thermogravimetric analysis (TGA), and vibrating sample magnetometry (VSM). The properties of immobilized cellulase on these functionalized magnetic nanospheres were investigated, including the amount of immobilized cellulase and its relative activity and stability.

2. Experimental method and characterization

2.1. Materials

Acremonium cellulase was purchased from Meiji Seika Pharma Co., Ltd., Japan. Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), tetraethylorthosilicate (TEOS), carboxymethyl cellulose sodium salt (CMC), were all extra pure reagent grade and purchased from Nacalai Tesque, Inc., Japan. 3,5-dinitrosalicylic acid (DNS) and ammonium hydroxide ($NH_3 \cdot H_2O$, 28 wt%) was obtained from Nacalai Tesque, Inc. The amino-silane coupling agents (APTES, AEAPTES and AEAPTMES) were purchased from Shin-Etsu Chemical Co., Ltd. Japan. The chemical structures and description are listed in Table 1.

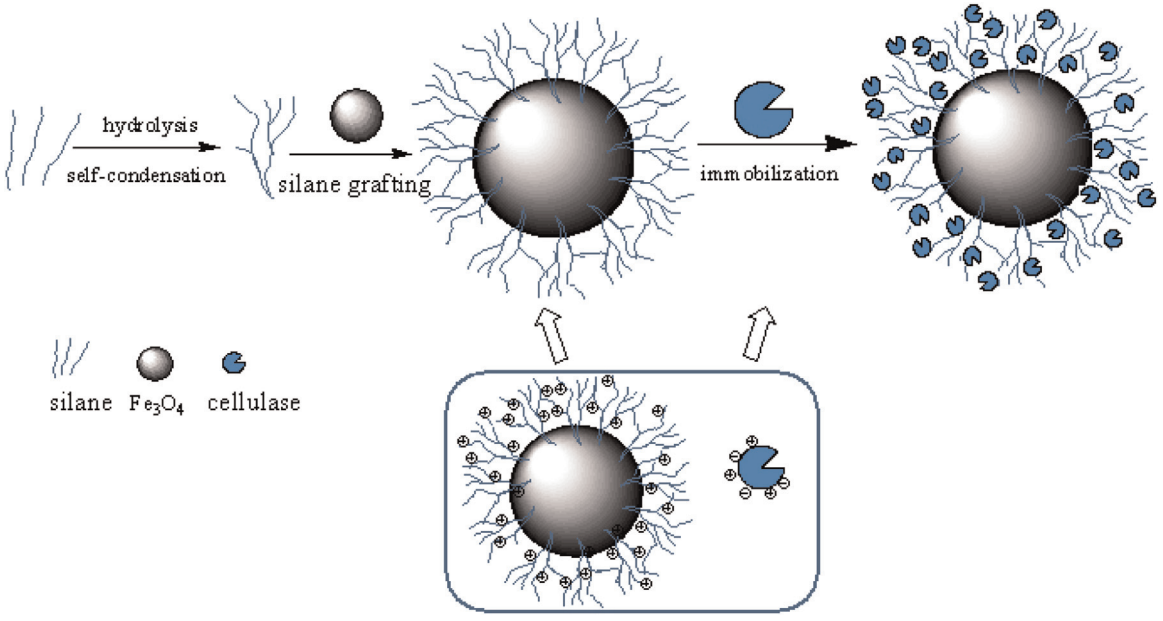
2.2. Synthesis of functionalized magnetic nanospheres

Magnetite (Fe_3O_4) nanoparticles were prepared by the conventional chemical coprecipitation [29]. Silica coated nanospheres were prepared by the Stöber method [30]. First, 2 g magnetite particles were mixed with 40 mL water, 160 mL alcohol and 5 mL $NH_3 \cdot H_2O$ by ultrasonic vibration for 1 h, and then 5 mL TEOS was added in the mixture under continuous vigorous stirring for 12 h at room temperature, silica was formed on the surface of magnetite nanoparticles through hydrolysis and condensation of TEOS. The resulting particles unfunctionalized magnetic nanospheres were washed ten times with distilled water and then dried at 50 °C under vacuum for 24 h.

Unfunctionalized magnetic nanospheres (containing 1 g Fe_3O_4) were added to 60 mL alcohol. The solution was treated by ultrasonic vibration for 30 min at room temperature. 6 mL $NH_3 \cdot H_2O$ and 4 mL amino-silane (AEAPTES, AEAPTMES and APTES) were added into the mixture and then the temperature was increased to 50 °C under nitrogen gas with vigorous stirring for 8 h [21]. The magnetic nanoparticles were washed ten times with distilled water and dried at 50 °C under vacuum for 24 h. The resulted functionalized magnetic nanospheres were denoted as S1, S2 and S3, respectively.

2.3. Characterization of functionalized magnetic nanospheres

The morphologies of functionalized magnetic nanospheres were characterized by TEM (Hitachi H-8100, Japan). For TEM observations, samples were dispersed in ethanol and then a small drop of the suspension was spread onto a 400 mesh copper grid. Zeta potential measurements were conducted at pH 3–7 using in 1 mM KCl solution by Zeta potential analyzer (Brookhaven instruments corporation, USA). Structures of functionalized



Scheme 1. Synthesis of functionalized magnetic silica nanospheres and immobilization of cellulose.