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Immobilization of inactivated microbial cells on magnetic Fe_3O_4@CTS nanoparticles for constructing a new biosorbent for removal of patulin in fruit juice

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ABSTRACT

Patulin contamination of juice is an important food safety issue throughout the world. In this paper, magnetic Fe_3O_4@CTS nanoparticles were coated with inactivated *C. utilis* CICC1769 cells and used as a new biosorbent for the removal of patulin in fruit juice. Prepared nanoparticles were characterized by X-ray diffraction and FT-IR spectra analyses and the results indicated that inactivated *C. utilis* CICC1769 cells were successfully immobilized on the surface of magnetic Fe_3O_4@CTS. Furthermore, this new biosorbent was found to reduce patulin by over 90% in the orange juice without any significant negative impacts on the quality parameters such as Brix, Vitamin C, and titratable acidity. This study shows that there are promising industrial applications of the as-prepared biosorbent for juice-detoxification.

1. Introduction

Patulin is a mycotoxin produced by certain moulds of the genera *Aspergillus*, *Penicillium* and *Byssochlamys* (Sewram, Nair, Nieuwoudt, Leggott, & Shephard, 2000). *Penicillium. expansum* is the most common producer of patulin in apples (Luo, Li, Yuan, & Yue, 2016). Patulin is a polyketide lactone and frequently found in rotten foods such as fruits, vegetables and grains (Appell, Jackson, & Dombrink-Kurtzman, 2011). At high doses, it is acutely toxic, and can cause serious immunotoxic and neurotoxic damages to human health, particularly to children (Prieta, Moreno, Diaz, Suarez, & Dominguez, 1994). Patulin contamination has become a severe food safety issue because of the remarkably increase in the consumption of fruits and juices throughout the world. Therefore, a number of countries have established maximum permitted levels of patulin in susceptible products. The World Health Organization (WHO) recommends a maximum concentration of 50 μg/L of patulin in apple juice, and the EU and China also have set a maximum limit for patulin of 50 μg/kg in fruit juices and in drinks containing apple juice or derived from apples.

Over the past decades, many approaches including chemical oxidation, physical absorption and biosorption, were developed to remove or degrade patulin for detoxification from contaminated foods and feeds. However, most of these approaches are not widely available because of chemical hazards, low detoxification efficiency, disposal of secondary wastes, impact on the quality of products, etc. (Moake, Padilla-Zakour, & Worobo, 2005; Sant'Ana, Rosenthal, & de Massaguer, 2008; Hatab, Yue, & Mohamad, 2012; Wang et al., 2015). Biosorption by inactivated microbial cells is a promising strategy due to high removal efficiency for patulin and minimal effects on product qualities (Wang et al., 2015). Numerous inactivated microorganisms including bacteria and yeasts have been tested for their adsorption capacity of patulin from aqueous solutions and various fruit juices. Yue, Dong, Guo, and Worobo (2011) examined 10 yeast strains and found that 2 inactivated yeast strains could reduce patulin levels by over 70% in apple juice within 24 h. Hatab et al. (2012) found that *Lactobacillus rhamnosus* 6224 and *Enterococcus faecium* 21605 could cause a decrease of patulin by 80.4% and 64.5%, respectively, in apple juice for 24 h. *Enterococcus faecium* M74 and EF031 also removed over 45% of the total patulin from liquid medium (Topcu, Bulat, Wishah, & Boyaci, 2010). Guo, Yue, Hatab, and Yuan (2012) investigated the adsorption

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removal of patulin from an apple juice using 2 types of inactivated laboratory-prepared yeast powders and 1 commercial yeast powder. As a conclusion, all these studies demonstrated that inactivated microorganism had potentials to be used as a novel and promising adsorbent to bind patulin with high efficiency.

There is a need for developing a new economic and eco-friendly biosorbent for absorptions of patulin, due to the fact that completely separation and recyclability of the free inactivated microorganism cells from the patulin absorption system are intractable in the practical applications. Magnetic nanoparticles can be used as ideal solid carriers in various immobilization strategies due to their unique high saturation magnetization for facile separation and ease of recyclability (Ansari & Husain, 2012). Additionally, chitosan has been widely used for coating magnetic nanoparticles to keep from agglomeration and thus increase their stabilities, and to provide functional groups for the applications of microbial cell immobilization (Leceta, Guerrero, & de la Caba, 2013). Therefore, magnetic nanoparticles coated by chitosan that immobilize inactivated microbial cells could provide a new separable and recyclable biosorbent, and thus solve the practical problems in patulin removal.

In this study, magnetic nanoparticles were firstly prepared by co-precipitation of Fe_3O_4 with protection of N_2 and PEG-2000 as a surfactant, and then chitosan was coated on Fe_3O_4 nanoparticles (Fe_3O_4@CTS) by suspension crosslinking method with glutaraldehyde as chemical crosslinker. A new magnetic inactivated-cell biosorbent was then constructed by immobilizing inactivated microbial cells on those magnetic Fe_3O_4@CTS nanoparticles by swelling-absorption method. The resulting nanoparticles were characterized in detail by X-ray diffraction and Fourier transform infrared (FT-IR) spectroscopy to determine the structure and validate the synthetic route of this absorbent. Finally, the ability of the new biosorbent to remove patulin from the fruit juices was evaluated.

2. Materials and methods

2.1. Chemicals

Reagents such as $FeCl_2 \cdot 4H_2O$ (analytical grade) and $FeCl_3 \cdot 6H_2O$ (analytical grade), chitosan powders and glutaraldehyde were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Standard patulin and acetonitrile (HPLC grade) were purchased from Sigma-Aldrich (St Louis, MO, USA). All other chemicals used in the experiments were obtained from local chemical reagent company. Distilled water was used for all the experimental process.

2.2. Bacterial strains

A total of 8 bacterial strains were collected to analyze their capacity of removing patulin, and the most efficient strain would be chosen for the next experiments. The name and source of each strain are represented in Table 1.

2.3. Preparation of inactivated freeze-dried bacterial powder

The strains of CICC 1769, CCTCC AY 93047, and CCTCC AY 93161 were cultured for 48 h in YEPD medium at 30 °C with a shaking rate of 120 rpm, and the rest 5 strains were cultured for 60 h in MRS broth at 37 °C with a shaking rate of 120 rpm. After incubation, bacterial cells were centrifuged at 4000 rpm for 20 min, and sterilized by high pressure steam at 121 °C for 20min for inactivation (Hatab et al., 2012). Then the inactivated cells were precooled at −20 °C for 12 h and freeze-dried under −54 °C and 5 mtorr for 26 h by a pilot scale freeze-dryer (LGJ-18S, Songyuanhuaxing Technology Co., Ltd. Beijing, China).

2.4. Synthesis of magnetic Fe_3O_4@CTS nanoparticles

The magnetic Fe_3O_4@CTS nanoparticles were prepared through a two-step wet chemical process using the method reported by Li, Jiang, Huang, Ding, and Chen (2008) and Unsoy, Yalcin, Khodadust, Gunduz, and Gunduz (2012) with slight modifications. Firstly, the synthesis of Fe_3O_4 nanoparticles was carried out, and then followed by coating these nanoparticles with chitosan. The first step involved synthesis of fine grained Fe_3O_4 nanoparticles prepared through a conventional co-precipitation approach at the presence of PEG-2000 as a surfactant using the following procedure. Ferric chloride (10.82 g) and ferrous chloride (4.00 g) were dissolved in 100 mL of distilled water with a molar ratio of 2:1 at 35 °C, and then mixed with 2 mL of hydrochloric acid solution (12 mol/L) and 0.8 g of PEG-2000. The pH of mixture was immediately adjusted to 9.5 by ammonia water with a concentration of 28%. The system was continuously aerated by compressed N_2 and stirred by a mechanical stirrer with a stirring rate of 800 rpm during the whole above procedure. After vigorous stirring for 15 min the mixture was heated by water bath at 70 °C for 30 min, and then the precipitates were separated by centrifugation and washed by anhydrous ethanol for times. Finally, the Fe_3O_4 nanoparticles were dried in an oven at 50 °C to until the weight was constant.

In the second step, chitosan was coated on the surfaces of magnetic Fe_3O_4 nanoparticles through suspension crosslinking method. Chitosan powders (2.00 g) were dissolved in 200 mL of 5% acetic acid and then mixed with 2.00 g of Fe_3O_4 magnetic nanoparticles, 200 mL of liquid paraffin and 5 mL of span-80. The mixture was processed by ultra-sonication for 20 min for even dispersion of magnetic Fe_3O_4 nanoparticles in the system. Then, 40 mL of 25% (w/w) glutaraldehyde solution was added in the system and the mixture was vigorously stirred for 4 h at 50 °C for crosslinking. The magnetic Fe_3O_4@CTS nanoparticles were separated from the system with a magnet and rinsed sequentially with petroleum ether, acetone and deionized water. After centrifugation (5000 rpm) the magnetic Fe_3O_4@CTS nanoparticles were finally dried in a vacuum oven at 50 °C.

Table 1
Names and sources of bacterial strains involved.

No	The name of strains	The source of strains
1	*Lactobcillus brevis* CICC 20023	China Center of Industrial Culture Collection (CICC, Beijing, China)
2	*Bifidobacterium bifidum* CICC 6071	CICC
3	*Lactobacillus acidophilus* CICC 6081	CICC
4	*Lactobacillus rhamnosus* CICC 6224	CICC
5	*Lactobacillus rhamnosus* CICC 21605	CICC
6	*Candida utilis* CICC 1769	CICC
7	*Hansenula anomala* var. *anomala* CCTCC AY 93047	China Center for Type Culture Collection (CITCC,Wuhan,China)
8	*Saccharomyces cerevisiae* CCTCC AY 93161	CITCC