Journal of Industrial and Engineering Chemistry 25 (2015) 138–144

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

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

Separation of isoflavone aglycones using chitosan microspheres from soy whey wastewater after foam fractionation and acidic hydrolysis

Wei Liu, Zhao Liang Wu *, Yan Ji Wang **, Rui Li, Na Na Yin, Jian Xing Jiang

School of Chemical Engineering and Technology, Hebei University of Technology, No.8 Guangrong Road, Dingzi Gu, Hongqiao District, Tianjin, 300130, China

ARTICLE INFO

Article history:
Received 22 July 2014
Received in revised form 6 October 2014
Accepted 11 October 2014
Available online 27 October 2014

Keywords:
Isoflavone aglycone
Industrial wastewater
Chitosan microspheres
Adsorption

ABSTRACT

The purpose of this work was to separate isoflavone aglycones from soy whey wastewater after foam fractionation and acidic hydrolysis using chitosan microspheres. The maximal equilibrium adsorption capacity could be obtained at pH 6.0. The adsorption isotherm fitted better to the Freundlich equation model and the adsorption was an exothermic and spontaneous physical process. The maximal desorption ratio of isoflavone aglycones could reach 94.82% by using 70% (v/v) ethanol as desorption solution. Moreover, the regenerated chitosan microspheres could be reused for separating isoflavone aglycones from the feeding solution up to five times without compromising their function.

Introduction

Chitosan, a natural linear biopolysaccharide is obtained by alkaline deacetylation of chitin, which is composed of β-(1→4) linked 2-amino-2-deoxy-D-glucopyranose units and residual 2-acetamindo-2-deoxy-D-glycopyranose units [1]. It not only keeps the favorable biocompatibility and biodegradability of chitin, but also possesses of the excellent antibacterial activity [2]. The structural formula of chitosan is showed in Fig. 1. From a structural point of view, chitosan can form numerous intramolecular and intermolecular hydrogen bonding for the existence of amino and hydroxyl groups. The free amino group is easily protonated under acidic condition leading chitosan molecule carries the positive charge, thus in turn reacts with many negatively charged surfaces/polymers and also undergoes chelation with metal ions [3,4]. Moreover, chitosan can also be developed as a bio-adsorbent for adsorbing various organic acids through hydrogen bonding or ion exchange interactions because of its outstanding characters, such as rich source, low price and friendly environment. Chitosan is generally made into cross-linked microspheres used as the carrier for drugs [5,6].

At present, adsorption technique has been widely used to separate materials from their crude extracts [7,8]. Active carbon, silica gel, kaolinitic clay and aluminium oxide are the most common adsorbents [9,10]. In addition, the emergence of synthetic polymer adsorbents further expands the applications of adsorption [11,12]. Some natural polymers are also developed as adsorbents for meeting the increasingly rigorous requirements on safety and efficiency in the food industry and the medical field [13,14]. Up to now, there are not any references on the separation of bioactive materials from industrial wastewaters using chitosan microspheres as adsorbents.

Isoflavones are a group of phenolic secondary metabolites found most in legumes and present in a concentration of 0.3–0.8% (dry biomass) [15]. In raw soybeans, isoflavones comprise of 12 forms, including three aglycones (i.e. daidzein, genistein and glycitein) and their corresponding glycosides (β-glycosides, acetyl- and malonyl-glycosides) [16]. Among of them, isoflavone aglycones are recognized as phytoestrogens because they are structurally similar to 17-β-estradiol. Thus, isoflavone aglycones can exert various pharmacological actions, such as anticancer, antihypertensive, antioxidative and antiallergic activities [17]. In our present work, foam fractionation can recover the total isoflavones from soy whey wastewater discharged during the process of soy protein isolate (SPI) production [18]. Furthermore, aglycones are the main forms of isoflavones existed in the foamate of foam fractionation after acidic hydrolysis. Therefore, it is necessary to explore an available technology to effectively separate isoflavone aglycones from the hydrolysate.

The objective of the present work was to separate isoflavone aglycones from soy whey wastewater after foam fractionation and acidic hydrolysis using chitosan microspheres as adsorbents. The

* Corresponding author. Tel.: +86 222656 4304; fax: +86 222656 4304.
** Corresponding author. Tel.: +86 222656 4294; fax: +86 222656 4294.
E-mail addresses: zhaoliangwu@163.com (Z.L. Wu), yjwang@hebut.edu.cn (Y.J. Wang).

http://dx.doi.org/10.1016/j.jiec.2014.10.024
1226-086X/

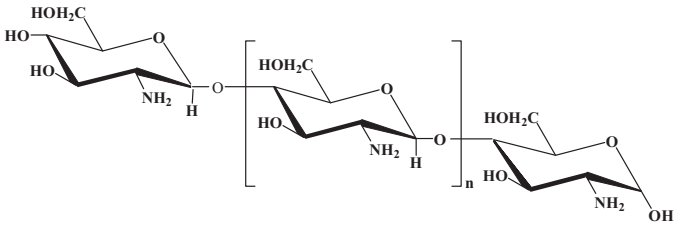

Fig. 1. Chemical structure of chitosan.

adsorption and desorption properties of chitosan microspheres for isoflavone aglycones were investigated. The discharged soy whey wastewater during the process of SPI production was employed as an actual research system. Foam fractionation and acidic hydrolysis were consecutively performed for obtaining hydrolysate, which was used as feeding solution of adsorption after pretreatment. This work is expected to provide a new insight into the separation of bioactive materials using bio-adsorbent and lay a foundation for the industrialization of recovering bioactive materials from industrial wastewaters.

Experimental

Materials and reagents

Soy whey wastewater was provided by Yu Xin Soy Protein Industry Co. Ltd. (Shandong, China). Chitosan powder (*Mr* 2 000 000) with a deacetylation grade of 87% was obtained from Hushi Chemical Co. Ltd. (Shanghai, China). The standard isoflavone aglycones of daidzein, glycitein and genistein were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The hydrochloric acid, disodium hydrogen phosphate, citric acid, glycine, sodium hydroxide, ethanol, acetic acid and epichlorohydrin were from Fengchuan Chemical Reagent Factory Co. Ltd. (Tianjin, China). All of above reagents were analytical grade. The ultrahigh-purity water was delivered using a Millipore Milli-Q system (Barnstead International, Dubuque, IA, USA). Acetonitrile (chromatographic grade) and acetic acid were used as the mobile phase for high performance liquid chromatography (HPLC) analysis.

Pretreatment of soy whey wastewater

According to the design in our present work, foam fractionation was first used to concentrate the total isoflavones from soy whey wastewater using a column under the optimal operating conditions of temperature 325 K, pH 5.0, volumetric air flow rate 100 mL/min and loading liquid height 400 mm [18]. The column of foam fractionation was constructed of a polymethyl methacrylate tube with an inner diameter of 44 mm and its length was 1200 mm. A porous polyethylene membrane with a pore diameter of 250 μm was mounted at the bottom of the column to serve as a gas distributor. Then, acidic hydrolysis was performed to hydrolyze β-glycosides into aglycones by using the foamate of foam fractionation as feeding solution under the optimum hydrolysis conditions of temperature 355 K, hydrochloric acid concentration 1.37 mol/L and hydrolytic time 90 min. The hydrolysate was first filtered using a 0.45 μm membrane filter. The filtrate was adjusted pH to 7.0 and then transferred into an Amicon Ultra-15 centrifugal filter unit which molecular weight cutoff was 3 kDa. Ultrafiltration was performed at the rotational speed of 4000 rpm for 20 min. The ultrafiltrate was collected and used as the feeding solution of adsorption.

Preparation of chitosan microspheres

Chitosan microspheres were prepared by the phase inversion method [19]. For preparation of microspheres, chitosan of 3 g was dissolved in an acetic acid solution (5%, v/v) of 100 mL to produce a gelatinous solution. Then, the gelatinous solution was pumped through a nozzle into a precipitation bath consisting of 2.0 mol/L sodium hydroxide solution. The reaction of acetic acid with the sodium hydroxide solution caused the precipitation of chitosan in solution, resulting in gelled microspheres. Microspheres were allowed to stand in the solution for 1 h and were washed with ultrahigh-purity water. The chemical cross-linked was carried out as follows: An aliquot of 12.5 mol/L epichlorohydrin of 25 mL was added into a suspension consisting of 100 g of microspheres immersed in water of 1000 mL and maintained at 325 K for 30 min. Subsequently, 0.1 mol/L sodium hydroxide solution of 700 mL was added and the system boiled for 2 h. The cross-linked chitosan microspheres were repeatedly washed with ultrahigh-purity water and finally freeze-dried by using a freeze dryer (Eyela Fdu-1200, Tokyo Rikakikai Co. Ltd., Tokyo, Japan).

Characterization of chitosan microspheres

The morphological characterizations of chitosan powder and chitosan microspheres were evaluated using a high-resolution field emission scanning electron microscope (SEM, Nova NanoSEM 450, FEI, Netherlands). Zeta potential and size distribution of chitosan microspheres were analysed using a Zetasizer Nano ZS90 (Malvern Instruments, England) at 300 K. Before each measurement, the sample solution was filtered with a 0.22 μm cellulose acetate filter and a thoroughly acid cleaned glass syringe.

Chromatographic conditions

The solution after adsorption of 5 mL was filtered using a 0.45 μm membrane filter to remove undissolved materials. The filtrate was freeze-dried and the resultant powder was extracted with 10 mL of 70% ethanol using ultrasonic concussion for 20 min in an ice bath and centrifuged at 4500 rpm for 10 min. The resultant supernatant was filtrated using a 0.45 μm membrane filter and it was used as the sample. The qualitative and quantitative analyses of isoflavone aglycones were performed by using an Agilent 1260 HPLC system with an Agilent Zorbax Extend C_{18} column ($250 \times 4.6\ mm^2$, 5 μm) under the following HPLC conditions: column temperature 315 K, mobile phase 0.1% acetic acid and acetonitrile (70:30, v/v), detection wavelength 260 nm, flow rate 1.0 mL/min and injection volume 20 μL. The isoflavone aglycones contents were calculated by HPLC peak areas compared with those of the standard isoflavone aglycones. The concentration of total isoflavone aglycones was determined by summing the concentrations of each isoflavone aglycones, as follows:

$$\text{Total isoflavone aglycones (TIA)} = \text{daidzein} + \text{glycitein} + \text{genistein}$$

Static adsorption and desorption experiments

Static adsorption and desorption experiments were performed as follows: Chitosan microspheres of 2.0 g was put into a 50 mL conical flask containing 25 mL of the feeding solution. Then, the flask was shaken by a shaker (100 rpm) at room temperature (300 K) for 300 min to reach adsorption equilibrium. The solution after adsorption was analyzed by HPLC. After adsorption equilibrium was reached, the chitosan microspheres were separated and desorbed with 25 mL of ethanol solution with different