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Cost-effective nanoporous Agar–Agar polymer/Nickel powder composite particle for effective bio-products adsorption by expanded bed chromatography

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ABSTRACT

In the present work a novel kind of dense nanoporous composite matrix for expanded bed application has been successfully first prepared with Nickel powder as a densifier and was covered with Agar–Agar layer as a skeleton, through the method of water-in-oil emulsification. Agar–Agar is a porous and inexpensive polymer. In order to fabricate cost-effective adsorbent with favorable qualities Agar–Agar polymer was used. Thereafter, the customized composite particle was modified by pseudo-affinity dye–ligand, Reactive Blue 4 (RB4), aimed at preparing a pseudo-affinity adsorbent (RB4-Agar-Ni) for bioprodut adsorption from aqueous solution. Bovine Serum Albumin (BSA) was selected as a model protein to investigate the adsorption behavior in batchwise and expanded bed chromatography, and the obtained results were evaluated with that of Streamline™ (Amersham–Pharmacia Biotech, Sweden). Spherical appearance and porous structure of composite particles were observed by the optical microscope (OM) and scanning electronic microscope (SEM). The results suggested that the matrices followed the logarithmic normal size distribution with the range of 65–300 μm and average diameter of 126.81–151.47 μm, proper wet density of 1.64–2.78 g/ml, water content of 62.74–34%, porosity of 98–90% and pore size of about 38–130 nm. For better comprehension of the impact of solid phase properties on the performance of the expanded bed, the expansion and hydrodynamic properties of a composite matrix with a series of densities was evaluated and estimated by the retention time distribution method (RTD) in an expanded bed and was compared with that of other matrices. According to obtained results the expansion factors under the same fluid velocity decreased by increasing the matrix density. Moreover, the axial dispersion coefficient (D_{ax}) is the most appropriate parameter for evaluating the stability of expanded bed, on various operating conditions, such as different flow velocity, bed expansion degree, viscosity of the liquid phase and the density of adsorbent. It was observed that the application of matrix with high density was proper for high operation, fluid velocity, since the addition of densifier improves the rigidity of the matrix. Three momentous factors, pH, ionic strength and initial concentration of BSA were analyzed. The best results showed that the adsorption equilibrium isotherms seems to follow a typical Langmuir isotherm and also the maximum adsorption capacity (q_m) of BSA on RB4-Agar-Ni (64.01 mg/ml adsorbent) was higher than that on RB4-Streamline commercial adsorbent (about 54 mg.ml adsorbent). Additionally kinetic adsorption processes were characterized by the pseudo-first-order and pseudo-second-order kinetics equations. The experimental data followed the pseudo-first-order kinetic equation. Also the breakthrough curves were investigated. It was found that dynamic binding capacity (DBC) decreased with increasing the flow rate and the values of DBC decreased from 21.08 to 11.15 mg/ml adsorbent when the density of composite beads increased from 1.64 to 2.78 g/ml. All results indicate that the prepared composite is promising for efficient bioproduct adsorption with good hydrodynamic characteristics, high stability and it is suitable for expanded bed usage as a cost-effective adsorbent.

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1. Introduction

Chromatography technology, with high performance and efficiency, has been one of the most powerful separation techniques for the purification of bio-molecules in the biotechnological industry since past few decades [1]. Expanded bed adsorption (EBA) is an innovative chromatography method, integrating clarification, concentration, and initial purification into a single step. Therefore, it provides an increased bed voidage with increasing fluid velocity. The larger interparticle void fraction creates it possible for crude feedstock containing particulate materials such as cells, cell debris and other possible fine particles to pass through without the risk of blocking the bed [1,2]. In general, EBA technology enables a reduction in the number of process steps, thereupon reducing processing time, increasing process yield and overall cost-effectiveness [3]. Compared with classical packed bed adsorption chromatography, EBA processes use the specially designed adsorbents which expand in the column with an upward liquid phase, forming perfectly classified fluidized bed (termed expanded bed) [4]. The column of EBA is designed to provide a uniform liquid phase distribution in the column inlet, and the top adaptor of the column is adjustable to allow the column to be operated at different bed heights [5]. The solid materials have been commercially available, such as the streamline series of adsorbents based on 6% cross-linked agarose containing a crystalline quartz core as the densifier. The particle size is in the range of 100–300 μm, and the mean density is about 1.2 g/ml. The physical properties of streamline adsorbent indicate that it can be used under a fluid velocity of 200–400 cm/h in water corresponding to a suitable expansion factor of 2–3 [6]. The growth of applications of expanded bed chromatography is achieved mainly by a particular design of the column, availability of solid matrix with a defined size and density, and ligand chemistry applied to the matrix [7–9]. The perfect adsorbents for EBA processes should be designed to give maximal productivity, which could be gained with the operation at high flow velocity, have high dynamic capacity, and allow minimal feed stock dilution. There are two ways to increase the appropriate operational fluid velocity of adsorbents in an expanded bed: larger particle size and higher density [6]. The higher density matrix is needed for the stable operation at higher flow velocity, and the appropriate size distribution contributes considerably to reducing the mixing in the column. The adsorption capacity and mass transfer characteristics should also be considered in the synthesis of matrices in order to certify bed efficiency [2]. To form a stable expanded bed, the adsorbents should be classified in the column and the expansion is normally kept in the range of 2–3 [10]. Also, other matrix was provided with an irregular shape that possibly leads to unstable expansion behavior, and with large particle size up to 600 μm indicating long mass transfer distance thus slowing down the adsorption kinetics [11]. Although the primary design of EBA column has not been changed in recent years, number of pellicular [7,12,13], macro-porous [14,15], high-density [16], and polymer-coated matrices [17] have been developed and applied in EBA. More excellent supporting matrices have been produced and further stabilized with various ligands to fabricate functionalized adsorbents such as ion-exchange [11,14,18], immobilized metal affinity (IMA) [19,20] and dye–ligand affinity [2,3,17,21,22] adsorbents [23,24].

The work herein reports the fabrication of nanoporous composite adsorbents by a water-in-oil emulsification method. We use Nickel powder with average diameter 10 μm and a density of 8.9 g/ml to prepare a high-density gel. Moreover, Ni powder indicates magnetic properties, and can be quickly separated in a magnetic field. Accordingly Ni powder could also be employed to prepare adsorbents for both expanded bed adsorption and magnetically stabilized fluidized bed (MSFB) [2]. Agar is one of the most abundant and cheapest natural hydrophilic polymer is used as the skeleton of adsorbent. It is a complex mixture of polysaccharides composed of two major fractions, agarose, a neutral polymer and agaropectin, a charged sulfuted polymer. Novel nanoporous composite matrices are called Agar-Ni. The physicochemical properties of the Agar-Ni beads such as shape, porosity, hydrophilicity and mechanical strength will be analyzed. The influences of matrix density, fluid velocity and liquid phase viscosity on the liquid mixing, expansion and hydrodynamic properties, in expanded bed will be studied and measured for demonstrating the potential EBA application. Then, activation and immobilization, were made with epichlorohydrin and chlorotriazine dye Reactive Blue 4 (RB4), respectively. The matrices were analyzed using protein adsorption equilibrium, kinetics, flow hydrodynamics, and rapid and high capacity adsorption in expanded bed operation. The breakthrough curves of BSA in expanded beds were examined as well. Finally, all results were compared with RB4-modified Streamline gel as the solid matrix.

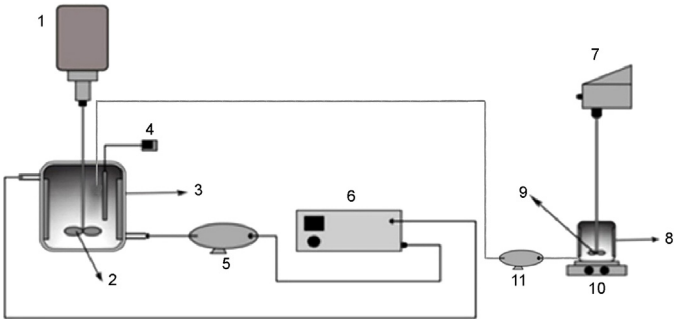

Fig. 1. Representation of apparatus for fabrication of Agar-Ni composite.1, 7: electric mixer; 2, 9: stainless steel propeller; 3: cylindrical glass reactor; 4: digital thermometer; 5: recirculating pump; 6: water batch; 8: glass vessel for slurry preparation; 10: heating plate; 11: peristaltic pump.

2. Experimental

2.1. Materials

Neutral agar powder with characteristic ambient gelling temperature, Reactive Blue 4 (RB4) and Bovine serum albumin (fraction V, purity 98%) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sorbitan monooleate (Span 80) and Nickel powder with the density of 8.9 g/ml and the mean particle diameter of 10 μm were ordered from Merck (Germany). Edible sesame oil was obtained from a local market. Commercial solid phase Streamline™ quartz-based matrix with nominal bead size 200 μm was provided by Amersham Biosciences (Uppsala, Sweden). Other chemicals were of analytical grade from local sources.

2.2. Fabrication of dense Agar-Nickel composite

The dense Agar-Ni matrix was fabricated through the method of water-in-oil emulsification, as described by the previous papers [7,12,13]. Experimental equipment is displayed schematically in Fig. 1. The emulsion was created in cylindrical glass reactor $H = 0.12$ m, $D = 0.09$ m equipped with four baffles of width 0.01 m, inserted into the reactor vertically. The reactor was stirred with stainless steel propeller, $D = 0.05$. The propeller was adjusted to the glass reactor bottom as near as possible for the agitation of the dense particles. A second glass vessel, $H = 0.07$ m & $D = 0.06$ m, with a stainless steel propeller, $D = 0.03$ m, was used for slurry preparation. Initially, 400 ml of sesame oil containing 15 g/ml Span 80 was poured to the cylindrical glass reactor and heated to 85 °C by circulating water. For preparing the agar solution, under continuous