Preparation and Adsorption Behavior of a Cellulose-Based, Mixed-Mode Adsorbent with a Benzylamine Ligand for Expanded Bed Applications

Dong Gao,[1,2] Shan-Jing Yao,[1] Dong-Qiang Lin[1]

[1]*Department of Chemical and Biochemical Engineering, Zhejiang University, Hangzhou 310027, China*
[2]*Institute of Modern Separation Science, Key Laboratory of Separation Science in Shaanxi Province, Northwest University, Xi'an 710069, China*

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ABSTRACT: A novel mixed-mode expanded bed adsorbent with anion-exchange properties was explored with benzylamine as the functional ligand. The cellulose composite matrix, densified with stainless steel powder, was prepared with the method of water-in-oil suspension thermal regeneration. High activation levels of the cellulose matrix were obtained with allyl bromide because of the relative inertness of the allyl group under the conditions of the activation reaction. After the formation of the bromohydrin with *N*-bromosuccinimide and coupling with benzylamine, the activated matrix was derived to function as a mixed-mode adsorbent containing both hydrophobic and ionic groups. The protein adsorption capacity was investigated with bovine serum albumin as a model protein. The results indicated that the prepared adsorbent could bind bovine serum albumin with a high adsorption capacity, and it showed salt tolerance. Effective desorption was achieved by a pH adjustment across the isoelectric point of the protein. The interactions between the cell and adsorbent were studied, and the bioadhesion was shielded by the adjustment of the salt concentration above 0.1*M*. Stable fluidization in the expanded bed was obtained even in a 2% (dry weight) yeast suspension. The direct capture of target proteins from a biomass-containing feedstock without extra dilution steps could be expected with the mixed-mode adsorbent prepared in this work, and this would be especially appropriate for expanded bed adsorption applications. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 674–682, 2008

Key words: chromatography; functionalization of polymers; ion exchangers; proteins; separation techniques

INTRODUCTION

Expanded bed adsorption (EBA) is a special chromatographic technique that allows for the adsorption of target proteins directly from unclarified feedstock, such as culture suspensions, cell homogenates, and crude extracts. A number of publications have demonstrated this technique, and various applications of EBA have been achieved for the recovery of target bioproducts.[1–4] In industrial applications, EBA processes have to face complex feedstocks that usually contain cells, debris, proteins, nucleic acids, endotoxins, and pigments. The competition between the target product and contaminants for the adsorption sites is potentially high, affecting the adsorption capacity and separation resolution. Therefore, ligand chemistry is one extremely relevant factor in terms of the efficient capture of target biomolecules for EBA applications. Ion-exchange ligands, such as sulfopropyl, carboxymethyl (CM), diethylaminoethyl (DEAE), and trimethylaminomethyl, of the most commonly used EBA adsorbents are dependent on low ionic strengths for efficient adsorption (generally below 5 mS/cm). However, for feedstocks based on high-density cell cultures, the conductivities are often between 10 and 30 mS/cm. Several-fold dilutions of feedstocks have to be performed to ensure efficient target capture with ion-exchange adsorption. On the other hand, hydrophobic interaction ligands (another widely used EBA mode) require high ionic strengths to promote the adsorption, so a large amount of an extra lyotropic salt is needed to increase the ionic strength of feedstock. All these extra steps result not only in longer processing times but also in increasing agent consumption and waste and even sometimes in the destruction of the target biomolecule. This is certain to reduce the process productivity and economics. The perfect solution, of course, would be to develop a new ligand with a salt-independent adsorption ability, which would thus improve the process

Correspondence to: S.-J. Yao (yaosj@zju.edu.cn).
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efficiency and reduce the cost of primary capture in the downstream process.[5,6]

Burton and coworkers[7,8] reported mixed-mode matrices for the adsorption of chymosin from both low and high ionic strength feedstocks. Upfront Chromatography A/S (Copenhagen, Denmark) and Amersham Biosciences (Uppsala, Sweden) have successfully developed mixed-mode EBA adsorbents, Fastline PRO and Streamline Direct HST, respectively.[9,10] The mixed-mode ligands have multiform binding functions, commonly combining hydrophobic and electrostatic interactions. The charged groups of the ligands at a low ionic strength perform the ion-exchange adsorption of the target protein; as the ionic strength increases, the aromatic ring of the ligand allows the hydrophobic interaction to target salt-tolerant adsorption. Therefore, high binding capacities can usually be achieved at moderate conductivities (10–30 mS/cm) without additional dilution steps in advance. Desorption in a mixed-mode adsorbent can be based on electrostatic charge repulsion and is often accomplished by changes in the pH of the mobile phase across the isoelectric point of the target protein. This technique is especially appropriate in EBA applications to directly capture the target protein from moderate ionic strength feedstock without the need for dilution or other additives. Hamilton et al.[11] reported the direct product sequestration of an extracellular protease from a microbial batch culture with the mixed-mode EBA process, which has the potential to significantly cut costs and process time. Lu et al.[6] used the mixed-mode EBA adsorbent Fastline PRO to capture nattokinase directly from a *Bacillus subtilis* fermentation broth. The purification factor could reach 12.3, and this demonstrated the advantage of mixed-mode EBA in enzyme separation. Upfront Chromatography A/S exploited a crosslinked agarose resin alkylated with epichlorohydrin and then chemically bound with benzylamine as the ligand for use in the chromatographic process, but the experimental and application details are limited.[12] Johansson et al.[13] reported several prototypes of multimodal ligands coupled to Sepharose 6 Fast Flow suitable for the capture of negatively charged proteins from high-conductivity mobile phases, and they found that the optimal ligands were nonaromatic amines, whereas with aromatic amines, the recovery was often low.

Because the two commercial mixed-mode EBA adsorbents, Fastline PRO and Streamline Direct HST, are both cation-exchange-function types and the details about anion-exchange functions such as benzylamine are very limited in the literature, a mixed-mode EBA adsorbent with an anion-exchange property was explored in this work, with benzylamine as the functional ligand. On the basis of our previous work,[14–17] spherical, densified cellulose composite particles were explored successfully for EBA applications. In this work, a high-density cellulose/stainless steel powder (SSP) composite matrix was chosen as the adsorbent base. After activation with allyl bromide (AB), bromohydrin formation with *N*-bromosuccinimide (NBS), and coupling with benzylamine, the matrix was derived to function as a mixed-mode adsorbent with both hydrophobic and ionic groups. To obtain a high ligand density, several reaction factors in the preparation process, such as the amount of AB, the concentration of NaOH, and the reaction time and temperature, were investigated in detail. The adsorption isotherm was studied to verify the mixed-mode properties of the prepared adsorbent.

EXPERIMENTAL

Materials

Cellulose xanthate viscose was prepared with a method published previously.[14,16] SSP with a density of 7.9 g/cm^3 and an average size of 20 μm was provided by Haining Metallurgy, Ltd. (Haining, China). Bovine serum albumin (BSA; A-7030) was obtained from Sigma (Milwaukee, WI). The pump oil was technical-grade and was supplied by Shanghai Chemical Reagent Co. (Shanghai, China). All other reagents were analytical-reagent-grade and were purchased from local suppliers.

Preparation of the composite beads

The composite matrix based on SSP-densified cellulose beads was prepared by the method of water-in-oil suspension thermal regeneration, as described in previously published articles.[14,16] SSP (15 g) was mixed thoroughly with 60 g of viscose. Then, the mixture was dispersed in 240 mL of pump oil in a 500-mL flask with agitation at 600 rpm for 0.5 h at room temperature. The suspension was heated to 90°C for 1 h under continuous stirring and then was cooled and filtered. The resulting particles were washed in turn with boiling water, methanol, and sulfuric acid (10% w/w). After sieving with standard test sieves in water, the composite beads (designated Cell-SSP) were obtained with a diameter of 60–180 μm.

Physical properties

The physical properties of the prepared matrices were characterized as follows. The definite size distribution of the Cell-SSP beads was determined with a Mastersizer laser particle size analyzer (Malvern Instruments, Ltd., Worcestershire, United Kingdom). The wet density of the beads was determined by the water displacement method in a 10-mL gravity bottle, whereas the water content was obtained by

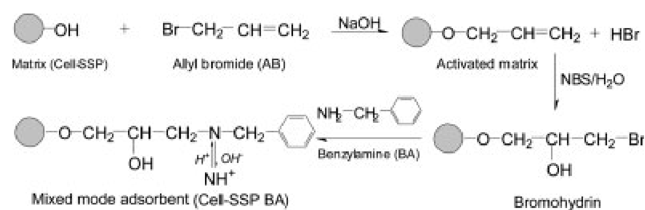

Figure 1 Preparation scheme for the mixed-mode adsorbent Cell-SSP BA.

dehydration at 105°C to a constant mass. Presuming that all the pores in the particles were full of water, we could roughly calculate the porosity (expressing the pore volume per milliliter of wet particles) and the pore volume (expressing the pore volume per gram of dried particles).[18] The specific surface area was defined as the accessible area of the solid per milliliter of wet beads, including external and internal porous surface areas, and it was determined by the method of adsorption of methylene blue.[19] The pore radius was estimated by a method used in a previous work.[16]

Activation and coupling of the matrix

After the Cell-SSP matrix was activated with AB, the brominated matrix was obtained by the use of aqueous NBS and then was attached to benzylamine to produce a mixed-mode adsorbent (Cell-SSP BA). The preparation scheme is shown in Figure 1. The activation with AB was carried out with the procedure of Burton and Harding.[8,20] Typically, the activation of the matrix was completed by the shaking of 10 mL of Cell-SSP and 1.5 mL of dimethyl sulfoxide (DMSO) in 4.5 mL of a 1.94*M* NaOH solution containing 0.7 mL of AB for 20 h at 25°C. After being washed with ethanol and water, the allylated matrix was suspended in an aqueous solution, a 1.2 molar excess of NBS over allyl groups was added, and the reaction was kept for 1 h at 25°C. Then, the brominated matrix was mixed with the same volume ratio of benzylamine to the matrix, and the mixture was shaken for 30 h at 25°C. The reaction product was washed with deionized water and stored in 20% (v/v) ethanol.

Protein adsorption

BSA was used as a model adsorbate for protein adsorption. In adsorption equilibrium experiments, drained adsorbents of different masses were added to 15 mL of a 5 mg/mL BSA solution in a 20 m*M* phosphate buffer (pH 7.0) containing different NaCl concentrations. The adsorption experiments were conducted at 25°C for 10 h in a shaking incubator. After adsorption equilibrium, the solid phase was separated, and the supernatant was analyzed for the protein concentration. The adsorbed mass of the protein was calculated from the mass balance.

Biomass adsorption

The biomass adsorption experiments were performed by the method published by Lin et al.[21] In the bath adsorption experiment, a diluted suspension of biomass was incubated with the adsorbent under investigation under defined fluid phase conditions (pH, salt concentration, etc.). Before the experiments, the adsorbents were washed with the appropriate buffer. A total of 25 mL of the suspension was added to the shaking flasks (each containing 6 mL of the adsorbent). The flasks were then sealed and incubated with gentle agitation (100 rpm). Samples (100 μL) were removed at 1–2-min intervals, and the cell concentration in the samples was determined by the adsorption at 600 nm (OD_{600nm}). The sample was immediately returned to keep the volume of the solution constant. The initial cell concentration was controlled at an OD_{600nm} value of 0.5. Yeast cells were suspended in the buffer to a final concentration of 0.03% (w/w, dry weight).

Expanded bed operation

A homemade column (1000 × 20 mm i.d.) was used for expanded bed experiments. About 2 mL of glass beads (0.3 mm in diameter) was added to improve the flow distribution at the column inlet. A movable top adapter was employed to adjust the position of the liquid outlet just above the bed surface. Proper column vertical alignment was confirmed in all experiments. A peristaltic pump (Lan'ge, Ltd., Baoding, China) was used for the fluid supply. The mobile phase was a suspension of various concentrations of yeast in 20 m*M* phosphate buffers with 0.2*M* NaCl. All operations were performed at 25°C with a settled bed height of 120 ± 2 mm. The degree of expansion was measured as H/H_0, where H is the expanded bed height and H_0 is the sedimented bed height. There, the degrees of expansion at a variety of flow velocities in different mobile phases were used to determine the expansion characteristics of the adsorbents.

Titration of the activated allyl groups

The titration experiments were performed by the method of Burton and Harding.[22] First, the activated matrix (1 mL, drained volume) was mixed with 120 μL of mercaptoacetic acid and 1 mL of water, including 25 mg of ammonium persulfate, and then the mixture was incubated for 8 h at 60°C. After the excess reagent was washed out with 20 mL of water,

TABLE I
Physical Properties of the Matrix

Matrix	Wet density (g/mL)	Water content (%)	Porosity (%)	Pore volume (mL/g)	Specific surface area (m^2/mL)	Pore radius (nm)	Particle diameter distribution (μm)
Cell-SSP	1.31	63.1	82.7	1.71	31.6	52.3	60–180

the acidic groups on the matrix were protonated with 5 mL of 0.1*M* HCl, and the excess acid was removed through washing with 20 mL of water. The matrix was transferred to a vial, and 5 mL of a 0.1*M* NaCl solution was added; then, the matrix was titrated with 0.1*M* Tris to pH 6.4. The concentration of allyl groups on the beads was expressed as micromoles per milliliter of the gel.

Titration for substitution efficiencies

The titrations were performed with 1-mL (drained volume) adsorbent samples suspended in 5 mL of a 0.1*M* NaCl solution. The amine-substituted adsorbent was washed with 5 mL of 0.1*M* NaOH and then with 20 mL of water. Finally, the suspended samples were titrated with 0.1*M* HCl to pH 4.0. The substitution efficiency was expressed as micromoles per milliliter of the gel.

RESULTS AND DISCUSSION

Physical properties of the matrices

Some physical properties of the matrices are listed in Table I. The weight ratio of SSP to viscose in the preparation was 15/100.

Optimization of the activation with AB

The method of epichlorohydrin activation is used often for immobilizing a wide range of hydroxyl, thiol, and amine groups. However, low activation levels have been found, usually because of side reactions (e.g., crosslinking and epoxide hydrolysis) competing with activation.[20,23] Higher activation levels can be obtained with AB, and this has been attributed to the relative inertness of allyl groups toward water and cellulose, limiting the crosslinking and hydrolysis. Under alkaline conditions, the bromide group of AB should be reactive, but the allyl group is expected to be comparatively inert. After allylation, the matrices could be further activated by aqueous NBS to form bromohydroxypropyl groups and then used to attach benzylamine. The effects of the solvent, the concentration of NaOH, the reaction time and temperature, and the amounts of AB were investigated to optimize the activation reactions.

Effect of DMSO in the reaction system

Adding a small amount of DMSO to the reaction system (e.g., mixing 10 mL of the matrix with 1.5 mL of DMSO) increased the activation level by about 13%. The activation reaction was performed between hydrophilic hydroxyl groups of cellulose and a hydrophobic reagent (AB), so there was a barrier to the mass transfer of AB from the organic phase to the aqueous solution. DMSO could be dissolved in water and in an organic solvent, so the introduction of DMSO promoted mass transfer between the two phases, and this benefited the activation reaction.

Effect of the amount of NaOH

Under alkaline conditions, the substitution reaction occurred between AB and the hydroxyl-containing matrix. Figure 2 shows the content of allyl groups in the activated Cell-SSP beads as a function of the molar ratio of the hydroxide to AB. The activation reactions were performed at 25°C for 24 h, and 9% AB was used. The maximum content was found with the molar ratio in the range of 1.05–1.12. With a low [OH^-]/[AB] molar ratio, more hydroxyl groups were activated by AB with an increase in the amount of NaOH. When the molar ratio was above 1.12, an obvious reduction of the content of allyl groups occurred. Because AB should not have caused a crosslinking reaction itself, the decrease in the content

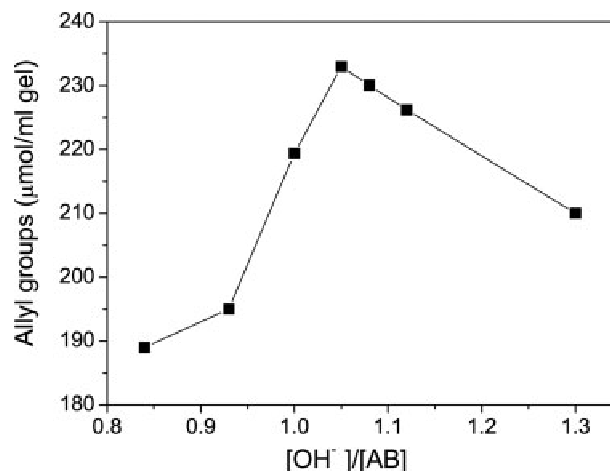

Figure 2 Effects of a molar excess of OH^- over AB on the degree of AB activation. The activation reactions were performed at 25°C for 24 h, and 9% AB was used.

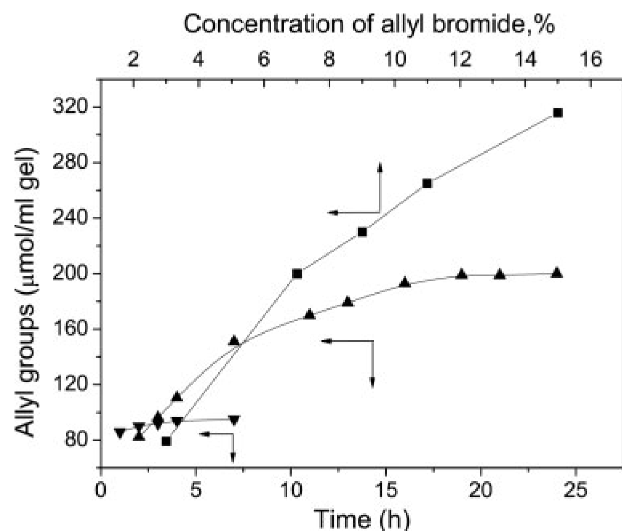

Figure 3 Effects of the temperature, reaction time, and AB concentration on the AB activation levels: (▲) 25°C, (▼) 60°C, and (■) different AB concentrations. For testing the different temperatures and reaction times, 7% AB was used.

might have been due to the hydrolysis of AB at higher hydroxide concentrations. Similar results were reported by Burton and Harding.[20] Consequently, the optimal molar ratio of the hydroxide to AB for the activation reaction was 1.05–1.12.

Effect of the reaction time and temperature

Figure 3 shows the available allyl groups in the activated Cell-SSP as a function of the reaction time and temperature. AB (7%) was used for testing the different temperatures and reaction times. When the reaction was carried out at 60°C, the number of activated allyl groups ascended to the maximum value in 7 h, but with low activation efficiency (ca. 94 μmol/mL of gel). On the contrary, although the reaction was performed at room temperature, an obviously high activation efficiency (ca. 200 μmol/mL of gel) could be obtained, but the reaction time was greater than 18 h. The boiling point of AB was 71.3°C. The low activation level at a high temperature was attributed to the reagent hydrolysis rather than a cellulose allyl substitution reaction.

Effect of the amount of AB

As Figure 3 shows, the activation level increased roughly linearly with the increase in the AB concentration. The allyl groups of AB were relatively inert under the activation conditions used in this work, and there were only limiting side reactions of hydrolysis and crosslinking, so there should have been a linear relation between the AB proportion and activation level. In fact, water in the reaction system was a kind of reactant that induced the hydrolysis reaction of AB under alkali conditions, and this yielded different activation levels because of the different contents of water according to the altered reagent amounts. Nevertheless, the results indicated that different ligand densities in the range of 0–300 μmol/mL of gel could be obtained through the control of the amount of AB in the reaction system.

Bromohydrin and coupling with benzylamine

The relatively inert allyl groups introduced by the AB activation process can be modified further into more reactive forms by the addition of a halogen, and then the halogenated matrix can be substituted with some nucleophiles such as amine ligands. Under alkaline conditions, the halohydrin groups are converted into epoxides by intramolecular etherification, and then the epoxides can react easily with amines or thiols.

NBS has been used for synthesizing a number of different functionalized molecules starting from simple alkenes, and bromohydrin is produced because of Markovnikov's rule.[24] When excess NBS is added to the halohydrin reaction of an allyl matrix, there is no Br^- available in the reaction solution, and thus bromohydrin rather than dibromide can be obtained.[8,24] Figure 4 shows the effects of the reaction time on benzylamine substitution after NBS addition; a 1.2 molar excess of NBS over the allyl groups of the matrices was used, and the volume of water was determined from the solubility of NBS (25°C, 14.7 mg/mL of H_2O). As shown in Figure 5, the reaction of the allyl activated matrix with at least

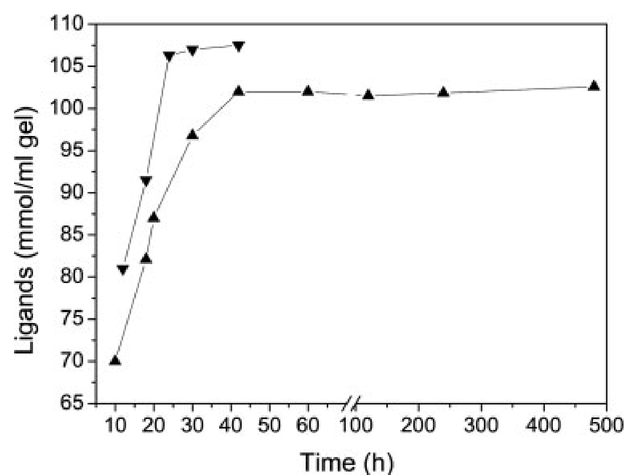

Figure 4 Effects of the temperature and time on the substitution levels after NBS addition: (▲) 25 and (▼) 40°C. AB (7%) and a 1.2 molar excess of NBS over the allyl groups of the matrices were used.

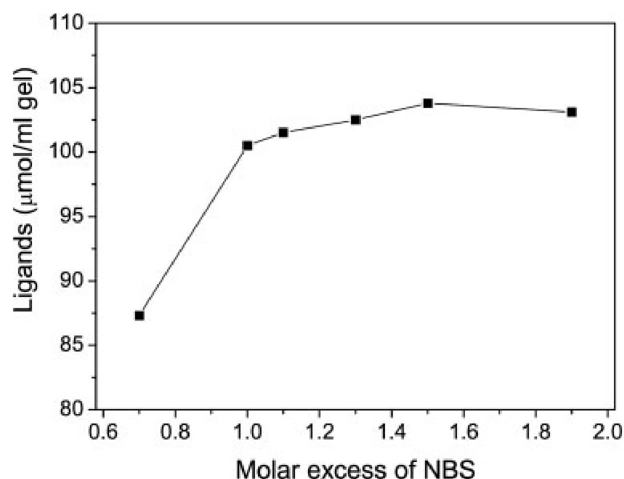

Figure 5 Effects of a molar excess of NBS over the allyl groups on the substitution levels. AB (7%) was used.

a 1.2 molar excess of NBS appeared to result in the complete reaction of the allyl groups, and then a maximum substitution level was found after 1 h of the reaction.

To optimize the substitution efficiency, a series of reaction factors, including the reaction temperature, pH value, and content of water, were investigated. The results are listed in Table II. Low substitution efficiencies (ca. 59–66%) were found when the reaction was performed with 1 mL of the allyl matrix, 0.1 or 0.9 mL of benzylamine, and 0.6 mL of water at various pHs. There was no significant effect of the reaction pH. The substitution efficiency could be improved to 74% by the omission of water. Compared to 25°C, a higher temperature (40°C) caused a slight increase in the substitution efficiency and a decrease in the reaction time, as shown in Figure 4.

TABLE II
Substitution of the Benzylamine Ligand on the Brominated Allyl Matrix

Reaction conditions	Ligand (μmol/mL)	Efficiency (%)
0.1 mL of benzylamine, 0.6 mL of H_2O, pH 7.0, 25°C	83.1	59
0.1 mL of benzylamine, 0.6 mL of H_2O, pH 11.0, 25°C	83.6	60
0.9 mL of benzylamine, 0.6 mL of H_2O, pH 11.0, 25°C	92.6	66
0.9 mL of benzylamine, no H_2O, 25°C	103.1	74
0.9 mL of benzylamine, no H_2O, 40°C	106.3	76
0.9 mL of benzylamine, no H_2O, 60°C	Flocculating	—

The brominated matrix was used, and the allyl groups were titrated by the addition of mercaptoacetic acid to 140 μmol/mL of gel.

However, some flocculation was found at 60°C. Therefore, the optimum reaction conditions were chosen to be the same ratio of benzylamine to drained beads (v/v) and 25°C. In fact, whether the ratio was 0.9 : 1 or 1 : 1, the result was the same because of the excess benzylamine over the bromohydrin groups of the matrices. The ligand density of Cell-SSP BA was determined to be 103 μmol/mL of adsorbent by titration, as described previously.

Protein adsorption

BSA was used as a model protein to test the adsorption characteristics of Cell-SSP BA. As normal ion exchangers, mixed-mode adsorbents possess ionic ligands, so the ionic strength and pH are two important factors influencing the target protein adsorption. First, the static adsorption behavior of BSA with Cell-SSP BA was studied under different salt concentrations at pH 7.0. The isotherm adsorption curves are shown in Figure 6. Increases in the salt concentration caused the adsorption ability of BSA to decrease slightly. Cell-SSP BA still showed some amount of BSA adsorption even with 0.50*M* NaCl, especially in comparison with Streamline DEAE (nearly no adsorption in 0.25*M* NaCl, as shown in Fig. 7). At a low ionic strength, Cell-SSP BA with the charged aliphatic amine group facilitated the ion-exchange adsorption of BSA; however, as the ionic strength increased, the aromatic ring provides a hydrophobic interaction for binding proteins. The salt-tolerance properties demonstrated that Cell-SSP BA functions as a type of mixed-mode adsorbent that achieves high binding capacities at moderate conductivities and allows for the application of undiluted feedstock for expanded bed processing.

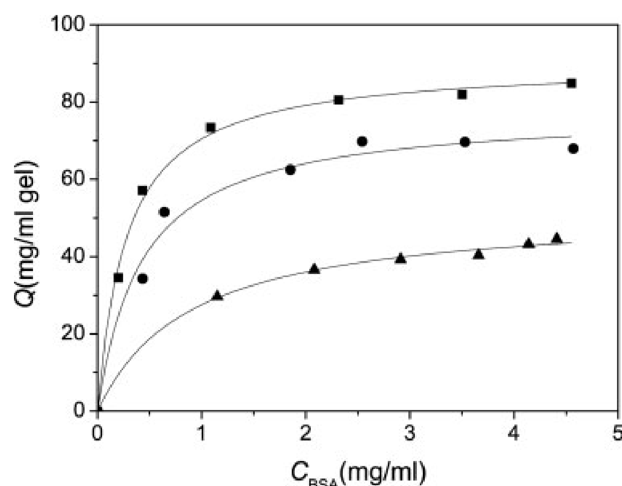

Figure 6 BSA adsorption isotherms on the mixed-mode adsorbent at different ion strengths (25°C): (■) 0, (●) 0.25, and (▲) 0.50*M* NaCl. C_{BSA} is the concentration of BSA, and *Q* is the protein adsorption capacity.

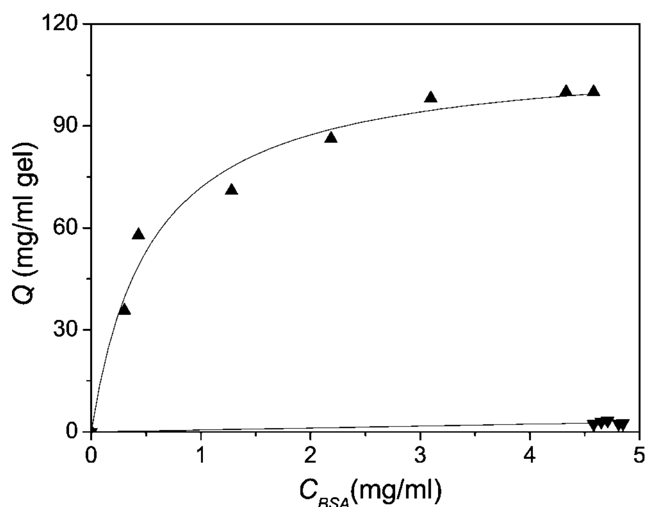

Figure 7 BSA adsorption isotherms on the Streamline DEAE adsorbent (25°C): (▲) 0 and (▼) 0.25*M* NaCl. C_{BSA} is the concentration of BSA.

The variation in the pH is another important factor with respect to mixed-mode adsorbents. Isotherm adsorption curves at various pH values are shown in Figure 8. The isoelectric point of BSA is 4.9. A pH change could effect the electrostatic interaction between the protein and adsorbent by altering the net charge of the protein. At pH 7.0, the adsorption was promoted under conditions that favored the electrostatic attraction and hydrophobic interaction between the protein and adsorbent. As the pH values decreased, the surface charge of BSA decreased, and thus the adsorption capacity for BSA obviously decreased. When the pH of the mobile phase was across the isoelectric point of the protein, an electrostatic charge repulsion interaction took place to induce the desorption of the protein.

Biomass adsorption

EBA is an integrated technology allowing the adsorption of proteins directly from unclarified feedstock. The interactions between the cell and adsorbent influence the bed stability and therefore have a significant impact on protein adsorption.[25–27] A bath adsorption experiment is easy to perform and can be used for the evaluation of cell–adsorbent interactions under various solution conditions.[21]

In Figure 9, the decrease of OD_{600nm} value is plotted as a function of the incubation time. In the potassium phosphate buffer (20 m*M*, pH 7.0), a strong decrease in OD_{600nm} could be found, and this meant that some amount of interaction between the yeast cells and adsorbent occurred. The adhesion of yeast cells onto anion exchangers can be explained by the assumption of electrostatic attraction between the negatively charged yeast cells and the positively charged anion exchangers. The electrostatic interaction between the cell and adsorbent could be reduced significantly by the NaCl concentration being increased above 0.1*M*, as shown in Figure 9(a). The same experiment was conducted with Streamline DEAE, as shown in Figure 9(b), in which a stronger interaction can be observed between the yeast cell and Streamline DEAE. This can be attributed to the higher ligand density of Streamline DEAE (130–210 μmol/mL) versus that of Cell-SSP BA (103 μmol/mL).

Expansion characteristics

The expansion characteristics were studied in an expanded bed under various mobile phase conditions, including the buffer and yeast suspension. The results are shown in Figure 10. Compared with that of Streamline DEAE, the bed expansion of Cell-SSP BA is considerably lower at identical flow rates, either in the buffer or in the yeast suspension. This means that the prepared matrix is suitable for higher flow rates. Typically, a twofold expansion could be achieved at close to 430 cm/h in the buffer for Cell-SSP BA and at 130 cm/h for Streamline DEAE.

As mentioned previously, the interactions between the cell and Cell-SSP BA adsorbent could be shielded by the addition of NaCl above 0.1*M*, so stable expansion and perfect clarified fluidization could be achieved even for 2% (w/w, dry weight) yeast suspensions. A stable expanded bed in biomass-containing feedstock would guarantee the Cell-SSP BA adsorbents prepared in this work for EBA applications.

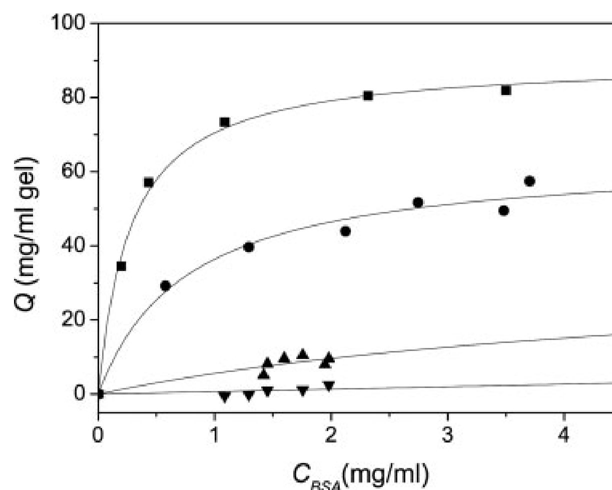

Figure 8 BSA adsorption isotherms on the mixed-mode adsorbent at different pH values (25°C): (■) 7.0, (●) 5.0, (▲) 4.0, and (▼) 3.0. C_{BSA} is the concentration of BSA.

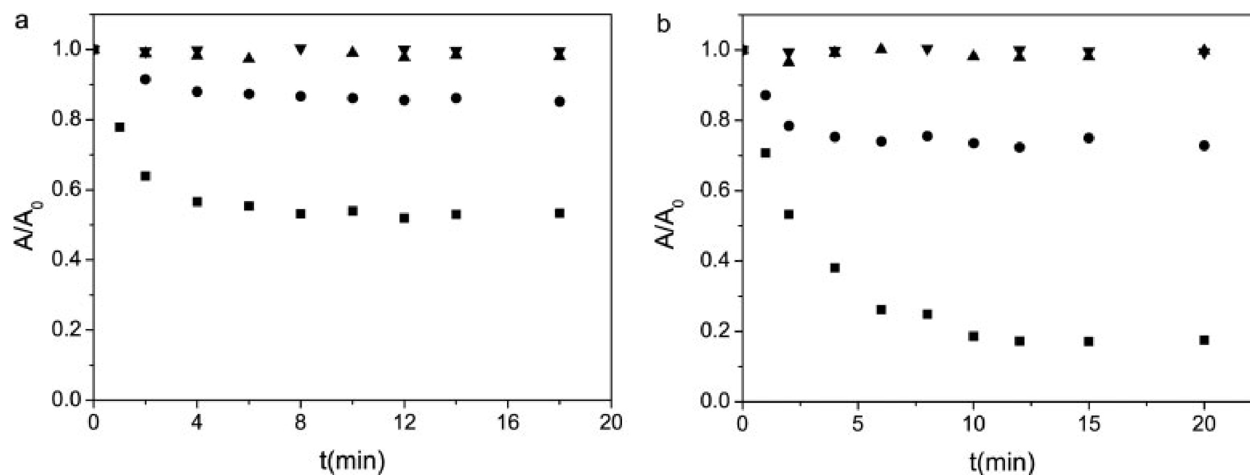

Figure 9 Bath adsorption of intact yeast cells to (a) Cell-SSP BA and (b) Streamline DEAE at pH 7.0 and various salt concentrations (25°C): (■) 0, (●) 0.05, (▲) 0.10, and (▼) 0.25*M* NaCl. *t* is the time, and A/A_0 is the ratio of OD_{600nm} with time to the initial OD_{600nm}.

In previous articles,[14,15] a type of spherical, TiO_2-densified cellulose composite particle was prepared with a similar method, and the composite particles were further derived to function as an anion exchanger (Cell-Ti DEAHP)[28] and a cation exchanger (Cell-Ti CM).[29] The dynamic adsorption capacity at 10% breakthrough in an expanded bed could reach 42.6 mg of BSA/mL of adsorbent for Cell-Ti DEAHP and 69.3 mg of lysozyme/mL of adsorbent for Cell-Ti CM; this strongly demonstrated that the densified cellulose composite matrices prepared could be used as EBA adsorbents. In addition, the addition of SSP to the cellulose skeleton showed little effect on the porosity, specific surface area, and pore radius in the composite particle,[16] and the Cell-SSP composite matrix showed suitable expansion and hydrodynamic properties for forming a stable expanded bed.[17] The results indicate that the Cell-SSP composite particles have excellent potential for expanded bed applications of biomolecule separation. Therefore, the adsorbent prepared in this work (Cell-SSP BA) may have practical use in expanded bed applications.

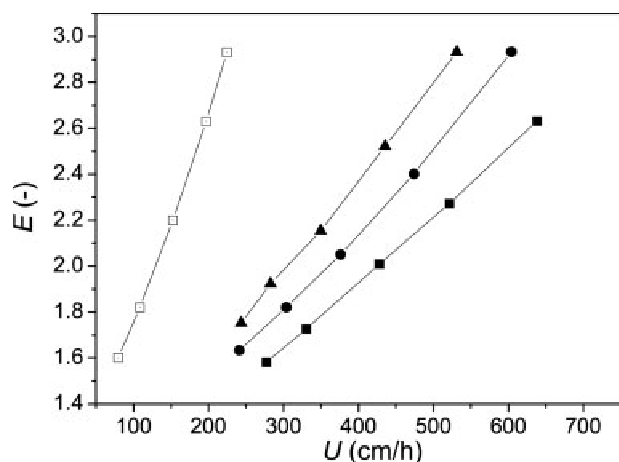

Figure 10 Expansion characteristics of Cell-SSP BA equilibrated with yeast suspensions of various cell concentrations at pH 7.0 and 0.20*M* NaCl: (■) yeast-free buffer, (●) 1% yeast, (▲) 2% yeast, and (□) Streamline DEAE yeast-free buffer. *E* is the degree of expansion, and *U* is the linear velocity of liquid.

CONCLUSIONS

A novel mixed-mode adsorbent with an anion-exchange property for EBA was prepared on the basis of SSP-densified cellulose composite beads as the matrix. The activation of the cellulose composite matrix with AB was investigated in detail, and the optimal activation procedure was determined to be as follows: 10 mL of matrix beads and 1.5 mL of DMSO in 4.5 mL of a 1.94*M* NaOH solution containing 0.7 mL of AB for 20 h at 25°C. After the modification of a 1.2 molar excess of NBS over allyl groups for 1 h at 25°C, the matrix beads were then mixed with the same volume of benzylamine for 30 h at 25°C. The coupling efficiency was above 75% under the given conditions. A series of Cell-SSP BA adsorbents with different ligand densities was achieved according to the control of the preparation procedure.

The prepared adsorbent (Cell-SSP BA) showed a relatively high adsorption capacity and salt-tolerance properties. Effective desorption was achieved by the adjustment of the pH across the isoelectric point of the protein. The interaction between the cell and adsorbent was reduced by the adjustment of the salt concentration above 0.1*M*. The fluidization experiments in the expanded bed indicate that the mixed-mode adsorbent prepared in this work has strong potential for EBA applications involving the direct capture of target proteins from high ionic strength feedstock without predilution. Because the desired

matrix was developed for EBA processes, more information about the adsorption behaviors with different ligand densities, adsorption kinetics, and dynamic adsorption behaviors in the expanded bed will be studied in another work.

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