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A Comparative Study of Agarose-Nickel Prototype Composite Adsorbent and Commercial Streamline Deae Adsorbent: Physical and Hydrodynamical Assessments

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Abstract: Expanded bed adsorption (EBA) is an integration technology for capturing target bioproducts directly from unclarified feedstock, designing and applying a good adsorbent affects its performance. In present work, a novel matrix has been developed for expanded bed adsorption process. The prepared matrix was manufactured using water-in-oil emulsification method. Desirable adsorbents tend to be of small size, high density as well as high porosity. Here, the prepared matrices had regular spherical shape, wet density of 1.95, followed logarithmic normal size distribution within the range of 50-200 μm, mean diameter of 126.4 μm and 91.93% prosily. The Richardson-Zaki equation expressed the bed expansion and operation flow rate for the prepared matrix and Streamline DEAE. In addition, the theoretical prediction of correlation parameters (Ų and n) was also measured. To characterize the hydrodynamic properties in the expanded bed, the residence time distribution method was used. The physical and hydrodynamic properties of the fabricated matrix were studied and compared with those of the commercial Streamline DEAE matrix. The results indicated that the prepared matrices showed good expansion and stability in the bed and are thus suitable for expanded bed applications.

Key words: Agarose-Nickel matrix · Expanded bed adsorption · Matrix · Agarose · Nickel · Hydrodynamic property · Streamline DEAE

INTRODUCTION

Expanded bed adsorption (EBA) has been introduced as a primary recovery step for protein purification to combine solid-liquid separation with an adsorptive operation. When adsorbent beads of suitable size and density are expanded, feedstock containing suspended biological material may be applied without prior clarification. Therefore, a reduction in the number of process steps is achieved with particular advantages with respect to processing time and overall yield [1]. In EBA, the chromatographic fluid enters the separation column from the bottom, causing the bed to expand, usually with a factor 2-3, dependent on fluid velocity, viscosity and particle properties. Significantly, the adsorbent particles have an increased density and show a size distribution, which leads to a classification of the expanded bed-the heaviest particles in bottom and the lightest particles on top. Normally, each particle will find its own place of equilibration in the expanded bed and will reside there during the separation process [2]. Due to fluidization process, the space between the adsorbent beads is increased, allowing feed material containing cells and/or cell debris to be pumped through the column without blocking it [3]. It is obvious that the use of EBA technique would be both technically and economically valuable. Adsorbent matrix is the principal "hardware" pillar supporting the successful application of EBA. The basic criteria of the adsorbents for EBA are formulated as being a sufficient density and a distribution of particle size. Some other physical properties of adsorbents, such as shape, porosity, hydrophilicity and modifiability are also important for efficient protein adsorption [4]. The adsorbent of EBA named Streamline were introduced to the market in early 1990s and were developed based on the demands from biotechnology industry in late 1980s.

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The Streamline series of matrices are based on 6% cross-linked Agarose containing a crystalline quartz core as the densifier. Until now, there have been quite a few new commercial adsorbents going into the EBA family. However, compared with those 10 years ago, new host cells, the expression level of the target product and the culture volume have increased significantly today. Thus, improved adsorbents are needed to match the fast development of biotechnology [5].

In this study, a novel Agarose-Nickel matrix was prepared with the method of water-in-oil emulsification. Fine Nickel powder with a size of 10 μm and density of 8.9 g/ml is used as the densifier. The physical properties of the prepared matrix, as well as expansion properties in expanded bed, are compared with commercial Streamline DEAE matrix. In addition, based on liquid mixing in expanded bed, the bed stability of fabricated matrix along with commercial matrix is investigated.

Experimental

Materials: Neutral Agarose with a low gelling temperature was purchased from Invitrogen (USA) and sorbitanmonooleate (Span 80) was sourced from Merck (Darmstadt, Germany). Nickel powder with a bulk density of 8.9 g/ml and a mean particle diameter of 10 μm was sourced from Merck (Germany). Silicone oil was obtained from Merck (Germany). Streamline DEAE adsorbent was purchased from Pharmacia Biotech (Uppsala, Sweden). All other chemical materials that used to analytical reagent grade were purchased from local suppliers.

Preparation of Agarose-Nickel Composite Matrix: Agarose-Nickel composite matrix was prepared by water in oil emulsification method based on our previous work [6, 7]. The emulsion was produced in a 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 a stainless steel propeller (D = 0.05 m). The propeller was adjusted in the glass reactor bottom as close as possible. 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.

The Agarose-Nickel composite matrix was prepared; the detail stated as follows:

Silicon oil (300 ml) containing 10 g/ml Span 80 in the glass reactor was heated to 85°C in a water bath. By heating an Agarose powder suspension to 90±1°C in the water bath, Agarose solution (4%, g/ml) was prepared. Subsequently, 18 g of Nickel powder were mixed and agitated for about 10 min at 800 rpm. The slurry was poured into the emulsion agitated at 900 rpm. After 20 min, the oil phase was cooled to 15°C by replacing the hot water with cold water in the water bath. During the cooling step, the Agarose solidified and when the emulsion temperature reached to 15°C, the stirring was terminated. Agarose-Nickel particles were recovered by centrifuging the oil. The particles were washed with acetone and water for three times, in order to displace entrapped oil. Then prepared particles were rinsed by distilled water. The resulting particles were screened by graded metal screen. The Agarose-Nickel gels were stored at 15°C in Tris-HCl (10 mM) containing 0.02% (g/ml) sodium azide at pH of 7.5.

Measurement of Physical Propertie: The size distribution of the matrix was determined with a particle size analyzer Fritsch A22 (Idar-Oberstein, Germany). An optical microscope Nikon YS 100 (Nikon Instrument, Japan) was used to observe the shape of the particles. Several physical properties of matrices were calculated as follows:

In brief, wet density, ρ_p (g/ml), was determined by water replacement in a 10 ml gravity bottle and was calculated the wet density of the composite particles by Eq.1:

$$\rho_p = \frac{M_{1\omega}}{M_1 + M_2 - M_3} \quad (1)$$

Where ρ_p and ρ_ω (g/ml) represents the matrix wet density and water density, respectively. M_1, M_2 and M_3 (g) represents the mass of wet matrix, a gravity bottle filled with water and a gravity bottle with wet matrix full of water, respectively. Water content, ω (%), was obtained by removing water of wet composite particles at 105°C to a constant mass and was calculated by Eq. 2 [7].

$$\omega = \frac{m_2 - m_3}{m_2 - m_1} \times 100\% \quad (2)$$

Where m_1, m_2, m_3 (g) represent the mass of weighing bottle, the matrix and weighing bottle before drying and the matrix and weighing bottle after drying, respectively. It is supposed that all pores in the matrix were filled with water. Porosity, P (%) representing the percentage of pore volume per volume wet matrix, can be calculated as Eq. 3 and pore volume, V (ml/g dried matrix) representing pore volume per gram dried matrix, can be calculated as Eq. 4 [8].

$$P = \frac{\rho_p \omega}{\rho_\omega} \times 100\% \quad (3)$$

$$V = \frac{\omega}{(1-\omega)\rho_\omega} \quad (4)$$

Determination of Expansion Properties and Liquid Mixing in Expanded Bed: A simple unique expanded bed contactor (NBG expanded bed column with internal diameter 0.013 and 0.25 m in height), which was equipped with a glass tube fitted with a simple sintered glass distributor comprising a 40 μm mesh, has been prepared and used herein [9, 10]. To regulate the position of the liquid outlet to the top of the expanded bed, a movable adapter was used. Normally the adsorbent particles were added to 0.06 m [11] sedimented bed height. In order to transport the fluid, a peristaltic pump (B-V Trat Acque, Etatron DS-Rome, Italy) was used. Proper column vertical alignment was positioned in all experiments. The experiments were carried out in deionized water at 25°C. The degree of the expanded bed was measured three times for each flow velocity after the equilibrium expansion for 30min. Then the mean value of degree of the expanded bed was used to estimate the bed expansion factor and bed viodage [12].

$$E = \frac{H}{H_0} \tag{5}$$

Where H and H_0 represent the expanded bed height and the sedimented bed height, respectively. In this study, H_0 was 6 cm. To describe the expansion properties, the well-known Richardson-Zaki equitation was used as follows [13].

$$U = U_t \varepsilon^n \tag{7}$$

Based on relation between the voidage of expanded bed (ε) with the superficial liquid velocity (U), the terminal settling velocity of particle (U_t) and the expansion index (n) could be calculated. Bed voidage was calculated by Eq. 7.

$$E = \frac{H}{H_0} = \frac{1-\varepsilon_0}{1-\varepsilon} \tag{7}$$

Where, the ratio of expanded bed height (H) to sediment bed height (H_0) is named the expansion factor (E) and the voidage of sedimented bed (ε_0) is commonly supposed as 0.4 [14].

Then, according to Eqs. (3) and (4), experimental values of U_t and n can be determined by the double logarithmic plot of ε vs. U.

Residence Time Distribution (RTD) measurements were performed using a negative step signal method. A bed of the adsorbent particles was fully expanded using buffer A at tested flow rates. A dilute acetone solution (10% v/v) was used as the input to the column in a system fluidised with buffer A. The UV absorbance of the acetone was measured by spectrophotometer (Jenway 6305 UV/VIS. spectrophotometer, Germany) at wave length of 280 nm [15] in stream output from the column using a UV monitor. The Bodenstein number (B_o) and axial dispersion coefficient (D_{ax}) which express the state of liquid dispersion and fluidisation behaviour, were calculated by the following equations [8]:

$$N = 5.54\left(\frac{t_R}{W_{1/2}}\right)^2 \tag{8}$$

Where t_R is the residence time and $W_{1/2}$ is the half peak width.

Eq. 9 calculates value of HETP as follows:

$$HETP = \frac{H}{N} \tag{9}$$

Equation below defines the B_o number relating convective transport of liquid to dispersion:

$$B_0 = \frac{UH}{D_{ax}\varepsilon} \tag{10}$$

In addition, Eq. 11 calculates B_o number as follows:

$$\frac{1}{N} = \frac{2}{B_0} + \frac{8}{B_0^2} \tag{11}$$

Thus, D_{ax} is defined by Eq. 7.

RESULTS AND DISCUSSIONS

Particle Appearance and Physical Characteristics: Figure 1 shows the morphology of the prepared matrix under the optical microscope. The prepared matrix was in a uniform spherical shape and no fragments were observed. The important physical properties of expanded bed matrices are considered, including sphericity, wet density, water content, size distribution and pore construction [16]. In the present work, Streamline DEAE was used for comparison purposes. The main physical properties of Agarose-Nickel matrix and Streamline DEAE were measured and listed in Table 1.

The size distribution of prepared matrix was measured and the results are illustrated in Figure 2. Using the size distribution data, the size range and mean particle size were investigated and listed in Table 1. Agarose-Nickel particles have the logarithmic symmetrical distribution. The mean size of Agarose-Nickel particles is about 126.4 μm, which is smaller than Streamline DEAE (203 μm).

Table 1: The physical properties of the Agarose-Nickel matrices and Streamline DEAE

Matrix	Size range(µm)	Mean size(µm)	ρ_p (g/ml)	ω (%)	P (%)
Agarose-Nickel	50-200	126.4	1.95	49.5	91.93
Streamline DEAE [17]	100-300	203	1.16	74.3	86.4

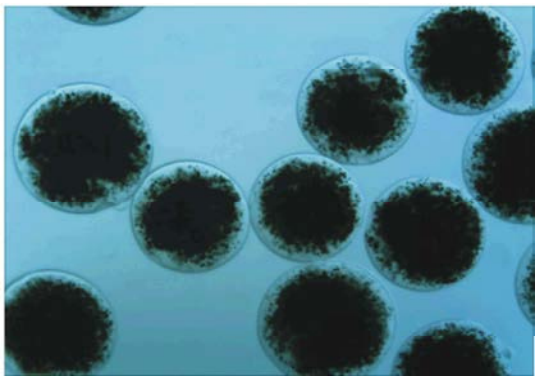

Fig. 1: Optical micrograph of Agarose-Nickel matrix

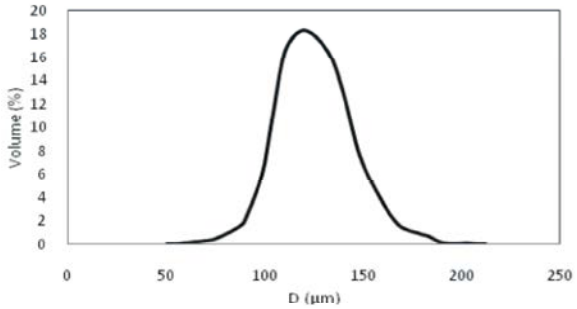

Fig. 2: Size distribution of Agarose-Nickel matrix

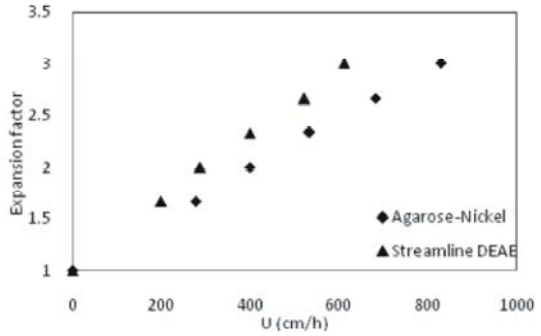

Fig. 3: Bed expansion for Agarose-Nickel matrix and Streamline DEAE

Generally, wet density of adsorbent plays an important role in performance of expanded bed [8]. Wet density of matrix determines the operation velocity of the expanded bed and high matrix density means high productivity of the separation process [5].

Based on physical properties of the Agarose-Nickel matrices and Streamline DEAE (Table 1), the wet density of prepared matrix was 1.95 g/ml, which is significantly higher than that of Streamline DEAE bead (1.16 g/ml) [17]. Considering the differences of densities between Nickel powder (8.9 g/ml) and quartz (2.4 g/ml), addition of Nickel powder at the same volume ratio produced a heavier matrix. The results indicated that prepared matrix can be used at higher flow velocity.

Hydrodynamic Characteristics

Expansion Characteristics and Correlations: The bed expansion contributes to the adsorption efficiency as a composite function of liquid distribution, liquid properties (e.g. viscosity, density and content of cell homogenate), particle characteristics and the configuration of the column in terms of wall and distributor effects [15]. The expansion characteristics of Agarose-Nickel matrix and Streamline DEAE were measured under the normal operating conditions for EBA. The results are shown in Figure 3. Obviously big and heavy particles caused low expansion at the same fluid velocity [18]. The Streamline DEAE matrices have shown bigger particle diameter and smaller wet density in compare to the prepared matrices of present work (Table 1). It can be deduced from Figure 3 that a matrix having a low wet density does not necessarily exhibit a high degree of bed expansion at a given fluid velocity (compare the prepared matrix and Streamline DEAE degree of bed expansion at the different values of fluid velocity) [15]. It meant that bed expansion of Agarose-Nickel matrix was lower than that of Streamline DEAE at the same flow velocity.

The perfect operation expansion factor of EBA is in the range of 2-3 and Agarose-Nickel matrix could be used for a fluid velocity of 398.72 to 829.77 cm/h and Streamline DEAE for a fluid velocity of 286.55 to 611.23 cm/h. Therefore, Agarose-Nickel matrix is beneficial for the separation with fast adsorption and high productivity.

The famous Richardson-Zaki equation (Eq. 6) has been used to correlate the expansion factor and bed voidage. It was observed that Richardson-Zaki equation could describe the relationship between the expansion voidage and the flow velocity for the prepared matrix and Streamline DEAE (Figure 4). The decision coefficients (R^2) were 0.992 for the prepared matrix and Streamline DEAE.

The correlated parameters are listed in Table 2. The expansion index (n) was 4.9 for the prepared matrices and 5.12 for Streamline DEAE matrices and consequently near to the theoretical value of 4.8 in the laminar flow

Table 2: Parameters of U_t and with Richardson-Zaki equation.

Matrix	U_t (cm/h)	n
Agarose-Nickel	2377.96	4.9
Streamline DEAE	1878.07	5.12

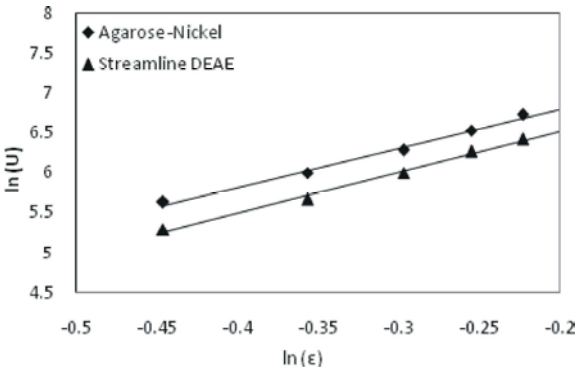

Fig. 4: Richardson-Zaki correlation between flow velocity and bed viodage

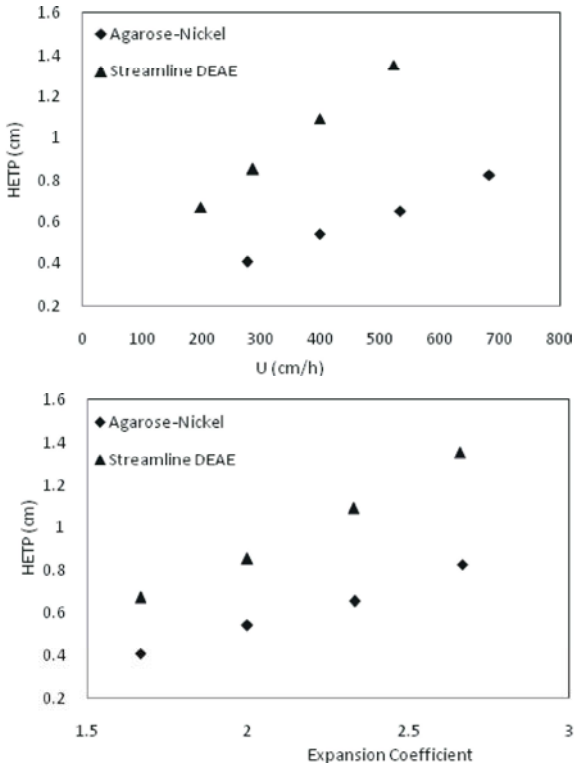

Fig. 5: HETP as the function of flow velocity and bed expansion

regime. In addition, it was observed that terminal settling velocity (U_t) of Agarose-Nickel n correlated matrix was higher than that of Streamline DEAE.

Mixing of the Liquid Phase in the Expanded Bed: The fluidization stability of adsorbent in the expanded bed is another important factor, which should be considered

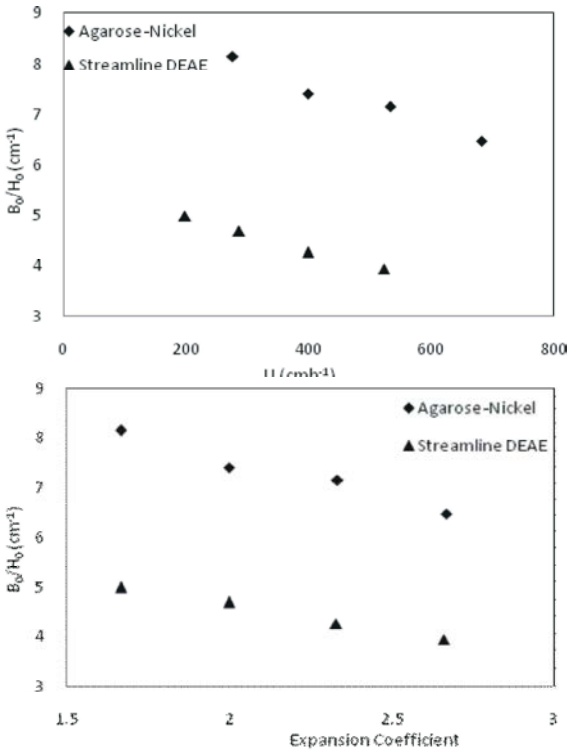

Fig. 6: B_0/H_0 number as the function of flow velocity and bed expansion

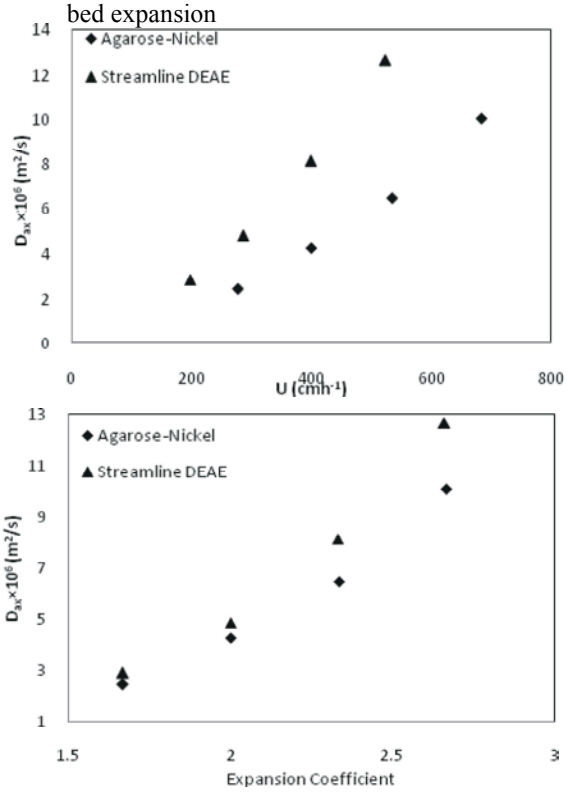

Fig. 7: Axial mixing coefficient (D_{ax}) as the function of flow velocity and bed expansion

in the EBA applications [20]. Measurement of the axial dispersion, i.e. the deviation from plug flow movement of fluid elements in an adsorbent bed (both packed and fluidized), is commonly performed by RTD analysis of step or pulse signals [11]. In this work, this method was employed in order to characterize and compare Agarose-Nickel matrix with a commercial matrix. To investigate the characteristic of liquid mixing in the column, the height equivalent of a theoretical plate (HETP), Bodenstein number (*Bo*) and the axial mixing coefficient (D_{ax}) are used.

As noticed in Eqs. (8) and (9), the number of theoretical plates (N) and then the height equivalent of theoretical plate for both the matrices under corresponding operation conditions were calculated. Figure 5 depicts HETP as the function of flow velocity and bed expansion. The prepared matrices showed smaller HETP than commercial matrix.

To calculate the B_0 numbers, Eq.11 was used. To eliminate the effect of different initial bed height, the parameter of B_0/H_0 was used [21]. Figure 6 shows the B_o/H_0 as the function of expansion factor and flow velocity. The values of B_0/H_0 ranged from 6.46 to 8.15 cm^{-1} for Agarose-Nickel matrix and 3.94 to 4.98 cm^{-1} for Streamline DEAE. It meant that the expanded bed with Agarose-Nickel composite matrix is more stable than that with Streamline DEAE.

The axial mixing coefficients (D_{ax}) were calculated in Eq. (10) and the results are shown in Figure 7. It was observed that under corresponding operation conditions, the D_{axl} values of the prepared matrix were lower than that of Streamline DEAE, which indicated that the prepared matrix had better hydrodynamic properties than commercial matrix. In this study, at fluid velocity of 276.49-829.77 cm/h, the D_{ax} values of the prepared matrix were between 1.0×10^{-6} and 1.0×10^{-5} $m^2.s^{-1}$. According to the literature, this range of the D_{ax} values will benefit EBA application [17]. In addition, for a normal operation velocity of 520 cm/h, the values of D_{ax} were lower than 6.5×10^{-6} $m^2.s^{-1}$ for the prepared matrix and 12.6×10^{-6} $m^2.s^{-1}$ for Streamline DEAE. The results indicated that Agarose-Nickel matrix can formed a better stable expanded bed than Streamline DEAE.

As illustrated in Figures 5, 6 and 7, with increasing fluid velocity, the HETP values increased, the B_o values decreased and the D_{ax} obviously increased. It indicated that the stability of expanded bed would be disturbed with the increase of flow rate.

CONCLUSION

A novel type of composite matrix was prepared with Nickel powder as the densifier and Agarose as the skeleton using the method of water-in-oil emulsification. The physical properties of Agarose-Nickel matrix were measured. The prepared matrix had the spherical appearance, suitable size and size distribution, appropriate wet density of 1.95 g/ml and porosity of 91.93%. The bed expansion factor in the range of 2-3 was demonstrated and correlated with Richardson-Zaki equation. In addition, the theoretical prediction of correlation parameters (the terminal settling velocity (U_t) and expansion index (n) was measured. The stability of expanded bed was studied by residence time distribution (RTD) experiments and the common quantities used to describe the expanded bed process were discussed (i.e. height equivalent of a theoretical plate (*HETP*), Bodenstein number (B_o) and axial dispersion coefficient (D_{ax}). The obtained results indicated that Agarose-Nickel matrix shown better feature of expansion, lower axial mixing than the commercial Streamline DEAE matrix. Furthermore, the results indicated that the stability of expanded bed decreased with the increase of fluid velocity.

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Notation:

B_o = Bodenstein number dimensionless
D_{ax} = Axial dispersion coefficient m^2/s
E = Bed expansion factor dimensionless
H = Height of expanded bed cm
H_0 = Height of sedimented bed cm
M_1 = Mass of wet matrix g
M_2 = Mass of a gravity bottle filled with water g
M_3 = Mass of a gravity bottle with wet matrix full of water g
m_1 = Mass of weighing bottle g
m_2 = Mass of matrix and weighing bottle before drying g
m_3 = The mass of matrix and weighing bottle after drying g
n = Richardson-Zakai parameter dimensionless

N = The number of theoretical plates dimensionless
P = Porosity %
t_R = Retention time min
U = Superficial fluid velocity cm/h
u_t = Terminal velocity of a single adsorbent particle in a volume of stagnant liquid cm/h
$W_{1/2}$ = Half-peak width min
V = Pore volume ml/g
$HETP$ = The height equivalent of theoretical plate dimensionless

Greek Letter:

ρ_c = Wet density of the particles g/ml
ρ_W = Density of water g/ml
ω = Water content %
ε = Voidage of expanded bed dimensionless
ε_0 = Voidage of sedimented bed dimensionless

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