Biotechnology and Bioprocess Engineering **2004**, 9: 323-325

Application of Modified Stokes Expression to Model the Behavior of Expanded Beds with Feed Streams Containing *E. coli* Homogenates

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Abstract To model the behavior of expanded beds with aqueous feed streams containing different amounts of glycerol, we previously developed a modified Stokes expression that correlates the terminal settling velocity of a particle of a solution with the properties of the particle (particle diameter and density) and the solution (density and viscosity). Two empirical parameters, the effective diameter of the poly-disperse resins for protein adsorption and an exponent of non-unity for $(\rho_p-\rho)/\mu$ term, are introduced in the modified Stokes expression. We applied the same type of the modified Stokes expression in combination with the Richardson-Zaki correlation to the published results [1], and found that the expansions of the beds with feed streams containing different amounts of *E. coli* homogenates can also be successfully described.

Keywords: expanded bed, modeling, Stokes expression, cell homogenates

Before selectively separating proteins by packed-column chromatography methods, such as gel-filtration chromatography or ion-exchange chromatography, traditional recovery of proteins from fermentation broths or cell homogenates starts with the clarification and concentration steps; protein solutions containing solid particles should go through centrifugation or filtration steps to remove cells or cell debris and then dialysis and concentration steps to remove untargeted small molecules and to decrease the solution volume [2-4]. These preliminary steps for protein recovery are the main causes for low yields, prolonged process time, and high operational costs. These problems become more serious as the scale of protein recovery process increases.

For expanded bed adsorption (EBA) technology, a feed stream is applied from the bottom of the column containing proper resins for protein adsorption at a flow rate enough to maintain the resins well expanded but with low back-mixing. This enables the simultaneous achievement of clarification, concentration, and selective separation [5-7]. Solid particles in the feed stream can pass freely through the expanded bed, therefore, the clarification steps become unnecessary.

EBA technology, however, lacks such thorough understanding of its hydrodynamic behavior as has been available for the conventional packed-column technology. Therefore, in order to utilize EBA technology for industrial-scale protein purification processes, a method to describe the behavior of the expanded beds in terms of the properties of resins and feed streams should be provided. Such a model will be utilized to design equipment and to preset the optimal values of operating parameters for EBA processes.

The Richardson-Zaki correlation, in which logarithms of the superficial fluid velocity (v_o) and the void fraction of the bed ($1-\phi_s$) are linearly correlated with each other by Eq. (1), has been widely used to describe the expansion of beds containing the resins as the adsorbents for protein recovery.

$$\log v_o = n \log (1-\phi_s) + \log v_t \quad (1)$$

The ϕ_s and v_t values are the solid fraction of the expanded beds and the Stokes terminal settling velocity of the particle at infinite dilution ($\phi_s = 0$), respectively [8]. The superficial velocity (v_o) is obtained by dividing the volumetric flow rate of the feed stream by the cross-sectional area of the column. The solid fraction, ϕ_s, of the expanded beds at a height H can be obtained by Eq. (2).

$$\phi_s = (\phi_s)_o H_o / H \quad (2)$$

In Eq. (2), $(\phi_s)_o$ is the solid fraction, and H_o is the height of the sedimented bed at zero flow rate of the feed stream. Combining Eq. (2) and Eq. (1) results in an explicit expression for bed expansion by Eq. (3).

$$H/H_o = (\phi_s)_o / [1-(v_o/ v_t)^{1/n}] \quad (3)$$

In theory, the Stokes terminal settling velocity is ap-

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plied when Reynolds number for the particle is less than 1 and expressed as a function of the properties of the particle and solution by Eq. (4).

$$v_t = g\,(d_p)^2(\rho_p-\rho)/18\mu \quad (4)$$

In Eq. (4), g is the gravitational acceleration, ρ_p and ρ are the densities of solid particle and solution, respectively, and μ the solution viscosity. Due to the polydispersity of almost all the resins used for the expanded beds, however, v_t values cannot be determined theoretically by Eq. (4) but are estimated by Eq. (1) using the experimentally measured values of v_o and ϕ_s. Though, the Richardson-Zaki correlation was successful in modeling the behavior of many expanded beds [1,9], until now no further attempts have been reported to correlate the estimated values of n and v_t to the properties of resins and solutions. Therefore, the Richardson-Zaki correlation cannot be utilized for the purposes of scaling up.

Previously, we studied bed expansion containing chelating excellose® (70~210 μm in diameter, 1.21 g/cm^3 in density, purchased from Bioprogen, Inc., Korea), which had Ni^{2+} ions that enabled the selective binding of histidine-tagged proteins [3] in a column (2.54 cm in diameter and 75 cm in length). The density and viscosity of the feed stream into the column varied because aqueous buffers (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 7) containing different concentrations of glycerol were used. To model the behavior of the expanded beds, we developed a modified Stokes expression, Eq. (5), incorporating two empirical parameters; effective diameter ($(d_p)_e$) and an exponent (a) for the $(\rho_p-\rho)/\mu$ term.

$$v_t = g\,(d_p)_e^2[(\rho_p-\rho)/\mu]^a/18 \quad (5)$$

According to Eq. (5), the intercepts, log v_t values, of the Richardson-Zaki correlation have a linear relation with log $(\rho_p-\rho)/\mu$ values as shown in Eq. (6).

$$\log v_t = \log (g\,(d_p)_e^2/18) + a \log (\rho_p-\rho)/\mu \quad (6)$$

Therefore, using Eq. (6), the $(d_p)_e$ value can be determined from the intercept and a value from the slope of the plot.

To ensure the general applicability of Eq. (6), we applied the modified Stokes expression to the results reported by Reichert *et al.* [1]. Fig. 1 shows bed expansion at various superficial velocities of the feed streams containing different concentrations of *E. coli* homogenates. Application of Eq. (1) to analyze the data in Fig. 1 gives the two parameters; n and v_t. The variation of value n upon the changes in the concentration of the *E. coli* homogenates of the feed stream was not large. For the four feed streams, 4.32 was the average value, n_{av}, of the n values. Fig. 2 shows the linear relation between the values log v_t and log $(\rho_p-\rho)/\mu$ with a slope of 0.86 and an intercept of 1.79. Because Reichert *et al.* [1] did not provide the density (ρ_p) value of the resins in the bed, we assumed the value of 1.2 g/cm^3, a typical density of resins

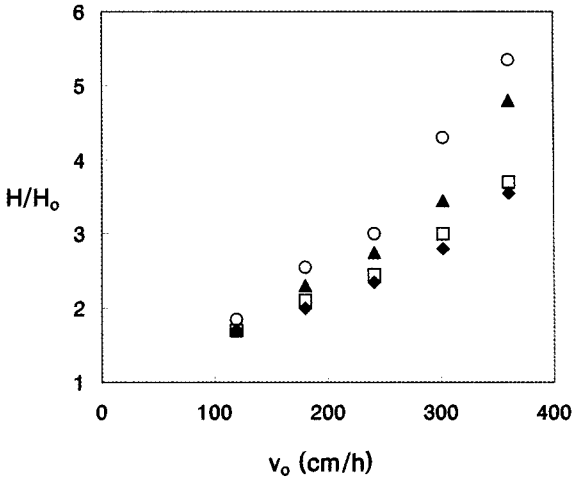

Fig. 1. Effects of the superficial velocities of the feed streams on bed expansion of the resins (Streamline Red). Aqueous buffers containing different biomass concentrations were used as the feed streams. The concentrations of the *E-coli* homogenates (wet weight %) are 0% (◆), 5% (□), 10 %(▲), and 15%(○). Data were reproduced from Reichert *et al.* [1].

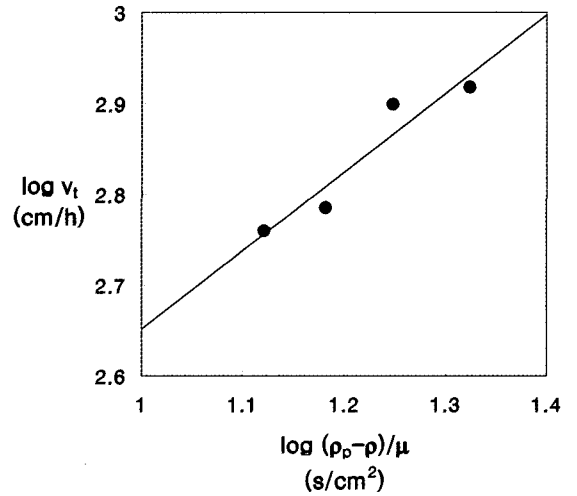

Fig. 2. A linear correlation between the log $(\rho_p-\rho)/\mu$ values with the log v_t values determined from the Richardson-Zaki plot.

used for EBA technology [6]. The density (ρ) and viscosity (μ) values of each feed stream containing different amounts of *E. coli* homogenates were determined by the linear interpolation of the data provided by Reichert *et al.* [1]. For the solution containing 15% wet weight of the *E. coli* homogenate, $\mu = 1.4 \times 10^{-2}$ g/(cm s) and $\rho = 1.015$ g/cm^3. The intercept and the slope of the linear plot in Fig. 2 helps calculate $(d_p)_e$ (177 μm) and the exponent, a (0.86), values, respectively.

Therefore, combined use of Eq. (3) and Eq. (5) is necessary to calculate bed expansion (H/H_o) as a function of the superficial velocity (v_o) of feed streams which vary in density and viscosity. Fig. 3 demonstrates that the calcu

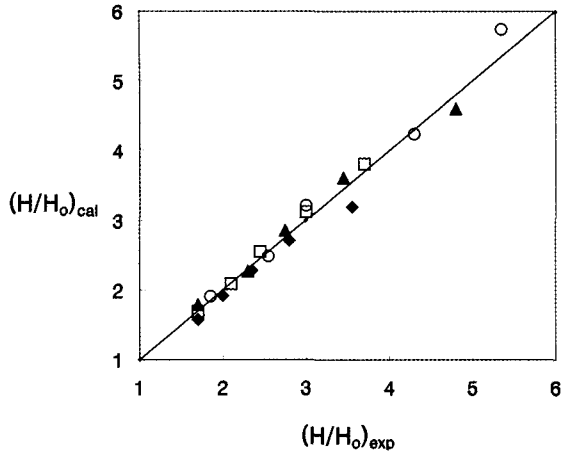

Fig. 3. Comparison of bed expansions calculated using the modified Stokes expression, $(H/H_o)_{cal}$, with those of the experimental value, $(H/H_o)_{exp}$. The same symbols from Fig. 1 are used.

lated bed expansions, $(H/H_o)_{cal}$, are in good agreement with experimental values of bed expansion, $(H/H_o)_{exp}$. The value n in Eq. (3) was replaced by n_{av} to calculate the $(H/H_o)_{cal}$ values. The solid fraction of the sedimented bed at zero flow rate of the feed stream, $(\phi_s)_o$, was found to be 0.58 from the analysis of the Richardson-Zaki parameters. In particular, the agreement between the two bed expansions in Fig. 3 was best when the bed expansion was from 1 to 3, which is the recommended range required for proper operation of the expanded beds [1].

Conclusively, the modified Stokes expression we developed is proven to be successful in describing the behavior of the expanded beds for a variety of resins and feed streams. In addition, because only the physical properties of resins and feed streams are incorporated into the modified Stokes expression, our approach will also be valuable for the scale-up studies of EBA technology.

Acknowledgements This work was supported by the Basic Research Program from the Korea Science & Engineering Foundation (grant number R01-2002-000-00050-0). Authors thank Mr. Hyun-Tae Sung for his experimental assistance.

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[Received April 23, 2004; accepted July 7, 2004]