Expanded Bed Adsorption: An Option For Energy Savings In Multiple Proteins Purification

Kavitha T C & B.S.V.S.R.Krishna
Department of Chemical Engineering, MIT, Manipal University, Manipal-576104, India
E-mail : krishna.bandaru@manipal.edu

Abstract **-** Expanded Bed Adsorption enables the protein recovery directly from cultivations of microorganisms or cells and preparations of disrupted cells, without the need for prior removal of suspended solids. The performance ofan expanded bed is comparable to a packed bed owing to reduced mixing of the adsorbent particles, clogging/plugging of solid particles in the column, while fluidized bed requires more velocity which may hinder the adsorption equilibrium. However,optimal operating conditions are more restricted than in a packed bed/fluidized bed due to the dependence of bed expansion on the sizeand density of the adsorbent particles as well as the viscosity and density of the feedstock. These difficulties can beovercomes in expanded-bed chromatography. In this work the steady state hydrodynamic behaviour of expanded beds studied experimentally.The present study focused on hydrodynamics of multiple particles separation and subsequently can be applied to the multiple proteins separation in a single expanded bed.

Keywords **-** *Expanded bed adsorption; packed bed; proteins; feedstocks; hydrodynamics;*

I. INTRODUCTION

Proteins are produced by genetically engineered microorganisms, yeasts and animal cells became a very important technique for the preparation of pharmaceuticals. The feed stocks are generally complex, which contains solids of various sizes and molecular masses, respectively. Despite the nature of the raw material the products have to be purified with a definite purity and potency. Many unit operations are involved in this purificationand account for the different separation necessities, as shown in the Fig.1. The targeted protein can be in the interior of cells or excreted; these proteins are brought out by celldisruption method that yields cell debris of different sizes besides some intact cells and proteins. These solids have to be separated from the desiredprotein. Microfiltration and centrifugation are the common methods for the separation of solids and soluble. It must be considered that, besides the cost, the required process time is typically several hours. Therefore, the target proteins are not in a safe environment, where oxidation or aggregation can be controlled. Expanded bed adsorption allows integrating solid –liquid separation, volume reduction by protein adsorption and partial purification in a single unit operation without compromising on separation efficiency, but saving considerably process energy, processing time and capital investment [1,2,3].

Expanded bed :This separation technique is commonly employed in stirred-tank processes using particles with large diameters (0.5–3 mm) and high inner surface area in order to separate adsorbent particles and biomass and to allow for a high capacity for a product. Also column processes are reported, such as for the adsorption of the secondary metabolite streptomycin [4]. Batch processes are composed of several consequent and usually manual operation steps with pouring or pumping the suspensions from one vessel into another in an open environment.

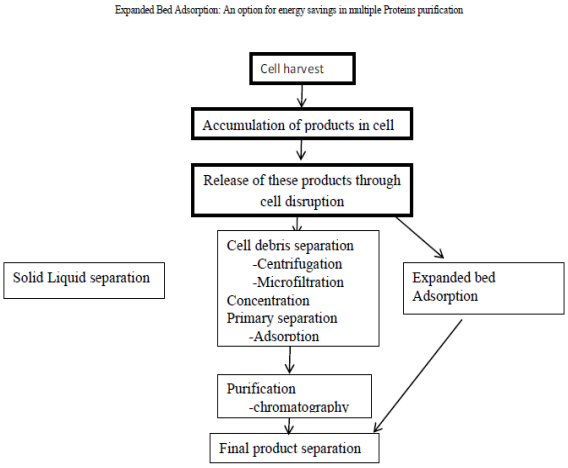

Fig. 1: Process pathways with conventional downstream processes and after inclusion of expanded bed adsorption

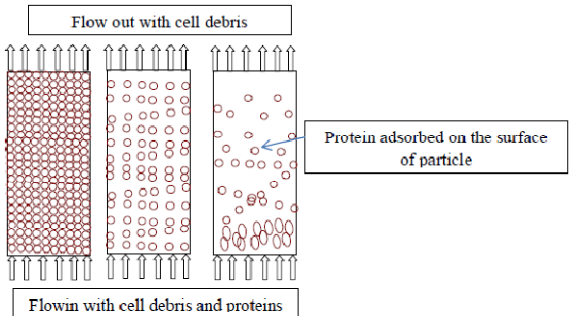

Fig 2 : Operation modes for protein adsorption in column beds with packed bed, fluidized bed and expanded respectively.

As adsorption is not instantaneous, a certain contact time must be designated in between the target protein and adsorbent, so that equilibrium can adjust. Fig 2 shows the feed stock passage through various modes of operation.

However, except for very large beads, a packed-bed column is not suitable for processing feed-stocks with suspended biomass, as particles become trapped in the voids of the bed. This results in the formation of a plug of trapped solids near the column inlet and finally to a complete blockage of the column, and leads to heavy pressure drop. A way out of this dilemma is to pump liquid upwards through a particle bed that is not constrained by an upper column adapter. Then the particle bed begins to expand, if a certain flow-rate is exceeded. Such fluidized beds are known from the heterogeneous catalysis of chemical reactions, where often a gas phase (bubbles) is added to obtain high rates of heat transfer and uniform temperatures within the bed. Mixing in conventional liquid fluidizedbeds is not as pronounced. However, it is characteristic also for these systems that the adsorbent particles are moving slowly, but constantly in all directions inside the column. Although the performance of a liquid fluidized-bed system is higher compared to the stirred-tank system, it is inferior compared to a packed-bed and may therefore still require recirculation of the feedstock. Besides rare exceptions, chromatographic particles are not monodisperse and are thus classified in a fluidized bed with the larger particles being locatedcloser to the bottom of the bed and the smaller particles distributed towards the top. If the particles have an appropriate size distribution, classification leads to layers of particles and a reduced mixing in the column. This contributes significantly to the stability of the bed, which then shows fluid dispersion characteristics similar to a packed bed [3]. The appropriate distribution requires a particle size ratio of about greater than 2.2 [5,6]. If also a distribution of the specific density of the particles can berealized, a further stabilization of particle classification is possible. In order to distinguish the dispersion characteristics of classified fluidized beds from those containing particles with relatively narrow size distribution, the term expanded bed was assigned [3,4,9]; expanded-bed adsorption and expanded-bed chromatography are coexisting synonyms.

Column requirements:

The column design must fulfill some basic requirements. The major differences of EBA and packed-bed column are the two adapters. The lower adapter holds a flow distributor — either a plate with holes or a bed of glass ballotini — that ensures a plugged flow in the column by assuring an even pressure drop. This, as a rule of thumb, should be of about the same value as experienced along the column. The distributor enables the passage of particulates without a blockage. An additional screen is placed on top of it; it retains the adsorbent in the column when flow is stopped or reversed. If the column is to be operated both in expanded and packed mode, altering the position of the upper adapter must be possible. This can be done manually, hydraulically or by using special floating devices. Perfect vertical alignment is mandatory for both the packed and expanded bed columns. Deviations inevitably lead to inhomogeneous liquid flow and consequently to unstable beds. This is to be specifically emphasized for laboratory-made equipment. Another column design completely circumvents a lower column adapter; the development of a plug flow is considered irrelevant in the lower part of the column [7]. Here the column is closed at the bottom, but contains laterally connected tubes close to the bottom and a stirrer blade inside. With closing the outlet tube and allowing liquid to flow from the inlet tube to the upper adapter, the bed starts to fluidize. With starting the stirrer, adsorbent mixing takes place in the lower part of the bed, whereas the upper part stays stable with a sharp transition between. Further operation is carried out in the same way as stated above with the difference that elution takes place through the outlet tube. This tube contains a screen in order to retain the adsorbent in the column. The concept reminds of a stirred-tank process at first; however, using adsorbents with appropriate size distribution, classification takes place in the upper part of the column also in this configuration. It remains to be shown whether the mixing and the elution through a laterally connected outlet affect the performance of this system.

II. PRACTICAL CONSIDERATIONS

Matrices for expanded-bed chromatography **:** Some fundamental work was done with chromatographic adsorbents being used in packed-bed chromatography [3,8,9]. However, besides the relatively unstable fluidized beds that develop with these matrices, their

size and specific density are not optimized for a fluidized bed operation. This means low flow-rates, long application times and hence low productivities. Denser particles, such as silica, are more appropriate in this respect [10]; however, they are not stable under CIP conditions. Generally, tailor-made particles for expanded-bed operation are the result of a compromise between the matrix characteristics (particle size, particle density, pore size), which determine the useful range of flow-rates, and adsorption kinetics, especially mass transfer limitations. Very big particles allow high flow-rates; however, then very high sedimented bed heights and long columns must be used in order to meet the residence time required for adsorption. Very dense particles, such as zirconia may be used at high flow-rates and can be small in particle size at the same time to reduce mass transfer restrictions. However, small adsorbent particles require a screen with narrow mesh size to retain them in the column, and such screens are prone to be blocked with cells or cell debris. Tailor-made matrices for protein adsorption in expanded beds are therefore available as hydrophilic polymers made of particulate cellulose, agarose or artificial polymers, such as trisacrylate. These are increased in density by the incorporation of heavier particles, such as quartz, glass, titania or zirconia or metal alloys. The sizes of these matrices are generally larger than those of packed-bed adsorbents used for protein purification, and consequently, column performances are low. Expanded-bed chromatography is to be seen as a primary adsorption process, where the target protein — among others — is to be adsorbed in a frontal chromatographic mode. Further purification of adsorbed proteins is not the main intention, though it can be done if it is easily accomplished. The process can therefore also be described as a capture step.

Limitations of expanded bed **:** As the expanded bed behaves in principle much like a packed bed, mixing in the liquid phase is not the major limiting parameter, as long as some requirements are met. One is the static bed height, which must have a minimal height for efficient protein adsorptionotherwise, dispersion is limiting and a high percentage of the target protein will be lost in early breakthrough fractions. Also, local turbulences created by the flow distributor are more effective at low bed heights. In practice, the dynamic capacity of an adsorbent is often taken as a measure to compare operation conditions. With the tailor-made adsorbents described above, a minimal sedimented bed or static bed height of 100 mm is required for the adsorption of small proteins, such as lysozyme on a cation-exchanger. For larger proteins may need to be increased. It is recommended to start with a bed height of about 150 mm during method development to avoid the risk of an early break through.

Experimental**:**

Materials: The matrices used in this experiment are particle 1, particle 2, particle 3,particle 4. The physical properties of the particles are presented in table 1. A plexi glass column was used for conducting experiments. Bed expansion characteristics are identified visually. All experiments are performed using high purity water.

particle	dp(mm)	Density(kg/m^3)	Av size
Particle1	100-300	1200	200
Particle2	100-300	1200	200
Particle3	80-165	1800	140
Particle4	40-105	3200	75

Experimental procedure: The column was loaded with known amount of material of different particles (at least two particles) with initial height of 10 to 15 cm as mentioned the above theory. For all particles an estimated porosity of 0.39 was obtained by draining the liquid volume occupied in the pores for a given volume of bed.

Separation of multiple particles: The separations of multiple particles were tried in the experimental setup. The preliminary experiments were conducted for identifying the suitable particles or matrices based on their physical properties. The expansion of these particles has shown in Fig 4.From these bed expansion experiments particle 1 and particle 3 were found suitable for the separation. The colour of the particle1 and particle 3 can make easy for visual observation, since the particle 1 is whitein colour and particle 3 is black in colour. For example equal volumes of particle 1 and particle 3 made homogeneous mixture and loaded to the column. The liquid was pumped through the column. This resulted in expansion of the mixed particles. A clear segregation of the two particles at steady state was observed under conditions when the velocity was larger than the minimum fluidization velocity of the bigger and denser particles and shown in Fig 3. The individual particles were seen to form two distinct beds. The interface was very clear at all liquid velocities above the minimum fluidization velocity of the bigger and denser particles. Fig 4 shows the expansion of individual particles of particle 1 and particle 3. The expansion of two particles with various ratios of individual particles was shown in the Fig.5. This phenomenon helps in separation of multiple protein separation in a single expanded bed.

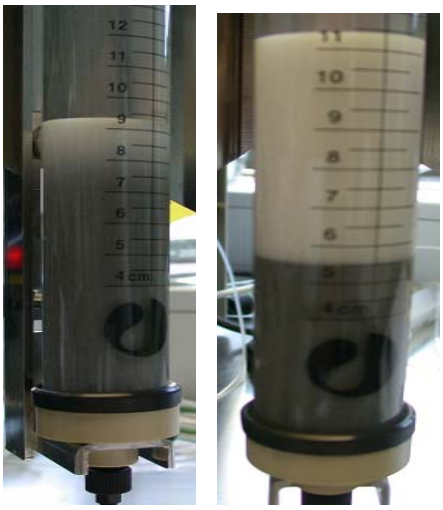

Fig. 3 : Binary mixture of particle 1 and 3 (Before separation and after separation)

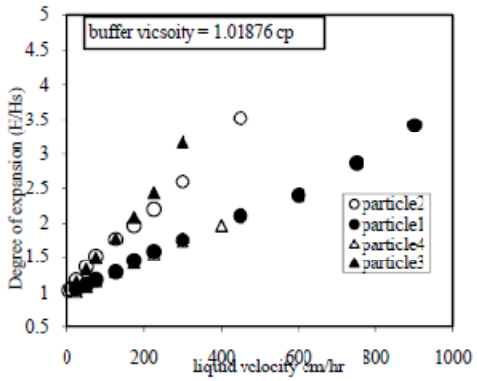

Fig 4. Variation of bed height with liquid velocity

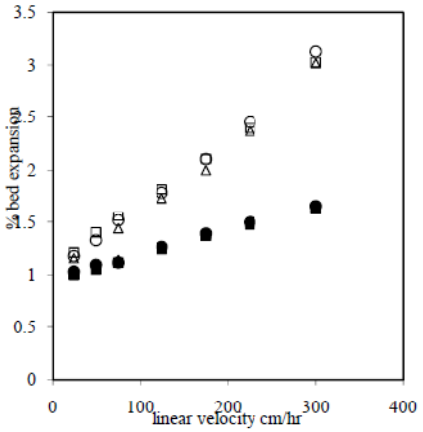

Fig. 5: Variation of bed height with linear velocity for different ratio's of settled bed in mixed particles with open solid as particle 1 and closed ones are particle 3

III. CONCLUSIONS

Expanded-bed chromatography is a solid/ liquidfluidized-bed technique with reduced mixing of adsorbent particles. It is an integrated method, combining particle separation, product concentration and partial protein purification in one equipment. Capital expenditure can be saved in those cases where large centrifuges and/or microfiltration units are not needed in cell separation. This reduces the energy requirement for a given separation. Saving of time is an additional advantage, which can amount to several hours in a large-scale process. Special column designs are necessary with a flow distributor in the lower adapter (to establish plug flow) and a movable upper adapter. The separation of multiple proteins can be done in single Expanded bed adsorption column. This reduces the operation time at the same time energy requirements for a given separation and improves the overall economics.

REFERENCES

[1] M. Weissenborn, B. Hutter, M. Singh, T.C. Beeskow, F.B. Anspach, Biotechnol. Appl. Biochem. 25 (1997) 159.

[2] P.H. Hodgson, G.L. Leslie, R.P. Schneider, A.G. Fane, C.J.D. Fell, K.C. Marshall, J. Membr. Sci. 79 (1993) 35.

[3] H.A. Chase, Trends Biotechnol. 12 (1994) 296.

[4] R. Hjorth, Trends Biotechnol. 15 (1997) 230.

[5] M.R. Al-Dibouni, J. Garside, Trans. Inst. Chem. Eng. 57 (1979) 94.

[6] A. Karau, C. Benken, J. Thommes, M.-R. Kula, Biotechnol. Bioeng. 55 (1997) 54.

[7] E. Zafirakos, A. Lihme, EBA '96, First International Conf.on Expanded-Bed Adsorption, Cambridge

[8] H.A. Chase, M. Draeger, Sep. Sci. Technol. 27 (1992) 2021.

[9] N.M. Draeger, H.A. Chase, Bioseparation 2 (1991) 67.

[10] G.M.S.Finette, B. Baharin, Q.M. Mao, M.T.W. Hearn, Biotechnol. Progr. 14 (1998) 286.

◈◈◈