Expanded Bed Adsorption (EBA) for Purification of Protein Product of Biotechnology

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A large percentage of the products emerging from the rapidly expanding biotechnology industry are proteins. Proteins represent the most useful and diverse class of biopolymers [1]. They are important as foods, food supplements, industrial catalysts, cleaning agents and pharmaceuticals. In the area of pharmaceuticals, their usefulness lies in their properties, such as their enzymatic activity, specific recognition interactions with other molecules or other therapeutic actions. Proteins may be formed in, or obtained from a variety of sources such as fermentation broths of bacterial, yeast or mammalian cells or preparations of naturally occurring tissues or fluid. If one considers the heterogeneity of courses from which these proteins are derived, their intended use and their diversity, the need for a great variety of recovery processes becomes apparent [2]. These recovery processes are usually collectively described by the term 'downstream processing' which in its narrowest definition is the processes which involve the recovery, purification and stabilisation of such products from conditioned media or fermentation broth.

Techniques suitable for the purification of proteins have to meet boundary conditions, which are different from those applied to traditional chemical processing. This is on the one hand due to the sensitivity of the native protein structure and function to factors such as temperature, pressure, proteases and interfacial contact [3]. The other important difference between the purification of proteins and the separation of chemically produced compounds is the medium from which the protein has to be isolated. Protein sources typically contain particulate material which has to be removed prior to further purification. To complicate matters further, the liquid itself contains a wide variety of compounds such as other proteins, released lipids and nucleic acids, all in comparatively low concentrations, the desired protein often being only a minor component in this mixture.

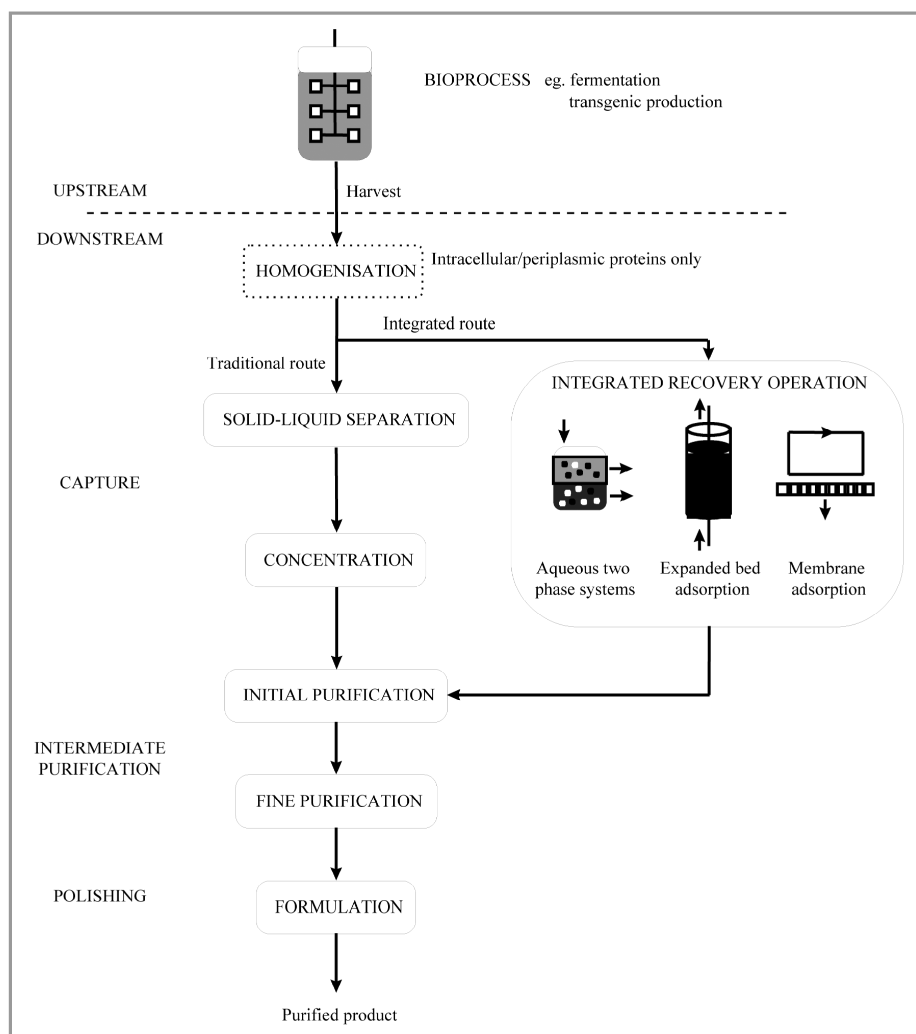

Figure 1: A typical downstream processing flowsheet with a possible site for the inclusion of an integrated unit operation such as expanded bed adsorption.

PROTEIN PURIFICATION FLOWSHEET

Due to the complexity of the separation and the high level of product purity required, the resulting purification schemes often contain multiple unit operations. In the past, cascades of 8–10 purification steps were normally used in the downstream processing flowsheet of protein production (Figure 1). Each step in the recovery process will affect the overall process economy by increasing operational cost and process time, and also by causing a loss in product yield [4]. Indeed, the purification process itself may contribute up to 80% of total production costs [5]. This highlights the

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need for bioprocess engineers to either achieve very high step yields of 92–98% or to reduce the number of steps for the downstream processing to be economical [6]. The downstream processes that comprises 8–10 unit operations typically exhibiting an average yield of 95% for each step will result in an overall purification yield of 50–80%. This represents 60–80% of the total cost of recombinant protein [7]. Therefore designing in the least possible processing steps offers the most efficient way of attaining high process economy in the overall production process.

A general scheme for the downstream processing flowsheet with a possible site for the inclusion of one or more integrated unit operations is shown in Figure 1. The initial phases of protein capture often involve the processing of feedstock containing particulate matter. In traditional downstream processing, removal of particulate matter from the feedstock is necessary before application to packed chromatography beds [9]. Two of the most widely used techniques for particulate removal are centrifugation and filtration.

Although the improved capacity of the centrifugation process has allowed larger volumes of process liquid to be processed at industrial scales [10], the efficiency of continuous centrifuges for clearing smaller particles remains poor. This is due to the lower centrifugal forces and the shorter residence times than those achieved by batch-operated centrifuges. Centrifugation is also damaging – the process may shear sensitive cells such as mammalian cells. The generation of a fine mist during centrifugation may also be unacceptable in certain cases, particularly for processes involving genetically-modified organisms [11,12].

Filtration is an alternative method for the clarification of the process liquid. The shear force in a filtration unit is gentler than centrifugation and is therefore more suitable for shear sensitive organisms. However, filtration rates are limited by filter fouling, in particular when smaller particles ranging in size between 0.1–1 m are to be filtered. Limitations arising from a dramatic decrease in the flux per unit area of membrane during operation

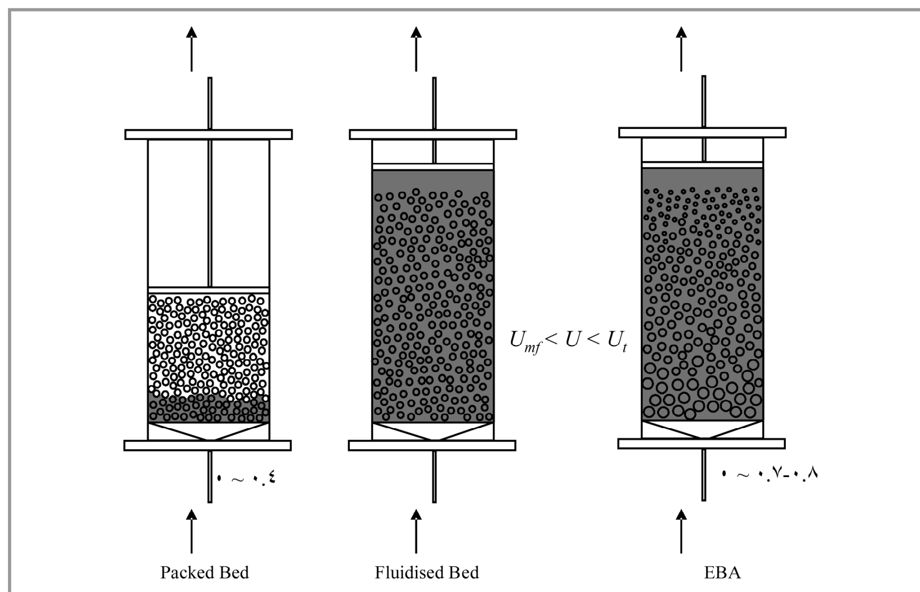

Figure 2: Comparison between packed bed, conventional fluidised bed and EBA. U is the adsorbent velocity, U_{mf} is the minimum adsorbent velocity, U_t is the terminal settling velocity and e is the bed voidage.

often result in a subsequent requirement for large membrane areas [13]. They often result in long processing times and the use of large units is often necessary [14].

In a simplified downstream processing flowsheet, the initial phase of protein recovery is integrated into a single step. This integrative recovery route (Figure 1) not only simplifies solid and liquid separation, but also combines originally independent steps to form new unit operations. This enables substantial improvements to downstream processing. Integrative protein recovery operations should be able to tolerate particles containing biological suspensions and deliver a clarified protein concentrate. Ideally a degree of purification of the target protein should also be achieved, thus combining clarification, concentration and purification in a single step. In a simplified integrated downstream processing flowsheet, the initial phase of protein recovery is integrated into a single step. This is of great significance since any reduction in the number of stages required in the flow sheets has always been of industrial interest. In fact, it is regarded as a tool for improving process performance and productivity. An integrated capture step is a challenging operation particularly when large quantities of contaminants and large volume of liquids are involved [15].

There are many different ways of achieving this integration of previously independent processes. Considering an adsorption solution to the integration problem, the conventional operating formats for the recovery of proteins are the packed bed, the stirred tank, the fluidised bed and the membrane. Packed beds cannot be challenged with particulate containing feedstreams as they act as depth filters, the voids of the bed rapidly blocking resulting in the pressure drop across the bed increasing until pump or pipe failure occurs. There are no occlusion problems with the stirred tank method, but the tank operates as just one equilibrium stage at best, resulting in inefficient capture at all but the highest partition coefficients. Problems can also arise with adsorbent handling and solid/liquid separation; however several benchscale batch and continuous applications have been developed [16, 17, 18, 19]. By fluidising the adsorbent, the average interparticle distance is increased such that any particles in the feedstock may pass freely through the interstitial voidage of the bed. Although there are a number of methods of achieving a stable fluidised bed [20, 21, 22, 23] it is evident that the basic structure of a fluidised bed might allow separation of proteins from unclarified feeds. Membrane-based chromatography has the advantage over

conventional chromatography on columns of porous particles due to the absence of pore diffusion [24]. Operation in crossflow mode can allow the adsorption of proteins to the inner surface of the through pores, whilst cellular debris is rejected [25, 26]. The only disadvantages of this method are the low surface area for adsorption and the dependence on the transmembrane flux, which can fail due to fouling with particulates.

Aqueous two phase adsorption is also capable of handling particulates; however the degree of purification attainable is usually lower than with chromatographic separations. Fluidised bed (FBA) adsorption would seem to have greater potential for efficient protein capture than stirred tanks, aqueous two phase extraction or crossflow microfiltration approaches.

INTEGRATIVE EXPANDED BED ADSORPTION (EBA)

A distinction should be made at this point between a packed, fluidised and an expanded bed (Figure 2). Liquid solid fluidised beds, with small density differences between phases, show particulate fluidisation behaviour, rather than the aggregative behaviour shown by gas solid systems. The mixing in particulate systems is less severe, however in many situations, particularly where there is uneven distribution of liquid across the cross section of the bed, there is still a significant and undesirable amount of mixing. This mixing leads to inferior adsorption performance within the fluidised bed as compared to the packed bed, where plug flow of liquid maximises the number of theoretical equilibrium stages (or plates). By introducing a distribution of both size and density into the solid phase, a classification occurs upon fluidisation eventually giving a stratified bed with the higher buoyant weight particles at the base and the lower buoyant weight particles at the top [4]. The reduction in solid and associated liquid dispersion results in adsorption performance similar to the packed bed. The term ‘expanded bed’ has been used extensively in the literature and by manufacturers to describe fluidised beds which have been stabilised by careful adsorbent and equipment design to give limited axial dispersion and higher plate numbers, and to distinguish them from well-mixed alternatives. A number of recent reviews of EBA have been published, covering operational and design aspects and more theoretical considerations [4, 14, 8].

OPERATIONAL PROCEDURE FOR EBA

EBA was introduced to meet the challenge of an increasing demand for fewer purification steps and shorter processing times. It integrates clarification, concentration and enables initial purification of the target proteins directly from unclarified feedstock in a single step [4, 14, 8]. In principle, expanded bed processes are operated in a similar fashion to packed bed processes, the main difference being the direction of liquid flow. The standard sequence of frontal chromatography is generally followed as in Figure 3, which are column equilibration, sample application, washing, elution and clean in place.

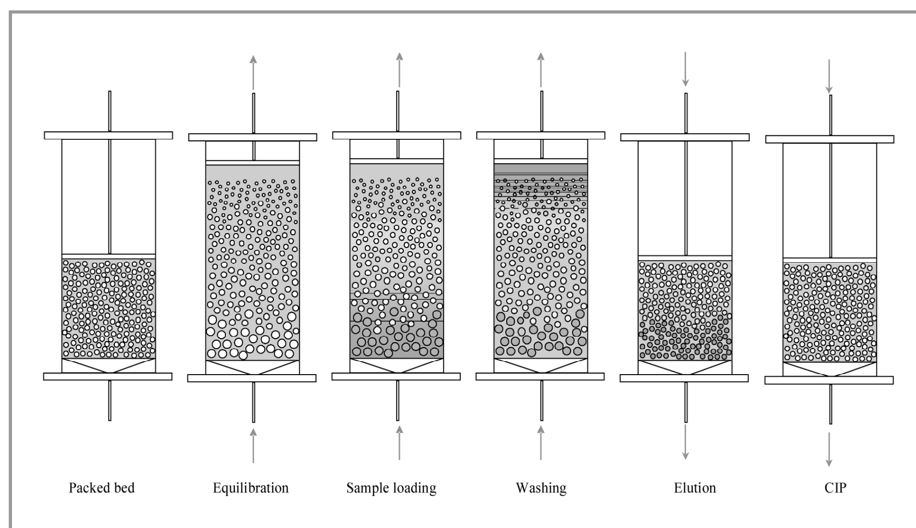

Figure 3: Schematic diagram of the stages in expanded bed adsorption. The arrows indicate the direction of liquid flow through the column (either upwards or downward).

APPLICATION OF EBA

There have been many successful applications of EBA for the purification of proteins and other biomolecules from crude (solid-containing) fermentation broth. Proteins have been captured from a wide range of particulate containing feedstock such as bacterial [9, 27, 28], yeast homogenates [29, 30, 31], mammalian cell cultures [32, 33, 34, 35], solutions of others renatured inclusion bodies [9, 36, 37] and milk preparations [38]. EBA has also been proven to be successful for the separation of E.coli and yeast cells [39], monocytes from human peripheral blood [40], nano-particles, such as plasmid DNA from E.coli cell lysates [41, 42] and calf thymus DNA [43]. Pilot-and production-scale processes have also been reported, including the purification of annexin V from E. coli homogenates using ion-exchange adsorbents [9], exotoxin A capture from E. coli BL21 (lDE3) homogenate using an anion exchanger adsorbent [15], IgG1 isolation from mouse hybridoma cells [35, 11] and the recovery of recombinant human serum albumin (rHSA) from Pichia pastoris suspension [44]. The same purity of monoclonal antibody from CHO cell culture is reported via a three-phase purification strategy in which Protein A adsorbent was used in an expanded bed capture step, as achieved by conventional processing routes [45]. These wide ranges of applications of EBA reported in the literature demonstrate this technology to be robust and economical for the early recovery of proteins directly from crude feedstock [2, 14,4 6].

CONCLUDING REMARKS

With many successful demonstrations and applications, EBA is becoming recognised as a realistic alternative to existing procedures for protein recovery. Indeed, examples of full scale industrial processes are beginning to appear. This may be attributed to the increasing number and diversity of successful applications at bench scale, the

development of purpose-designed adsorbents [2] and equipment and increasingly tighter process control and understanding. Such processes are proving to be efficient and compliant with the tough requirements of regulatory bodies such as the Food and Drug Administration (FDA). Validation considerations have been reviewed with regard to EBA applications and the first fully commissioned processes are beginning to appear [47]. ■

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