EXPANDED BED ADSORPTION: CHALLENGES FOR THE CHOICE OF TECHNOLOGICALLY ADVANCED ADSORBENT MATERIALS

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Bioprocessing is the technology for biologics production, separation, and purification. It is comprised of upstream and downstream bioprocesses. Upstream bioprocesses deal with the manufacturing of a desired product on a great scale, while the downstream bioprocesses deal with the separation and polishing of a preferred product. In recent years, the upstream bioprocesses have become efficient in producing very high titers of therapeutic molecules, an impossible feat just a few decades ago. As culture yields continue to increase, the downstream processing (DSP) has become the major bottleneck for bioproduct generation.

In bio-production, many multi-variable factors can affect the process efficiency. In the case of downstream processing, most often the fermentation broth contains a complex mixture of product, by-products, suspended cells, and debris, as well as many additional undefined contaminants.

Traditional DSP methods such as clarification, concentration, and primary recovery have reached their limits and are unable to meet current product demand, not only in terms of space requirements, but also in terms of cost, resource consumption and process time. Consequently, technologies that offer process integration, that is, a reduction in the number of unit operations in the initial isolation and purification steps while maintaining high product recovery and purity, would not only increase process economy but also reduce process time.

Expanded bed adsorption (EBA) is an integrated technology for the primary recovery of proteins from unclarified feedstock. A method which allows a qualitative and quantitative understanding of the main mechanisms governing the interaction of biomass with fluidised resins is reported in literature[1]. A pulse response technique was used to determine the adsorption of various cell types (yeast, Gram positive and Gram-negative bacteria, mammalian cells and yeast homogenate) to a range of commercially available matrices for EBA[1].

A typical EBA operation involves the following steps: bed stabilization/equilibration, sample loading, washing, elution, regeneration and cleaning, and re-equilibration as shown in Figure 1. The settled EBA bed is first expanded by applying upward flow that is sufficiently fast to fluidize the media beads. Particulate containing feedstock will be directly applied into the column after equilibration. The target proteins or smaller molecules will be binded to the EBA absorbent while other contaminants such as nucleotides and lipids pass through. A wash will be applied using fresh buffer to remove loosely bound contaminant molecules after the sample loading. Elution step is usually run as a packed bed by lowering the top adaptor and applying downward flow. The target proteins or molecules that were binding on the adsorbent will be eluted form the column and collected. The column will then be cleaned/regenerated using stronger agents such as sodium hydroxide and re-equilibrated using the starting buffer for the next loading cycle.

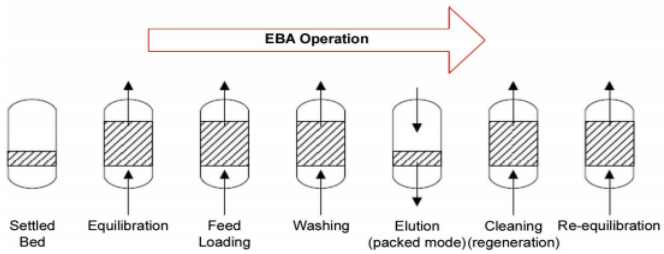

Figure 1. Concept of EBA operation

A wide range of applications of the EBA have been reported from lab-large scale protein purification using various host systems[2]. EBA has been successfully applied to the purification of a variety of biomolecules of commercial interest, from crude unclarified source materials, such as: Escherichia coli homogenate, yeast, fermentation, mammalian cell culture, milk, and animal tissue extracts[3].

The aim of this PhD Thesis work is the optimization of EBA processing using surface modified adsorbent beads. The primary objective of the work is to shield EBA beads from biomass interactions, so as to improve the process performance, and to investigate the effect of shielding, on process yield, product purity, and system hydrodynamics/bed stability.

Phase 1.

The PhD thesis is primarily centred on the study and preliminary characterization of several generations of the EBA adsorbents. Mostly, expanded bed adsorbents are manufactured in two different ways. In the first method, a high-density porous material such as cellulose, silica gel, or zirconium oxide particles are used. Alternatively, polysaccharide beads such as agarose, or dextran, are built with high density cores. Most commonly, high density cores are made of crystalline quartz, stainless steel, glass, tungsten carbide, kieselguhr particles, Nd–Fe–B alloy powder, zirconia-silica, nickel, zinc, titanium oxide, and zirconium oxide. The main goal is to understand the binding capacity that these adsorbent materials are able to have towards contaminants such as cells, cell debris, particulate matter.

Phase 2.

Numerical simulation modeling could be used to understand many aspects about the EBA adsorbents:

- The relation between the biomass type, adsorbent material, ligand and size of cell-adsorbent interaction
- Modelling of biomass transport in EBA, as **porous media (like silica media)**
- Evaluation of the process performance of the **cell repellant polymer-coated bead**

	6 months	12 months	18 months	24 months	30 months	36 months
Phase 1						
Phase 2						

Figure 1. Gantt Chart of the PhD Thesis

References:

[1] J. Feuser, J. Walter, M.R. Kula, J. Thömmes (1999)"Cell/adsorbent interactions in expanded bed adsorption of proteins", Expanded Bed Chromatography, *Bioseparation* **8**, pp 99-109.

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[3] Jörg Thömmes, AndreaBader, MarkusHalfar, AndreasKarau, Maria-Regina Kula (1996) "Isolation of monoclonal antibodies from cell containing hybridoma broth using a protein A coated adsorbent in expanded beds" *Elsevier,* pp 111-122

[3] Vikas Yelemane, Martin Kangwa, Roy N. Dsouza & Marcelo Fernández-Lahore *(2021)* "Surface energetics to assess influence of biomass-type and biomass–adsorbent interactions in expanded beds" *Bioresources and Bioprocessing, Article number: 29*

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