DOWNSTREAM PROCESSING OF PROTEINS USING FOAM FRACTIONATION

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Summary

Foam fractionation, as a low cost and effective separation technique in downstream processing, has been studied experimentally and theoretically. The effectiveness of foam fractionation has been demonstrated. The various experimental studies on protein foam fractionations focus on qualitatively investigating the effect of important parameters (pH, initial protein concentration, superficial gas velocity, liquid pool height and foam height) on the enrichment ratio, recovery rate, or separation ratio, as well as the biological activity of proteins. Modeling of foam fractionation of proteins is very dependent on understanding protein adsorption at gas-liquid surfaces and understanding of the hydrodynamics of foaming process. There has been some progress to date in qualifying the adsorption behavior of some proteins (β-casein, bovine serum albumin and lysozyme). There is need, however, for the development of better predictive models, for describing both the single and the more difficult binary/multi-component foam fractionation systems. The application of foam fractionation to downstream processing, therefore, is still at an early stage.

The experimental study presented in the latter part of this paper showed the advantage of foam fractionation in treating liquid mixtures of dilute protein solutions. In this case study of a mixture of BSA and cytochrome c, the best separation occurred at pH 4.9 using a low gas velocity. BSA is the more hydrophobic molecule and cytochrome c is the more hydrophilic molecule. This led us to conclude that, choosing the right pH with a low V_{gas} value, we can separate and concentrate a more hydrophobic protein molecule initially present in a mixture by using a very simple setup for foam fractionation.

1. Introduction

Recent developments in genetic engineering and cell culture, particularly in recombinant protein technology, has stimulated new research into downstream processing, which focuses on separation and purification of biological materials. Since the present separation techniques are quite expensive, new downstream processes are needed to reduce the manufacturing cost.

Downstream processing is commonly classified into four distinct steps: broth conditioning and removal of insolubles; isolation of the desired product (including clarification and extraction); purification with high-resolution techniques; and polishing. Among these steps, isolation and purification receive the most attention in order to reduce the high cost of producing proteins. The techniques used for bioseparation include aqueous two-phase, reversed micelle, liquid membrane, membrane filtration, precipitation, chromatograph, and electrophoresis systems (see also chapter Physical Methods Applied to Biotechnology). A high purity protein product generally requires a number of these steps and techniques, often including multiple column chromatography operations. The replacements of even one column chromatographic operation by a simple, inexpensive yet effective alternative process has the potential to considerably reduce the cost of protein production.

Foam fractionation is an adsorptive bubble separation technique. The basis of separation in a foam is the difference in surface activity of molecules in a bulk liquid protein mixture, and with the high surface to volume ratio of the foam enhancing that separation. The surface activity of proteins in foams has long been recognized thus foam fractionation of proteins has been studied since 1937. Here the development of foam fractionation of proteins, along with a case study, is presented.

2. General Aspects of Foam Fractionation

Foam is a type of gas-liquid dispersion system, with gas bubbles forming the inner non-continuous phase and liquid forming the continuous phase. Typically, the volume fraction of gas in the foam is more than 95 percent. Foam fractionation is a bubble adsorptive separation method, based on the differences in surface activity of its solutes. As bubbles pass through a liquid solution, surface-active substances preferentially adsorb onto a bubble surface. The surface-active substances can be carried out of the liquid phase by these bubbles into a foam phase, which can be formed when these bubbles accumulate above the gas-liquid pool interface. The most strongly surface active component or that component with the largest bubble net adsorption rate in the liquid solution will have the highest relative adsorption in the foam phase. When the

foam phase collapses to form a new liquid phase, a liquid solution can be produced with a solute concentration several times higher than the original solution due to the large prior bubble surface area and small liquid content of the foam.

There are two key variables that measure the concentration/separation in the foam phase. The first is the surface excess (mass of solute per unit area at the defined interface, in excess of mass per unit cross-sectional area in the bulk solution, Γ, mg cm^{-2}) of the target solute. The second is the large specific interfacial area (a, cm^2 surface area cm^{-3} foam). Γ is determined by the adsorption properties of the solute on the gas-liquid surface and a is determined by the bubble size (d) and liquid hold-up (ε_l; generally, $\varepsilon_l < 0.05$) in the foam phase. Therefore, all of those factors that influence these two properties will affect the performance of the foam fractionation process, such as the solution conditions (solute concentration, pH, ionic strength) and the column conditions (pore size of the sparger, superficial gas velocity, liquid pool height and the foam height).

Foaming has long been employed in the purification and concentration of conventional surfactants (see also chapter Production of Biosurfactants) as well as in ion flotation. The strong amphiphilic nature of proteins and enzymes, resulting from their polar and non-polar molecular structures, makes them surface-active, allowing them to adsorb preferentially at a gas-liquid interface. Thus, since proteins become richer at a gas-liquid interface, a foam fractionation process would be expected to be a good method for concentrating/separating proteins. Compared to other conventional protein separation techniques, such as chromatography, ion exchange, electrophoresis and filtration, foam fractionation typically has the advantage of being lower in costs (capital, operating and labor). It is generally also easier to scale-up. Therefore, foam fractionation offers great promise in the development of cost-effective purification and recovery processes for proteins in complex mixtures, which include incubation broths, biological waste effluents, fermentation broths, plant extracts and fruit juices. The efficiency of foam fractionation is generally high for dilute solutions. With higher efficiency and lower costs for dilute solutions, foam fractionation, when applied in the early stage of a downstream purification regime, can reduce the total production cost of biological materials.

The first use of protein foam fractionation (in 1937 by Ostwald and Siehr) was to separate albumen from potato and beet juices. Subsequently, numerous foam fractionation experimental studies have been carried out on single-protein and multi-protein mixtures. The current renewed interest in using foam fractionation to separate proteins and enzymes, is motivated by the need for a low cost, highly effective purification method for biological products, particularly in the first concentration step that can be used to remove about 90 percent of water from dilute solutions.

Three main reasons that widespread use of foam fractionation for commercially concentrating and separating proteins has not been forthcoming are:

- The lack of understanding of adsorption of various proteins at gas-liquid interfaces (especially in competitive adsorption of multi-component systems).

- Limited understanding of the hydrodynamics of foaming, which makes prediction of the process performance difficult.
- Denaturation (the change of tertiary structure, usually unfolding) of biologically active molecules (enzymes and other proteins) during the foaming process.

The general objectives of a foam separation process can be characterized by the following relationships:

1. Maximize the protein enrichment ratio, defined as

$$\frac{C_f}{C_{i0}}, \text{ or } \frac{\text{protein concentration in foamate}}{\text{protein concentration in initial solution}}$$

2. Maximize the protein recovery

$$\left(\frac{\text{mass of protein in the foam}}{\text{initial mass of the protein}} \times 100\% = \frac{m_f}{m_i} \times 100\% \right)$$

3. Maximize the partitioning of one component from a multi-component mixture into a new recovered phase, relative to the original bulk liquid phase, compared to another component. Partition ratio (A from B) of a foaming system = $\frac{(C_f)_A/(C_i)_A}{(C_f)_B/(C_i)_B}$, or the ratio of two enrichment ratios A and B.

4. Retain the activity for enzymes by maintaining the native structure.

In our laboratories, several systems have been studied using foam fractionation, such as cellulase and egg albumin aqueous solutions, as well as waste process streams, such as kudzu plant solution and sweet potato solutions. It can be concluded that one potential application of foam fractionation is the extraction of useful proteins from wastewater.

Various experimental studies on protein foam fractionations have qualitatively investigated the effect of several parameters (pH, initial protein concentration, superficial gas velocity, liquid pool height and foam height) on the four performance criteria stated above. These studies indicate that those conditions for optimal enrichment, maximal recovery, separation, and biological activity retention may conflict with each other. Therefore, a general optimizing approach to the choice of column parameters and protein solutions conditions by modeling is necessary to balance these conflicting goals. An ideal optimizing model should be based on the physical characteristics of the foaming column and physicochemical properties of the components of the feed solution. But because of the complexity of the adsorption process and the hydrodynamics, such an ideal model is so complicated that it is difficult to solve rigorously. Some empirical, semi-empirical, and theoretical models are in the literature to characterize these systems but they have limited predictive ability. It is necessary, therefore, to develop better predictive models for both the single and the more difficult binary/multi-component system.

The following sections (3, 4, and 5) will separately discuss the foam fractionation of proteins from three aspects: foam fractionation of proteins, protein adsorption at a gas-liquid interface, and foam models. Then, an experimental study (section 6) on the foaming separation protein from a binary system: BSA-Cytochrome c is presented to illustrate the theory with a special example.

3. Foam Fractionation of Proteins

Foam fractionation can be carried out in a batch, a semi-batch, or a continuous mode, as illustrated in Figure 1. A batch process is like a semi-batch process except the gas is turned off when the foam is established and no foamate is collected.

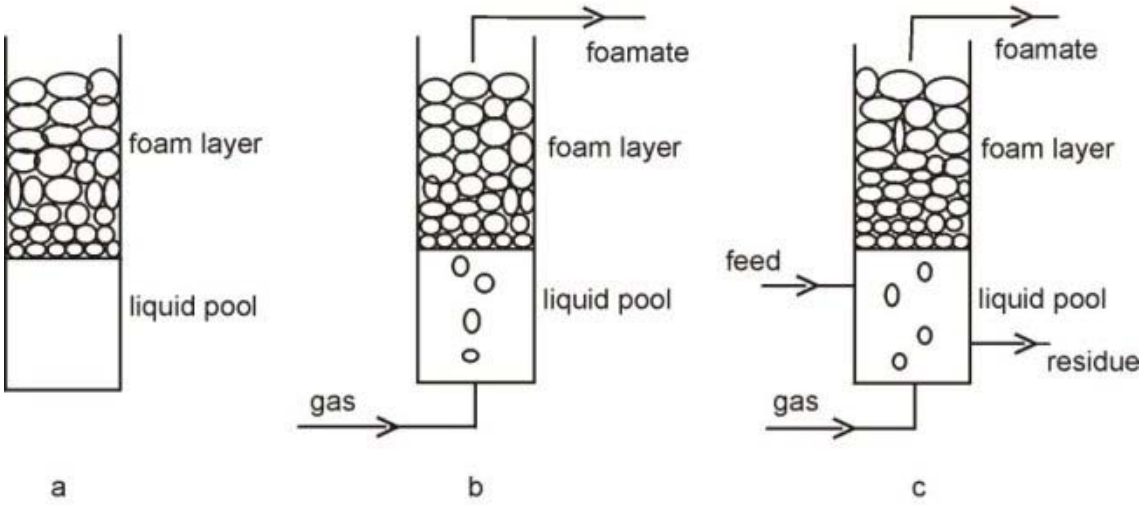

Figure 1. Three simple modes of a foam fractionation process: a. batch, b. semi-batch, c. continuous.

In a foam fractionation process, a gas (such as air, carbon dioxide, or an inert gas) is pumped to the bottom of a foam fractionation column, such as those depicted in Figure 1, which contains a given volume of protein solution. Bubbles are generated when the gas passes through a porous plate sparger installed at the bottom of column. As the bubbles rise through the solution, the surface-active substances (e.g. proteins) adsorb onto the gas-liquid surface. Foam is formed when the bubble films are stable enough for bubbles to accumulate above the solution surface. Foam is comprised of polyhedral bubbles, dodecahedrons, with liquid distributed in the films between neighboring bubbles and Plateau border channels (formed when three bubbles meet, Figure 4).

When the foam rises along the column, the liquid in the film is sucked into the Plateau border channels under the action of capillary pressure caused by the curvature of the Plateau border. As the film thickness becomes less than 100nm, the disjoining pressure (resulting from the electrostatic repulsion, the attractive Van der Waals force and the steric force) will dominate. The result is that the film reaches an equilibrium value when these forces are balanced. If the film thickness is less than the critical value before it reaches an equilibrium value, it will rupture, causing coalescence of neighboring bubbles. After the film thickness reaches its equilibrium value, whether it ruptures depends on whether there is an interruption of external mechanical forces. At the same

time, the liquid in the Plateau border will flow down the channels due to gravity until it returns to the bulk solution. On the other hand, because of the difference in bubble sizes, the gas in the smaller bubbles will diffuse into the bigger ones due to the Laplace pressure. This diffusion then causes the larger bubbles to increase in size at the expense of the smaller bubbles. In other words, coalescence occurs and some of the smaller bubbles disappear.

At the top of the foam, where the film first ruptures, the bubbles may collapse (burst outwards) and the entrained liquid may be released into the foam. A certain height of foam above the liquid pool is necessary to achieve concentration and/or separation. Hence, the effective operation of the process requires that only a certain fraction of the foam bubbles collapse. The concentration and properties of proteins on the gas-liquid surface affect both the viscosity and the elasticity of the surface. Changes in the viscosity and elasticity, in turn, affect the drainage, coalescence and collapse processes, and, thus, the stability of the foam. When the foam is collected from the top of the column and collapsed by a mechanical method (such as mechanical stirring or pressing it through a screen), a concentrated protein solution can be recovered. This concentration is generally several times that of the feed solution.

3.1 Single-Protein

For a single protein solution, foam fractionation is used mainly as a concentration method. For a solution with only one surface-active protein, foam fractionation is not only a concentration method, but also an excellent separation procedure. The main objective of this method then becomes one of maximizing the enrichment or recovery, while retaining the biological activity (when the protein is an enzyme) and concentrating the desired protein. The variables that are investigated usually include: pH, feed or initial protein concentration, superficial gas velocity, feed flow rate (for continuous separation), bubble size, ionic strength, liquid pool height, foam height, and foaming time.

Table 1 summarizes the work that has been done using single-protein foam fractionation. Most studies focused on the model system of BSA, some on β-casein and a few on other proteins. Because different proteins have different physicochemical properties due to their particular amino acid sequences and three-dimensional structures, the optimized experimental condition for one protein is generally not optimal for others.

Protein	Source	Operating mode	Math Model	Feed concentration range
BSA	Aqueous solution	Semi-batch with Cycling	no	0.05~0.5wt%
BSA	Aqueous solution	Continuous	yes	0.01~0.2 wt%
Cellulase*	Aqueous solution	Semi-Batch	no	0.01 wt% (100mg/l)
BSA	Aqueous solution	Continuous	yes	0.05 wt% and 0.1wt%

gel	Aqueous solution	Continuous	yes	0.1~0.8wt% (1~8 mg/ml)
Soybean protein*	Aqueous solution	Continuous	yes	0.005~0.024wt% (0.05~0.24g/l)
BSA	Aqueous solution	Semi-Batch	no	0.02wt%
BSA or HBB	Aqueous solution	Loop Bubble Column	no	0.02wt% (200μg/ml)
Sodium Caseinate or β-casein or BSA or β-lactoglobulin or α-lactalbumin or chymotrypsinogen	Aqueous solution	Semi-Batch	no	0~0.012 wt% (0~about 120 mg/l)
BSA	Aqueous solution	Semi-Batch and Continuous	no	0.008~0.05wt% (0.08~0.5mg/ml)
β-casein	Aqueous solution	Continuous	no	0.004~0.023 wt% (about 0.04~0.23 mg/ml)
Cellulase* or Egg Albumin*	Aqueous solution	Semi-Batch	yes	0.004~0.05 wt% (40~500mg/l)

* Actually, a mixture of proteins, but considered to be one protein in the study.

Table 1. Literature survey on single protein foam fractionation.

For the foam fractionation of BSA, the pH is one of the most important variables. Sometimes it was found that the largest enrichment ratio occurred at its isoelectric point, pI, (at a pH of 4.7 or 4.8), while other times at the pI, the enrichment ratio was at a minimum. These opposite results can be explained by the fact that the effect of pH is coupled with other variables, such as the bubble size.

Thus, drainage and coalescence can change the separation in a complicated manner. An increase in superficial gas velocity leads to a decrease in the enrichment ratio. The effect of liquid pool heights may be related to the adsorption kinetics. At a low pool height, very little protein is adsorbed onto the bubbles because of the short residence time. This can lead to lower surface viscosity, and, hence faster drainage from the foam as well as formation of unstable bubbles, resulting in coalescence. Coalescence increases the internal reflux of the protein and also the bubble size. Both increases are helpful in increasing the enrichment. So the shorter the pool height, the larger the enrichment is at that low pool height. As the residence time of bubbles in the liquid pool increases with increasing liquid level, more protein is adsorbed at the interface. This results in an increase in surface viscosity and more stable foam, and can also lead to an

increase in enrichment. At very large pool heights, the surface concentration may be close to the equilibrium concentration and the enrichment can't increase further. Protein enrichment depends on $\Gamma a/c$, with a being the interfacial area per unit volume of the foam.

Since the experimental surface concentration Γ is close to its equilibrium value for BSA, Γ/c for BSA is largest for lowest protein concentrations. As a result in this case, the protein enrichment is higher for the lowest concentrations, a relationship, which generally holds for all proteins.

In addition to BSA, the foam fractionation of gelatin, soybean protein and β-casein have also been studied. The key control variables, such as the pH, superficial gas velocity and the initial protein concentration have been investigated. Similar results to BSA have been obtained.

Brown and coworkers carried out a statistical study (factorial design) to establish the optimum operating conditions for the continuous foam separation of β-casein. The best enrichments were found at low levels of initial feed protein concentration, gas flow rate and the feed flow rate, and for high foam levels.

High protein recovery values were generally found for high levels of initial feed protein concentration, gas flow rate, feed flow rate and low foam levels.

Literature relating foam fractionation to other methods of downstream processing for a given biomolecule recovery is very rare. An outstanding exception is the work of a research group at the Technische University at Munchen dealing with the recovery of a bacterial lipase.

The main results of that work, displayed in Table 2, serves as a guide for selecting the recovery methods to meet different processing goals for that enzyme and indicate that foam fractionation is a study candidate for future development of large scale economic processing of proteins.

Criterion	Precipitation	Chromatography	Foam Fractionation
recovery	98%	98%	not known
purification factor*	2.4	4	1.5
enrichment factor**	10	40	4-80
operation expenditure	low	high	low
investment costs	moderate	high	low
running costs	high	moderate	low
environmental pollution	high	moderate-high	low

***** U/mg protein , in relation to initial values

** U/ml , in relation to initial values

Table 2. Comparison of recovery operations investigated with lipase (Wenzig et al.)

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Biographical Sketches

Dr. Cesar C. Santana is a professor in the School of Chemical Engineering, State University of Campinas, Campinas, SP, Brazil since 1987. He holds B.S. degree in Chemical Engineering, M.Sc. degree and D.Sc. degrees in Chemical Engineering from COPPE/Federal University of Rio de Janeiro. Post doctoral studies on Bioseparation were performed in North Carolina State University. His research interests are: Downstream Processing in protein recovery and purification by adsorption in solid-liquid and gas-liquid interfaces and foam fractionation of proteins.

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