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The Effect of External Reflux on the Foam Fractionation of Proteins

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ABSTRACT

Foam fractionation of proteins is especially advantageous in treating dilute solutions where other separation methods run into economical limitations. Improvement in separation efficiency can be obtained by adding external reflux to the column. This work studied operational variables, such as superficial gas velocity and solution concentration, on the enrichment of the foam phase when external reflux was added to the separation. Bovine serum albumin, cytochrome C, and barley malt α-amylase were the proteins fractionated in a column 3.2 cm in diameter and 105 cm in height operated by bubbling nitrogen in pH-controlled solutions. The determination of solute enrichment and purification factor for several external reflux ratios demonstrated the importance of this operational variable on the efficiency of foam fractionation of proteins.

Index Entries: Foam fractionation; bubble separation; reflux; protein separation; proteins.

INTRODUCTION

Foam fractionation is a technique that has long been used in the laboratory to recover and purify dilute solutions of proteins, including enzymes. Also, this technique can be an inexpensive way to separate two proteins present in an aqueous solution. This technique is based on the ability of surface-active compounds, such as proteins, to adsorb and concentrate in a gas–liquid interface. Therefore, a gas bubble rising through a pool of aqueous protein solution can have its interface enriched in protein. The formation of foam as the bubble reaches the top of the liquid phase provides a large gas–liquid interface where the adsorption of proteins can occur. Also, the liquid draining down through the foam bed is another important mechanism in which the proteins are exposed once more to the gas–liquid interface. This internal reflux is a second chance for proteins to adsorb at the interface of bubbles that are rising through the foam bed.

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Foam fractionation was first used by Ostwald and Sierh in 1937 to separate albumin from potato and beet juices *(1)*. The possibility of protein denaturation owing to foaming was always a drawback for broader use of foam fractionation of proteins, especially enzymes. However, although some proteins are very sensitive to foaming, others are not and can be processed by this technique with high activity recovery *(2)*. Recently, researchers have revisited this technique owing to the need to develop cost-effective downstream separation techniques of valuable products and to remove pollutants from waste streams *(3–5)*.

Environmental factors that affect protein structure and, consequently, its surface activity, like pH, ionic strength, and the presence of organic solvents, are important operational variables. Other important variables are superficial gas flow rate, temperature, length of the liquid pool, length of the foam bed, and type of gas.

As mentioned, the draining of liquid from bubbles that have collapsed inside the column allows proteins a renewed contact with the gas phase. It gives the adsorption process a chance to move toward completion, resulting in higher loadings and selectivity. An enhancement of this phenomenon can be achieved by the introduction of external reflux into the column: The liquid resulting from the foam that collapses outside the column is partially returned to the column. It drains down the column, adding to the internal reflux. Studies of the introduction of external reflux have been done for the case of the fractionation of surfactants *(6,7)*. In an extensive literature search, no work on the effect of external reflux on the foam fractionation of proteins was found. This work had the objective of studying the effect of external reflux on the foam fractionation of different proteins, including an enzyme.

MATERIALS AND METHODS

Materials

Bovine serum albumin (BSA) and a crude preparation of barley malt α-amylase (BMA) were purchased from Sigma (St. Louis, MO). All other chemicals were reagent-grade. Solutions of BSA (50–100 mg/L) and cytochrome C (100 mg/L) were prepared in 0.1*M* phosphate buffer, pH 7.0. BMA solutions (2 g/L) were prepared with 0.027*M* acetate buffer, pH 5.1. Since BMA preparation had an insoluble fraction, its solutions were filtered through a 1.2-μm pore membrane prior to use.

Column Setup and Runs

A scheme of the experimental setup is shown in Fig. 1. The system was composed of a column, a foam breaker, and a reservoir for protein solution. The gas for the separation (nitrogen) was provided from a gas cylinder and humidified before entering the column. Peristaltic pumps were used to introduce external reflux at the top of the column and to recirculate protein solution from the external reservoir to the liquid pool in the column.

The glass column was 105 cm in height and 3.2 cm in diameter. Temperature was controlled by a water jacket and measured at two points: at the top of the column (foam) and at the external reservoir (protein solution). Bubbles were formed when humidified nitrogen gas flowed through a fritted glass disk at the bottom of the column. The foam breaker consisted of a stirrer whose paddles were located just at the column exit.

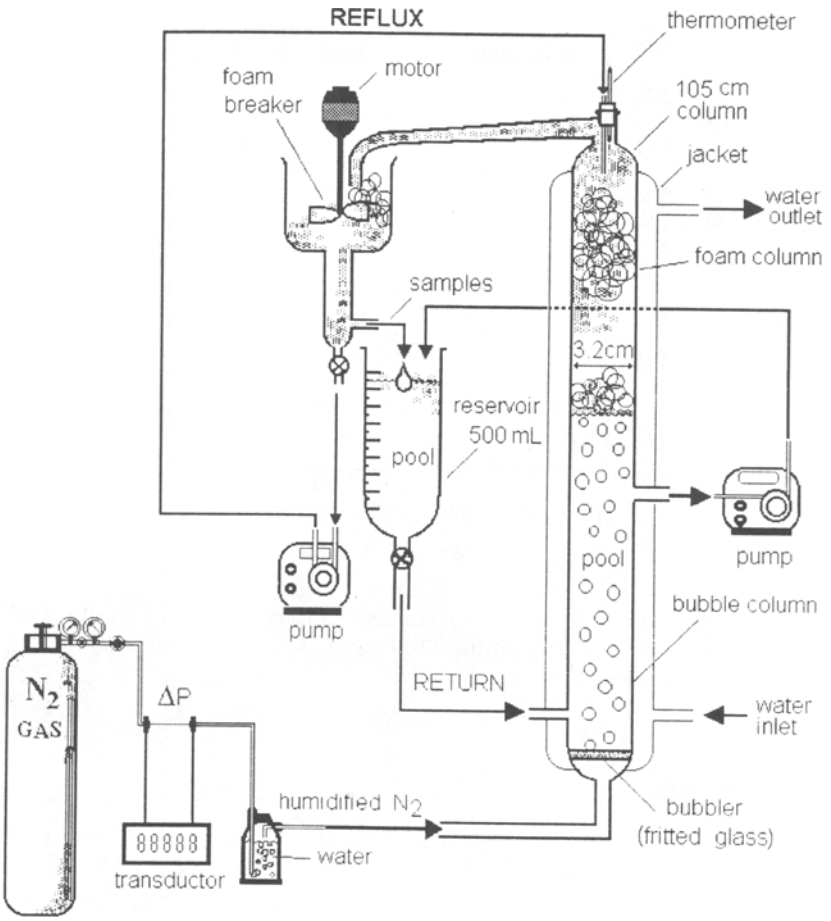

Fig. 1. Scheme of the experimental setup for fractionation of proteins in foam columns with external reflux.

Prerun operations consisted of turning the temperature control system on (recirculation of water in the column jacket), loading the column with 1 L of protein solution through the external reservoir, and turning the protein solution recirculating pump on. When the solution temperature at the external reservoir achieved 22°C, gas was introduced into the column at a fixed flow rate. Timing of the actual run was started when the first bubbles achieved the top of the liquid pool and started forming foam. When the foam reached the top of the column (initial height of foam bed of 48 cm), the foam breaker was turned on. External reflux was introduced at the top of the column by a peristaltic pump as soon as liquid overflowed from the foam breaker to the external reservoir. Samples of the foamate (collapsed foam) drawn at different times and samples of protein solution before and after fractionation were also taken for protein concentration determination and activity analysis. Protein enrichment was defined as the ratio of protein concentration in the sample to the protein concentration in the initial solution. Similarly, activity ratio was defined as the ratio of the activity of the sample to the activity of the initial enzyme solution (activity in U/mL).

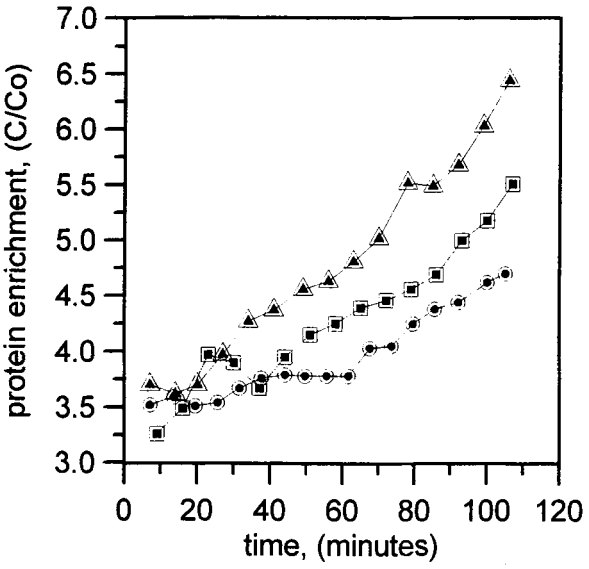

Fig. 2. Effect of external reflux ratio on protein enrichment from BSA solution with superficial gas velocity of 0.30 cm/s. BSA solution, 100 mg/L. ●, without external reflux; ■, external reflux ratio, 0.31; ▲, external reflux ratio, 1.10.

Analytical Methods

Protein concentration was determined by the Coomassie blue assay using a commercial kit based on the method developed by Bradford in 1976 (Bio-Rad [Hercules, CA] LIT33 Rev B) with insulin as the standard protein in the case of fractionation of α-amylase. BSA concentration was determined by the difference in absortion at 280 and 320 nm. When measuring protein in the binary mixture of cytochrome C and BSA, solution absorption was taken at 280, 320, and 409 nm. This procedure enables the setup of two algebraic equations relating the extinction coefficients of proteins with the concentration and the absorbances at 280 and 409 nm. Measurements at 320 nm were used as the baseline to subtract turbidity interference possibly present in the protein solutions. Enzyme activity was measured with 1% soluble starch solution, as substrate, at 20°C for 30 min, and the total reducing sugar released during reaction was measured by the 2,3-dinitrosalicilic acid method *(8)*. One unit of activity was defined as 1 mg of reducing sugar released as maltose equivalent/min at the assay conditions.

RESULTS AND DISCUSSION

Figures 2 and 3 show the results of BSA concentration by foaming at two different superficial gas velocities (0.30 and 0.45 cm/s, respectively). In both cases, the introduction of the external reflux increased the protein concentration in the foamate up to two times when compared with the case with no reflux. Also, the larger the external reflux ratio, the larger the enrichment in protein. In the case of no reflux, the increase in the superficial gas flow rate resulted in a lower enrichment of the foamate. Certainly, a shorter residence time of the gas in the column resulted in a

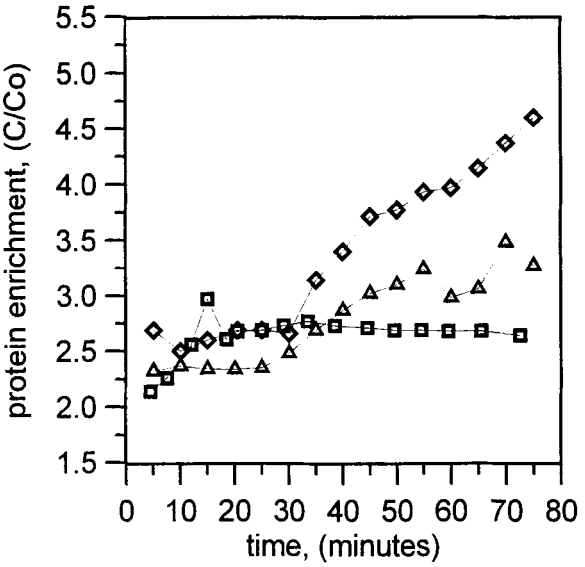

Fig. 3. Effect of external reflux ratio on protein enrichment from BSA solution with superficial gas velocity of 0.45 cm/s. BSA solution, 100 mg/L. External reflux started after 30 min of run. ◇, external reflux ratio, 0.68; △, external reflux ratio, 0.21; □, without external reflux.

lower loading of protein at the gas–liquid interface. Although the reflux ratios used at the two different superficial gas velocities were not the same, the overall enrichment seemed higher for lower superficial gas velocities when external reflux was present than it was for the case of no external reflux.

The effect of the initial protein concentration in the protein enrichment was evaluated at two different superficial gas velocities (Fig. 4). Protein enrichment showed an inverse relationship with initial protein concentration: Higher enrichments were obtained at lower protein concentrations. This is an advantageous feature of foam fractionation, since it enables the processing of diluted streams with high efficiency. The effect of superficial gas velocity on protein enrichment at different initial protein concentration was the same as verified in Figs. 2 and 3: The higher the superficial gas velocity, the lower the enrichment.

The fractionation of BSA in the presence of a second protein, cytochrome C, resulted in the same magnitude of BSA enrichment as before (Fig. 5). Also, the external reflux increased the enrichment of BSA in the foamate. However, cytochrome C enrichment was not strongly affected by the introduction of the external reflux. The fact that this protein is strongly hydrophilic may be the reason for this behavior: It does not strongly interact with the hydrophobic gas phase.

The fractionation of the α-amylase contained in the BMA preparation resulted in an increase in protein enrichment much higher than the increase in activity ratio (Fig. 6). However, the total activity recovery was only from 60 to 85%. Experiments showed that around 15% of the total activity loss was owing to the pumping necessary for the recirculation of protein solution between the external reservoir and the liquid pool in the column. Therefore, up to 25% of the initial activity was lost owing to foaming and foam breaking. The protein preparation used in these experiments was a crude α-amylase preparation where the enzyme was a small fraction of

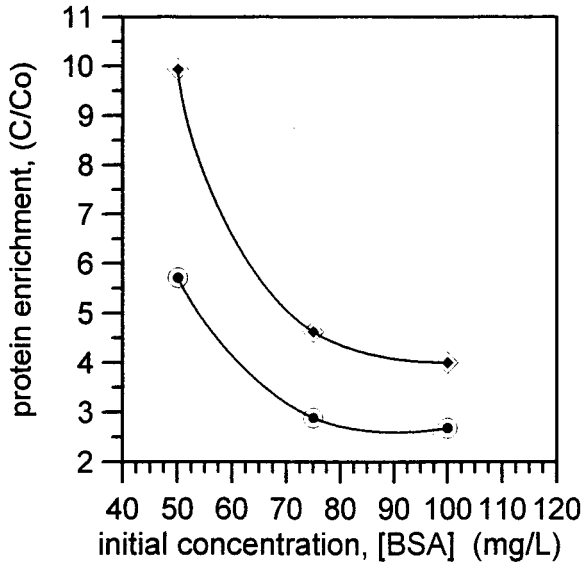

Fig. 4. Effect of initial protein concentration on protein enrichment from BSA solutions at two superficial gas velocities (V_{gas}) without external reflux. Processing time 60 min. Without external reflux: ◆, V_{gas}, 0.30 cm/s; ●, V_{gas}, 0.45 cm/s.

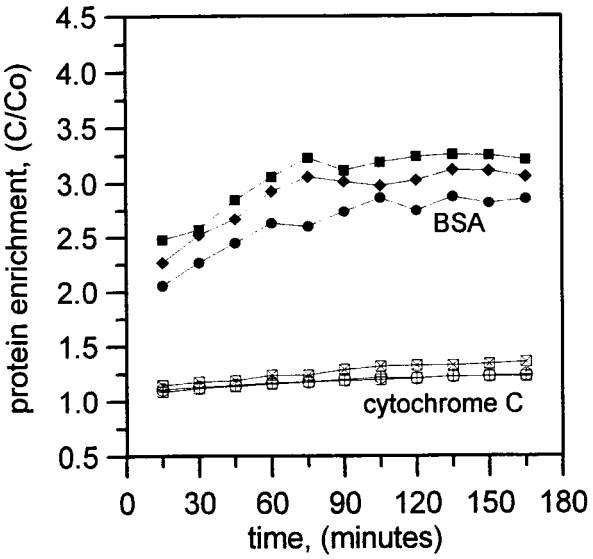

Fig. 5. Effect of external reflux on the BSA and cytochrome C enrichments from a solution 100 mg/L in both proteins. Superficial V_{gas}, 0.45 cm/s. ●, ⊕, without external reflux; ◆, ⊞, external reflux ratio, 0.15; ■, ⊠, external reflux ratio, 0.29.

the total protein (SA 1–3 U/mg of solids, whereas pure α-amylases have SAs in the range of thousands of U/mg of protein). Consequently, the denatured α-amylase contribution for the total protein enrichment in the foam was assumed small and negligible. Therefore, the results suggested that contaminant proteins

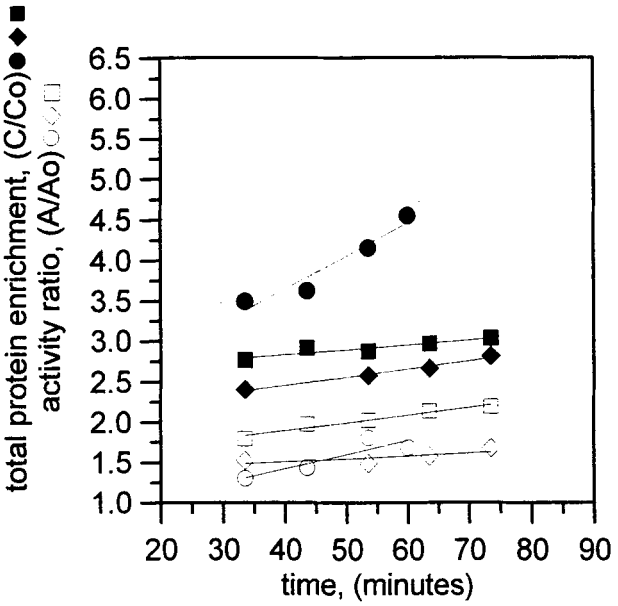

Fig. 6. Effect of superficial V_{gas} on the fractionation of BMA without external reflux. Without external reflux: ●, ○, V_{gas}, 0.20 cm/s; ■, □, V_{gas}, 0.30 cm/s; ◆, ◇, V_{gas}, 0.45 cm/s.

were preferentially adsorbed in the gas–liquid interface in comparison with α-amylase. The contaminants seems to be more hydrophobic than the α-amylase contained in the BMA solution.

The foaming of BMA with no reflux confirmed that total protein enrichment is a function of the superficial gas flow rate (higher enrichment for lower velocities). However, the effect of changing the superficial gas velocity on the activity ratio (ratio between activity of the foamate and activity of the initial solution) was not well defined by the experimental results; no significant changes in the activity ratio were verified with the change in the superficial gas velocity. The same behavior was observed when external reflux was introduced in the column (Figs. 7–9). The lack of sensitivity of the activity enrichment for changes in superficial gas velocity is probably owing to the lower hydrophobic character of the α-amylase at the experimental conditions as already mentioned. Since we expect no strong interaction between the gas phase and the enzyme based on its hydrophobicity, changes in the former are not to affect the latter.

CONCLUSION

The introduction of external reflux in the foam fractionation of proteins resulted in an increase in the protein enrichment in the foamate. This increase was more evident for proteins with strong hydrophobic characteristics, like BSA. The superficial gas velocity had the same effect on the protein enrichment with external reflux as for the case of no reflux. The larger the superficial gas velocity, the smaller the protein enrichment. The external reflux proved to be an important variable in the search for optimum operational conditions in the foam separation of proteins.

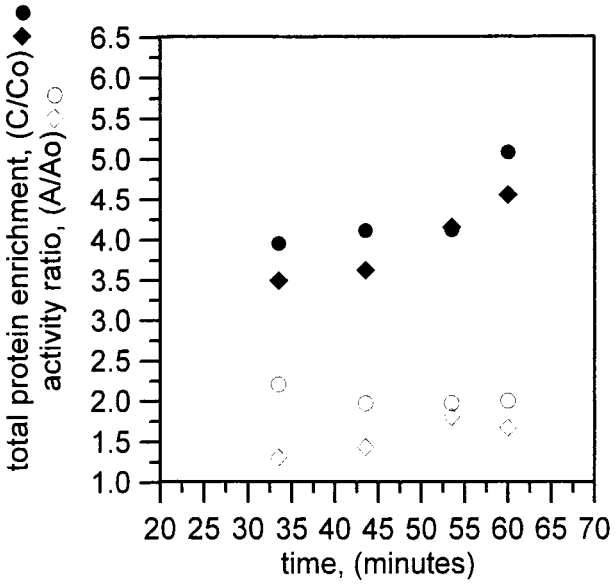

Fig. 7. Effect of external reflux on the foam fractionation of BMA. Superficial V_{gas}, 0.20 cm/s. ◆, ◇, without external reflux; ●, ○, external reflux, 0.69 mL/min.

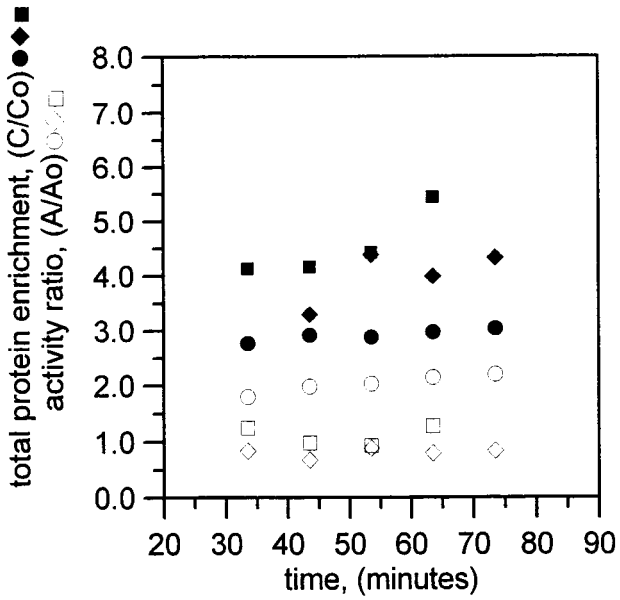

Fig. 8. Effect of external reflux on the foam fractionation of BMA. Superficial V_{gas}, 0.30 cm/s. ■, □, external reflux, 1.25 mL/min; ◆, ◇, external reflux, 0.78 mL/min; ●, ○, without external reflux.

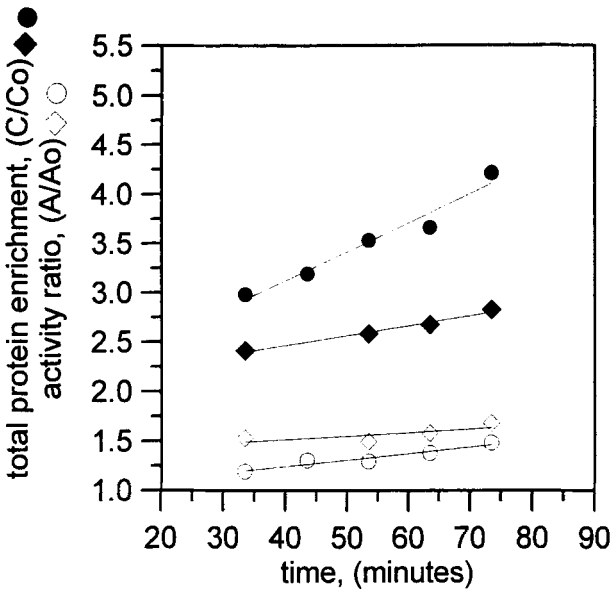

Fig. 9. Effect of external reflux on the foam fractionation of BMA. Superficial V_{gas}, 0.45 cm/s. ●, ○, external reflux, 0.95 mL/min; ◆, ◇, without external reflux.

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