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Batch Foam Fractionation of Kudzu *(Pueraria lobata)* Vine Retting Solution

JIRAWAT EIAMWAT, VEARA LOHA, ALEŠ PROKOP AND ROBERT D. TANNER*

Department of Chemical Engineering, Vanderbilt University, Nashville, TN 37235

ABSTRACT

The aqueous protein solution from kudzu *(Pueraria lobata)* vine retting broth, without the addition of other surfactants, was foam-fractionated in a vertical tubular column with multiple sampling ports. Time-varying trajectories of the total protein levels were determined to describe the protein behavior at six positions along the 1-m column. The lowest two trajectories of this batch process represented a loss of proteins from the bulk liquid and tended to merge and decay together in time; the other trajectories displayed a gain in proteins in the foam phase. These upper column port protein concentration trajectories generally increased in time up to 45 min, followed by a decrease, reflecting the removal of proteins from the column ports. The foam became dryer as it passed up the column to the top port. The protein concentration was about 5–8× higher in the top port foam than in the initial bulk solution, mainly as a result of liquid drainage from the foam along the column axis. This concentration increase in the collected foam was dependent on the initial pH of the bulk solution. The mol-wt profile of the proteins in the concentrated foam effluent was determined by one-dimensional gel electrophoresis. An analysis of the gel electropherograms indicated that the most abundant proteins could be cellulases and pectinases.

INTRODUCTION

Foam fractionation is an attractive technique for protein concentration and separation, because it is simple, relatively inexpensive, and easy to scale-up. Moreover, this technique has significant potential for reducing the high cost of protein recovery in commercial practice, particularly in the first recovery step in which much of the water is removed. When compared to other separation methods, such as precipitation by means of

* Author to whom all correspondence and reprint requests should be addressed.

salts or solvents, foam fractionation offers negligible product contamination during processing. The only additive in this processing technique is air or another gas such as carbon dioxide, with the possible exception of the addition of an acid or a base when a pH change is required. Typically, foam-fractionation technique is one in which air or an inert gas is introduced at the bottom of the fractionation column through a porous ceramic sparger or a nozzle. Above the sparger (nozzle), bubbles rise through the column and carry a surface-active substance (surfactant such as protein) on the bubbles at the air–liquid interface. When a protein, itself a surfactant, is in the solution, then no exogenous surfactant may need to be added to effect the separation. Above the liquid surface, the air bubbles may, under certain circumstances, become a foam, and the foam layer continues to rise in the column. Foam is collected and collapsed to be a liquid foamate, often using a mechanical stirrer. While the foam travels up the column, the liquid on the foam drains because of gravitational forces and the capillary forces at the complex foam interface (the plateau border). The residual surfactant material remains attached to the foam and is concentrated in the foamate (which is continuously removed).

Previous studies on foam fractionation *(1–3)* have demonstrated its feasibility for concentrating and separating proteins that foam. Proteins that do not foam can also be separated and concentrated by bubble fractionation alone, but to a lesser degree *(4)*. By itself, however, this first concentration step does not generally purify proteins to their desired specifications. In the present study, the initial pH and concentration of the bulk solution are shown to play an important role in both concentrating and separating these proteins. It is found that, even though the foam and the bulk solution are comprised of the same proteins, the individual protein concentrations varied between the two different phases, indicating that fractionation occurs.

In general, foam fractionation is a very promising method for removing soluble proteins *(5)* from dilute solutions, e.g., potato protein wastes in the potato processing industry *(6)*. Another candidate for foam fractionation is the extracellular protein-rich waste fermentation broth from the kudzu vine retting process *(7)*. Although this anaerobic fermentation process usually produces a foul smell and an aesthetically unpleasant-looking liquid waste stream (similar to liquid from a swamp), the aeration of this broth can create attractive foam (similar to beer), which may have economic value as a low-cost source for cellulases and pectinases. This unappealing waste stream is markedly improved in appearance and odor by the foam-fractionation procedure.

The natural retting process (on the ground or in water) allows for development of microorganisms that have an affect on the digestion of cellulose–pectin sheath of the phloem-fiber plants, such as hemp, flex, ramie, and jute. This process is slow and may take about 2 wk. The cellulose fibers in such plants are bounded together in bundles within the phloem

surrounding the stem of the plant. In order to recover fibers from such plants, the cellulose fibers must be separated from the phloem, and then from each other within the bundles. This separation of fibers requires special enzymes (cellulase and pectinase) to digest the plant. The anaerobic bacteria, by virtue of their content of cellulases and pectinases, are capable of digesting the cellulose and pectin that bind the bundles of fibers *(8)*. The separation of fibers can subsequently be refined by using mechanical force, as for typical textile industry.

The retting process (to recover fibers from kudzu vines) promises to be of commercial interest, since the flax-like cellulosic fiber may be in demand for natural fiber clothing *(9)*. The addition of a foam-fractionation step can serve as an attractive way of dealing with the waste, and may possibly lead to a commercial outlet for the recovered enzymes. It would be interesting to explore what enzymes are present in both the foam and the residual broth, and whether these are of sufficient value to be marketable at present.

MATERIALS AND METHODS

Retting Procedure

Kudzu vines of different diameters (0.3–0.7 cm) were harvested locally, near Gay Street and 1st Avenue S, on the west bank of the Cumberland River in downtown Nashville, Tennessee. Approximately 1.3 kg of vines were cut at a time, washed, and placed in a 10-L plastic container, which was then filled with 7 L of tap water (~ pH 5.9) *(10)* to give a solid concentration of 15.5% wt. These cut vines of various lengths (13–16 cm) were kept submerged in that water at ambient temperature of 23 ± 1°C. The retting was considered completed when the vines became loose and easily separated into fibers (~ 2 wk).

Sample Preparation

Sampling was performed every 1–2 wk until retting was completed. A 400-mL sample filtered through Whatman (Kent, UK) No. 4 filter paper was used for each foaming experiment. The solution to be foam-fractionated was adjusted initially to a desired pH by adding 1 *N* HCl or 1 *N* NaOH prior to aeration.

The total protein concentration was assayed using the Coomassie blue (Bradford) method *(11)* to measure the absorbance with a spectrophotometer, which is $A_i = K_i C_i$ (where A_i, C_i, and K_i are the absorbance, protein concentration, and the conversion value). The actual protein concentration was determined by evaporating 1 mL of sample solution in the hot-air oven for 10 hr and then weighing it. The calculated conversion value (K_i) with the concentration/absorbance is about 1.81 mL/mg.

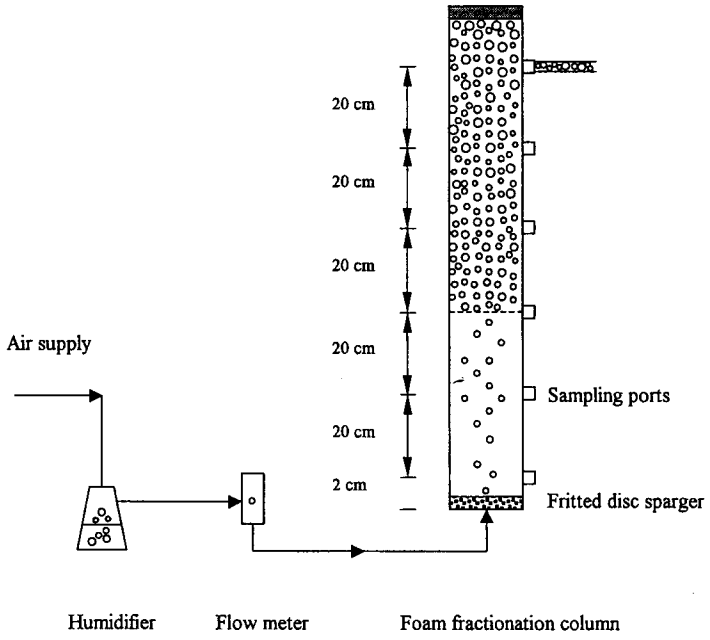

Fig. 1. Schematic diagram of the experimental apparatus.

Experimental Procedure

The foam-fractionation experiments were carried out in a 1-m glass column (*see* Fig. 1 for the schematic) with an id of 35 mm. A porous fritted glass sparger was fitted flush with the id of the bottom of the column. The column included six sampling ports located along its length axis. Each of them was covered with a rubber septum, to allow sample collection with a hypodermic syringe. Ports were 20 cm apart, starting with the first port, which was located 2 cm above the sparger, as illustrated in Fig. 1. To eliminate evaporation, the air supply was bubbled through the water to humidify it before entering the bottom of the column. The air-flow rate was monitored by a rotameter at 0.14 vvm, or 0.056 L/min, and, throughout this study, only one air-flow rate was used. Using 10-mL hypodermic syringes, 1 mL of samples were withdrawn every 15 min throughout the experiment from each of the six side ports by inserting the needle into the septum until it entered the bulk solution or the foam. The liquid sample contents were dispensed into 10 × 65-mm test tubes for subsequent determination of the total protein concentration, for gel electrophoresis samples, and for the absorption spectral characteristic study.

Analytical Methods

Total Protein Assay

The total protein concentration was quantified spectrophotometrically at 595 nm, using the Coomassie blue (Bradford) method *(11)* with a

Spectronic 20 spectrophotometer (Bausch and Lomb, New York). The samples were assayed 2 min after the addition of the Coomassie blue dye, and were compared with a zero reference of deionized water under the same conditions.

Gel Electrophoresis

The liquid foamate was resolved to identify the primary proteins by electrophoresis on 11% (w/v) sodium dodecyl sulphate-polyacrylamide gel, according to the Laemmli procedure *(12)*. The standard Sigma kit (Sigma, St. Louis, MO.) provided the mol wt markers. Coomassie blue R-250 (Bio-Rad, Richmond, CA) was used for staining. Protein markers (Sigma) were used for calibrating the gel and calculating R_f values for the approximation of mol wt as follows: bovine serum albumin (A-4378), mol wt 66,000; ovalbumin (A-7641), mol wt 45,000; and pectinase from *Aspergillus niger* (P-9932). The fourth marker, cellulase, was derived from *Trichoderma reesei* (MVA 1284; Gist-Brocades nv, Delft, Netherlands).

Absorption Spectral Characteristics

The absorption spectra were also used as an indicator of the characteristic proteins. The spectra of the known cellulase, known pectinase, and liquid foamate samples were measured at ambient temperature between 200 and 600 nm for the proteins in a deionized water solution in a 1-cm cuvet using a Hitachi Model 100-40 UV spectrophotometer (Hitachi, Tokyo, Japan). Each of these solutions was prepared as follows: A 0.1 mL of pectinase was added to 1.4 mL of deionized water (.067 dilution); 0.075 g of cellulase was mixed with 5 mL of deionized water, centrifuged at 1073*g* for 30 min, and further filtered through Whatman No. 40 filter paper; a 5-mL sample of liquid foamate was centrifuged at 1073*g* for 30 min, and then filtered through Whatman No. 40 filter paper.

RESULTS AND DISCUSSION

When air bubbles rise in the aqueous protein solution (from the kudzu vine retting broth) to the upper surface, foam is formed at the air–liquid interface. The enriched protein liquid leaving the bulk liquid solution is carried with the rising bubbles (bubble size ~ 0.6–1.0 mm) into the open air space as foam (foam cell ~ 0.6–2.0 mm), resulting in an enriched protein concentration in the foam phase. As the foam passes upwards, some air bubbles in the foam phase coalesce into larger bubbles, and remain attached because of capillary forces. At the same time, some unstable foam collapses. Following the operation of the foam-fractionation process over a given period of time, the amount of foam produced is lessened as the protein in the bulk solution becomes depleted. The collapsed foam has been collected; liquid volume was around 80–100 mL.

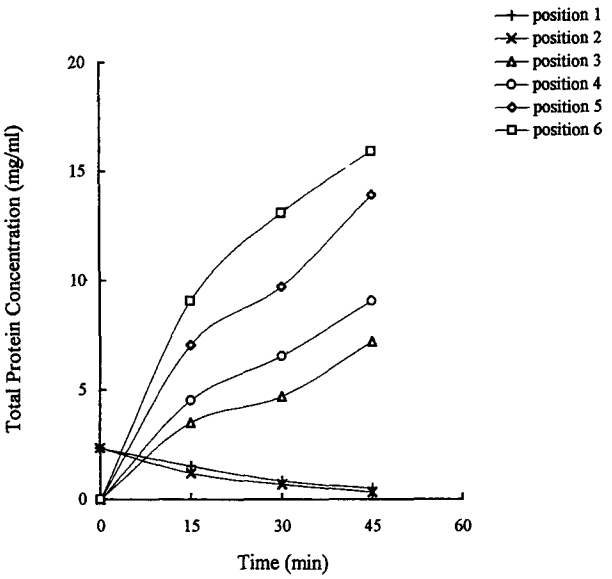

Fig. 2. Time varying trajectories of the total protein concentration at pH 3; and air flow rate 0.056 L/min.

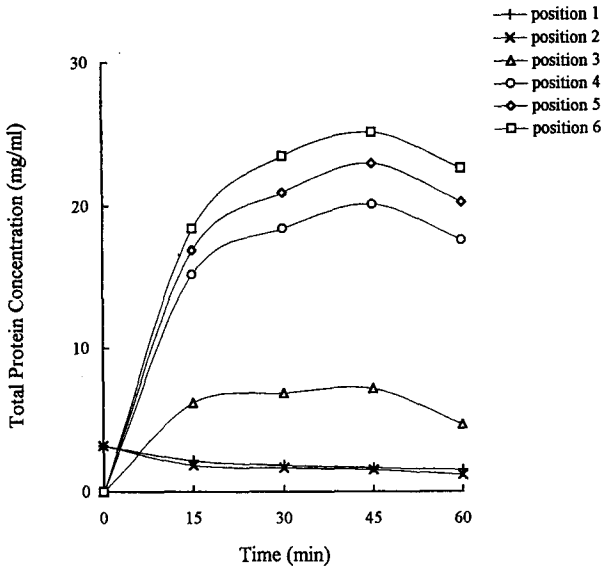

Fig. 3. Time varying trajectories of the total protein concentration at pH 5; and air flow rate 0.056 L/min.

Figures 2–4 show the protein concentration profiles for the liquid phase (positions 1 and 2) and the foam phase (positions 3 to 6) at various pHs. It is noted that since no foam is present at time zero, no protein concentrations were measured then. It could just as reasonably be argued

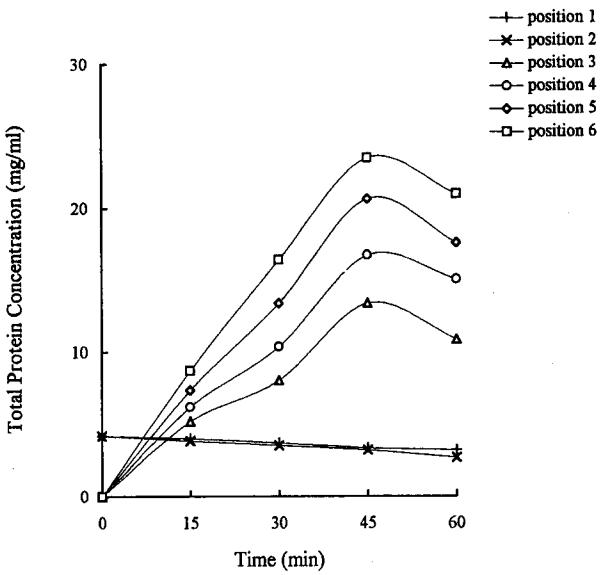

Fig. 4. Time varying trajectories of the total protein concentration at pH 7; and air flow rate 0.056 L/min.

that the concentration at time zero is the bulk concentration, rather than zero as shown on the three graphs. Although the total protein concentration of the foam phase increases appreciably as time increases, the total protein concentration of the much larger mass-liquid phase decreases gradually. This indicates that the loss of proteins from the bulk liquid is reflected in the gain of proteins in the foam phase. The total protein concentration increases up to approx 5–8× (compared to the initial concentration) at 45 min. Concomitantly, the volume of foam formed was observed to decrease beyond that time, because of the removal of foam in the samples. The pH of foamate usually differed from those of the initial pH. For the initial pH of bulk solution below 7.0, the foamate pH typically increased (by 0.2–0.3 pH units), and for the initial pH of bulk solution above 7.0, the foamate pH decreased (by about the same degree).

Analytical SDS-PAGE electrophoresis was performed in order to identify the primary proteins presented in the liquid foamate. The visualized protein bands on the gel are shown in Fig. 5. It is observed that the liquid foamate consists of at least three different proteins. The main constituent proteins may very well be cellulases and pectinases as expected, since their gel mol wt were close to the cellulase and pectinase markers. These foamate proteins, however, were very weak in color on the gel, in comparison to the protein markers. This may be the result of the relatively low concentration of proteins in the diluted vine retting solution foamate sample. The fainter bands detected on the gel apparently represent the minor protein constituents in the broth. The standard Sigma kit for mol

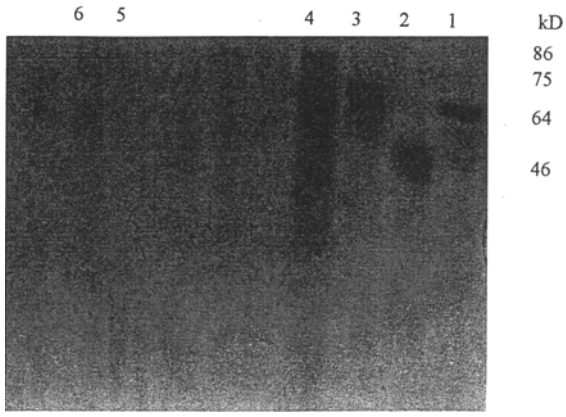

Fig. 5. SDS-Polyacrylamide gel electrophoresis results for markers (Lanes 1–4) and foam fractionation of kudzu vine retting solution (Lanes 5–6). Lane 1: bovine serum albumin; Lane 2: ovalbumin; Lane 3: cellulase; and Lane 4: pectinase.

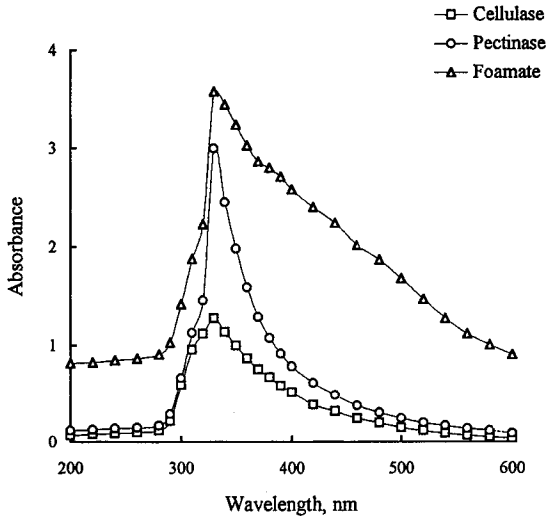

Fig. 6. Comparison of UV-VIS spectra for cellulase from *T. reesei* (15 mg/ml), pectinase from *A. niger* (3.8 mg/ml), and diluted kudzu vine retting solution foamate.

wt of the unknown proteins on the SDS-PAGE gel gave mol wt estimates of 86 kDa for one apparent pectinase band and 75 kDa and 62 kDa for two apparent cellulases *(13)*.

Another direct analysis of the absorption spectra provided another means of characterization of proteins. It can be seen from Fig. 6 that the cellulase, pectinase, and liquid foamate exhibited the same absorption peaks at 330 nm wavelength. In fact, although proteins generally exhibit an absorbance at 280 nm, an absorbance at 340 nm has also been reported

(14). This, however, apparently supports the gel electrophoresis result that the liquid foamate could be composed of cellulases and pectinases.

CONCLUSION

Foam fractionation of the kudzu vine retting solution causes removal of soluble proteins present within the solution, and substantial increase (5–8×) in the total protein concentration. Fractionation longer than 45 min results in a progressive decrease in the volume of the foam. The soluble proteins are apparently cellulases and pectinases. The kudzu vine retting solution foams, perhaps as a result of the foaming ability of cellulases and their hydrophobicity.

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