Batch Foam Separation of a Soluble Protein

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Abstract

Removal of protein dissolved in water by batch foam separation was conducted with using ovalbumin (OA) as a model protein in the light of wastewater treatment reducing organic loading. The removal efficiency had a maximum value near the i.e.p. of OA (pH 4.6), thus, most experiments were conducted at pH 4.6. Typical experimental conditions; superficial gas velocity, $U_g$: $1.97 \times 10^{-2} - 5.37 \times 10^{-2}$ cm/s; initial bulk concentration of OA, $C_i$: ca. 0.05 - 0.25 g/L; liquid volume, $V$: 600 cm$^3$. A model estimating bulk concentration profile was proposed by taking into account a mass balance of the present system. The model predicted that OA could be removed perfectly, however, was not all removed experimentally. The residual OA concentration of the bulk liquid within the column reached plateau value, which correspond to ca. 18 % of the initial OA concentration. The plateau value of the bulk concentration was attained for ca. 100 - 500 min with $U_g = 1.97 \times 10^{-2} - 5.37 \times 10^{-2}$ cm/s. Foaming ability test revealed that the foaming limit concentration of OA at pH 4.6 was $9.72 \times 10^{-3}$ g/L. These results suggested that OA molecules could be damaged by interaction of bubble surface in the dispersed phase, since there were the residual OA concentrations over the limit concentration. To take account of this phenomena and correct the model, average surface density, $X_d$, which should convert protein molecule into the denatured protein molecule, was introduced. The corrected model could explain well time profile of OA bulk concentration.

Keywords: Foam separation; Batch operation; Ovalbumin; Protein removal modeling; Wastewater Treatment
1. Introduction

Foam separation is an available method for the enrichment or the removal of dilute surface-active substances dissolved in water. This technique has been studied in many fields, such as chemical/biochemical engineering (Sarker et al., 1987; Brown et al., 1990; Bhattacharya et al., 1991; Mohan et al., 1992; Montero et al., 1993; Maruyama et al., 2000; Wong et al., 2001; Du et al., 2002), analytical chemistry (Schoen, 1966; Karger and Miller, 1969), wastewater treatment (Ng et al., 1976; Huang et al., 1995; Beheir and Aziz, 1996) and so on.

From the viewpoint of wastewater treatment, it is important to remove solid or soluble organic substances from aqueous environments since these substances cause high organic loading in the effluent and can become resources of un-ionized ammonia, ammonium, nitrate and nitrite ions which are well-known as toxic substances for human and vertebrates. Therefore, removal of these substances before disposing to wastewater treatment can reduce the organic loading. Foam separation method has been employed for this purpose as one of effective and low cost techniques.

However, studies on protein denaturation by this technique have been reported. It appears that there are three possible mechanisms by which proteins may be damaged in foam separation process: (i) unfolding at the gas-liquid interface (Graham and Phillips, 1979; Clark et al., 1988; Varley and Ball, 1994; Clarkson et al., 2000), (ii) high shear stress rates (Maa and Hsu, 1997), and (iii) chemical damage may occur by oxidation (Montero et al., 1993; Liu et al., 1998). Maa and Hsu (1997) examined the combined effect of shear stress and gas-liquid interface on protein denaturation. They concluded that shear plays only a minor role with the damage being mainly due to interaction with
gas-liquid interface even though their estimation of shear stress in foaming were lower. Monteo et al. (1993) reported denaturation of cellulase in foam and assumed that this damage was caused by oxidation due to sparging gas. Liu et al. (1998) investigated denaturation of enzymes in foam separation and reported that oxidative deactivation of enzymes was identified and the deactivation could be reduced by applying N$_2$ or CO$_2$ gas as sparging gas. They suggested that reducing the contact time of bubble and enzyme proven mild and effective recovery in foam separation using loop bubble column.

In batch foam separation process, protein molecules in bubble dispersed bulk phase seemed to be damaged by some factors described above. It is necessary to consider influences of these damages for estimation of protein removal from aqueous phase by foam separation. This consideration and the knowledge about this will be also important for enrichment operation of proteins/enzymes for biochemical engineering/biochemical industries.

In this paper, the authors conducted batch foam separation. Ovalbumin was used as a model protein in a batch foam separation process. A model is proposed estimating the bulk concentration profile of ovalbumin during batch foam separation.
2. Model description

Protein contained in the foam liquid is assumed to originate from that adsorbed onto the bubble surface and from that entrained bulk liquid. According to the material balance between the foam bed and the pool liquid in the column, the following relation was given as

\[-V(dC_b/dt) = S_b X + W_{f0} C_b,\]  

(1)

where, \(C_b\), \(S_b\), \(W_{f0}\), \(V\) and \(X\) are the protein concentration of the bulk liquid, the production rate of bubble surface area in the column, the volumetric flow rate of the liquid in foam at the liquid–foam interface, liquid volume within the column and the adsorption density onto bubble surface, respectively. We employed \(S_b\), \(X\) and \(W_{f0}\) as the following relationship.

The production rate of bubble surface area in the column, \(S_b\), is expressed by the following equation (Suzuki et al., 1995; Suzuki et al., 1996).

\[S_b = 6 A \varepsilon_G (1-\varepsilon_G)^{4.65} \{(4/225)(\rho_L-\rho_G)^2 g^2/(\mu_L \rho_L)\}^{1/3},\]  

(2)

where \(A\), \(\varepsilon_G\), \(g\), \(\rho_L\), \(\rho_G\) and \(\mu_L\) denote a cross–sectional area of the column, the gas holdup, the gravitational acceleration, the density of liquid and gas and the viscosity of the liquid, respectively.

In our previous study (Maruyama et al., 2000), we reported that adsorption of protein onto bubble surface was subjected to Langmuir's isotherm.
where $K$ and $\gamma$ are the equilibrium constant and the saturated surface density, respectively.

The volumetric flow rate, $W_{f0}$, of the liquid in foam at liquid-foam interface can be correlated using the Ohnesorge number, $Z$, (Suzuki and Maruyama, 2001),

$$W_{f0} = 2.53 A U_g Z^{0.533},$$

In Eq. (4), the Ohnesorge number, $Z$, is defined as follows:

$$Z = \mu_L / (\rho_L \sigma d_b)^{0.5},$$

where, $\sigma$ and $d_b$ are surface tension and the average bubble diameter in the pool liquid in the column. The average bubble diameter, $d_b$, was estimated by the following equation (Suzuki and Maruyama, 2001).

$$d_b = U_g/[\varepsilon_0(1-\varepsilon_0)^4.65 \{(4/225)(\rho_L-\rho_0)^2 g^2 / (\rho_L \mu_L)\}^{1/3}].$$

Eqs. (2) and (6) were derived from the following three assumptions: (i) bubbles are almost homogeneous spheres in aqueous solution including surface-active substances, (ii) their terminal velocity can be calculated by Allen’s equation (Allen, 1990) in our experimental range, and (iii) an equation for voidage compensation proposed by Lewis.
et al. (Lewis et al., 1949) was employed. This was reported in our previous studies (Suzuki et al., 1995; Suzuki and Maruyama, 2001).

$C_b$ is calculated numerically by combining Eq. (1) and Eqs. (2)-(6) with the Runge-Kutta method using a spreadsheet program on a personal computer. The calculated values will be compared with the experimental values.
3. Materials and method

3.1 Materials

Ovalbumin from egg white was purchased from Difco Laboratories and was used without further purification. All the other chemicals were analytical grade reagents. The pHs of solutions were adjusted with aqueous HCl or NaOH to several desired values.

3.2 Experimental setup

A schematic diagram of an experimental setup for batch foam separation is shown in Fig. 1. A bubble column of \(4.4 \times 10^{-2}\) m inside diameter and 0.4 m height was employed. The column was made of transparent acrylic resin. Sintered glass filter, which had pores of \(10 \times 10^{-6} - 15 \times 10^{-6}\) m mean diameter, was installed as a gas distributor at the bottom of the column. Nitrogen gas was supplied to the column through a distributor. Two pressure taps for measuring gas holdup in the column were along the wall installed at intervals of 0.25 m.

3.3 Batch foam separation experiment

All experiments were operated in batchwise with respect to liquid. Ovalbumin (OA) was used as a model water-soluble protein. The OA solution prepared at desired concentration was charged into the column without foaming. After that, nitrogen gas was supplied and was dispersed as bubbles through the distributor. The aspiration of foam was started at \(2 \times 10^{-2}\) m height from the foam-liquid interface within the column to prevent back flowing the collapsed foam liquid to the column. The bulk liquid in the column was sampled from a sampling tap at the bottom of the column. The
concentrations of protein solution were measured by Lowry method (Lowry et al., 1951). The pH of the bulk liquid in the column was measured with a pH meter (ORION Model SA 520). The gas holdup was determined from the difference in static pressure between the clear and aerated liquids using a differential pressure transducer (Tem-Tech Lab. Inc., JAPAN). Voltage signals were recorded by a personal computer (NEC PC-9801VM) via A/D converter (CONTEC).

3.4 Foaming ability experiment

The experiment was carried out according to a modified version of Japanese Industrial Standard K2518 method (1991). 200 mL of protein solution prepared at desired concentration and pH was charged into the column, and the nitrogen gas was dispersed into the solution as bubbles at $U_g = 9.86 \times 10^{-4}$ m/s. The foam height from the liquid–foam interface within the column was measured by a scale along the outside wall of the column during aeration.
4. Results and discussion

4.1 Dependence of removal efficiency on pH

Protein molecules are well known as an amphoteric molecule, therefore, properties of protein molecule are greatly affected by pH value of solution. Influence of pH on removal efficiency of ovalbumin (OA) is shown in Fig. 2. The removal efficiency profile had a maximum peak near the i.e.p. (iso-electronic point) of OA (pH 4.6). In case of BSA (bovine serum albumin), same tendency has been reported in literature (Schnepf and Gaden, 1959). Therefore, we carried out all subsequent experiments at pH 4.6.

4.2 Concentration profile of bulk liquid

Typical results of time course of OA concentration in the bulk liquid within the column are shown in Fig. 3. Superficial gas velocities, \( U_g \), greatly affected the time course profiles. The values of gas holdup and \( S_b \) calculated from Eq. (2) are shown in Figs. 4a and 4b as a function of \( U_g \), respectively. In the present experiments, \( S_b \) is proportional to \( U_g \) linearly. In the case of Fig. 3, the liquid properties of the protein solutions are not so different because pH and initial concentration of OA were almost same, removal rate would be dominated by the surface area production rate, \( S_b \).

4.3 Comparison of experimental results and model calculation

Solid lines in Fig. 3 represent the calculated values of bulk liquid concentration by Runge-Kutta method using spreadsheet program on a personal computer. In these calculations, the values of gas holdup was employed the experimental values, \( K \) and \( \gamma \).
were employed $1.01 \times 10^4$ cm$^3$/g and $3.39 \times 10^{-7}$ g/cm$^2$, respectively, which were determined experimentally in our previous study (Maruyama et al., 2000). The calculated values have good agreement with the experimental ones in the initial region ($t < 80$ min). The difference between both values became larger in the later region ($t > 80$ min). The calculated lines could reach at zero finally, i.e., the calculation predicted that OA in the bulk liquid was removed perfectly. On the other hand, the experimental value of bulk concentration of OA could not yet become zero. The residual concentration of OA seemed to reach at an apparent equilibrium concentration (plateau value) in this experimental condition.

4.4 Influence of initial concentration on residual concentration

Various initial concentrations of OA were employed to investigate whether the residual concentrations were influenced by the initial concentrations of OA. The result is shown in Fig. 5 as a function of the initial concentration. The residual concentration was proportional to the initial concentration. A least-square regression gave this slope as 0.182. Therefore, the residual concentration became ca. 18% of the initial concentration. When air or oxygen gas were used as aeration gas in place of nitrogen gas, there were not so large difference in concentration profiles and the residual concentrations in comparison with the case of nitrogen gas (data were not shown).

The authors considered that the residual concentration would be caused by (i) Corresponding the residual bulk concentration to the foaming limit concentration of OA or (ii) protein denaturation which made OA molecules not adsorb onto bubble surface. Therefore, we conducted some additional experiments to explore these possibilities and discussed in the following sections.
4.5 Determination of foaming limit concentration

The foaming profiles are shown in Fig. 6a. The foam height increased immediately up to 2 min. The foam height of all concentrations employed in this experiment reached at each maximum height at 3 min. The foam height at 3 min. was chosen to determine the foaming limit concentration by the extrapolation graphically. Fig. 6b shows a plot of the foam height at 3 min. versus the initial concentration of OA in logarithmic scale. The straight line in this figure was obtained by a least square method. The intercept of x-axis gave the foaming limit concentration. The value of the intercept was $9.72 \times 10^{-3}$ g/L.

This result conflicts with the result of Fig. 5. As can be seen in Fig. 5, there were obviously higher residual concentrations than the foaming limit concentration. This experimental result suggests that when the initial concentration was employed more than ca. 0.1 g/L, OA molecules in the bulk solution within the column might be damaged and denatured by aeration. The damage might make OA molecules be not adsorbed onto bubble surface.

The authors consider that the gas-liquid interface of bubbles in foam layer was not only the ground of the denaturation but that of the dispersed bubbles in bulk liquid also play a role with protein denaturation, since the foam bubbles were originated from the dispersed bubbles in the bulk liquid within the column. It will be necessary for preventing proteins from denaturation to shorten residence time of column liquid or to conduct with smaller air-liquid interface area.

4.6 Correction and verification of the model
The authors assumed that a part of the OA molecules adsorbed onto bubble surface (gas-liquid interface) should be damaged and the damaged OA could not adsorb onto bubble surface at all. Thus, they would be remained in the bulk liquid phase. The bubble surface generated within the column would play an important role in the denaturation. The authors assume an average adsorption density, $X_d$, which was denatured and became residual OA molecules in bulk liquid. Eq. (1) can be also rewritten as:

$$-V(dC_b/dt) = S_b (X - X_d) + W_{f0} C_b.$$  \hspace{1cm} (7)

$X_d$ will be determined by fitting the data to Eq. (7) using a least-squares regression.

Typical results of fitting of the data to Eq. (7) shows in Fig. 7. The corrected model could explain well the concentration profiles in batch foam separation by determination of one parameter, $X_d$, using a least-square regression. The determined parameter, $X_d$, in Eq. (7) is shown in Fig. 8 as a function of $S_b$. The $X_d$ increased with increasing $S_b$ up to ca. 30 cm$^2$/s and reached at plateau level in a region of $S_b >$ ca. 30 cm$^2$/s. Thus, the value of $X_d/\gamma$ indicates the degree of OA denaturation. The maximum value of $X_d/\gamma$ (denatured fraction) is about 0.15 in the present result.

4.7 Residual concentration of the OA mixture solution

To confirm whatever the OA in the residual solution could be removed, a batch experiment using a mixture solution was carried out. The mixture solution was consisted of prepared newly and residual OA solutions. The mixture solution included $4.71\times10^{-2}$ g/L of fresh OA and $2.89\times10^{-2}$ g/L of the residual OA, respectively. Thus, the initial concentration of total OA was $7.60\times10^{-2}$ g/L.
The time course of total OA concentration in the mixture solution is shown in Fig. 9. Solid curve in Fig. 9 was obtained by calculation with Eq. (7). The calculation was done with the following conditions for the prepared fresh OA solution: \(C_i = 4.71 \times 10^{-2} \text{ g/L}, U_g = 0.0537 \text{ cm/s}, V = 500 \text{ mL}\) (these are experimental condition) and \(X_d = 4.57 \times 10^{-8} \text{ g/cm}^2\) (from Fig. 8). The total OA concentration was calculated by the addition of the concentration of fresh OA and that of the denatured OA, \(2.89 \times 10^{-2} \text{ g/L}\), since it was assumed that the denatured OA originated from the residual solution would not be removed from bulk liquid. As seen in Fig. 9, the profile of the calculated total OA concentration corresponded to the experimental one. The concentration profiles could be estimated by the corrected model calculation (Eq. (7)) within ca. 10 % error (average value). The experimental result suggests that OA molecules originated from the residual bulk solution would not adsorbed onto bubble surface.

Many investigators reported adsorption of protein at liquid/air interface and surface-induced unfolding of protein molecules (MacRitchie and Alexander, 1963a-c; Graham and Phillips, 1979a-c; Guzman et al., 1986; Hunter et al., 1990; Hunter et al., 1991; Narsimhan and Uraizee, 1992). Graham and Phillips (1979a-c) reported adsorption of casein, BSA and lysozyme at air/water as well as oil/water interface and concluded that the extent of unfolding proteins depended on the surface pressure and that the time scale of the surface rearrangement in much larger than that of adsorption. The conformational change due to unfolding has been reported (Damodaran and Song, 1988; Clark et al., 1988 and so on).

Due to recent developments in techniques that can be exploited to obtained insight in the molecular details of these surface-unfolded proteins and their surface activity, many progress have been made in the understanding of the process. Rodriguez Patino et
al. (2004) studied adsorption of soy globulin at air/water interface and its adsorption kinetics by means of surface tension measurement coupled with Brewster angle microscopy. The result showed that the adsorption kinetics at the beginning of the adsorption was diffusion-controlled, however, the mechanism that controls the long-term adsorption is the penetration and unfolding of the protein. Lu et al. (2005) reported structural conformations of human lactoferrin adsorbed at air/water interface by neutron reflectivity. The result showed that unfolded lactoferrin was characterized by two main regions, a top layer of 10-20 Å with a high polypeptide volume fraction of 0.5 on the air side and a bottom layer of 50-80 Å with a low polypeptide volume fraction of 0.2 immersed in water. The top layer is predominantly exposed to air, indicating the strong hydrophobic nature of the polypeptide chain in this region.

Clarkson et al. (1999a) pointed out that protein denaturation in foam can be directly correlated with the interfacial exposure area and that shear stress and oxidation did not significantly contribute denaturation of protein. They also reported that in foam, protein molecules would undergo changes in tertiary and in some particular cases secondary structure and that some proteins were found to form aggregates after foaming (Clarkson et al., 1999b). Wierenga et al. (2003) studied influence of exposed hydrophobicity on kinetics of protein at air/water interface using native and caprylated ovalbumin. The adsorption kinetics of the caprylated ovalbumin follow the calculations form diffusional transport more closely, which shows that the energy barrier for adsorption of caprylated ovalbumin is much lower than for the native ovalbumin. Bramanti et al (2006) investigated surface activity of chemically denatured proteins by using dynamic surface tension detector. The results showed that surface pressure of some denatured proteins generally increased as denaturants (urea, GdmSCN and GdmHCl). Lechevalier, et al.,
(2003) investigated structural modification of ovalbumin, ovotransferrin and lysozyme at air-water interface by SDS-PAGE, intrinsic and ANS fluorometry and circular dichroism techniques and reported that ovalbumin undergoes secondary and tertiary structure modifications that are weaker than those for ovotransferrin, however, because of its free sulfhydryl groups, these changes are enough to enable intermolecular sulfhydryl-disulfide exchanges, leading to aggregation of ovalbumin.

The results in this study suggest that the denatured OA did not adsorbed on bubble surface and had lower surface activity rather than that of native OA. However, the present result is insistent with experimental facts reported by Wierenga et al. (2003) and Bramanti et al. (2006). If denatured OA formed aggregates by means of binding with exposed hydrophobic functional groups between unfolded OA molecules, resulting that the aggregated proteins exposed their hydrophilic functional groups (polar groups) to bulk liquid, thus, the aggregates might not adsorb on bubble surface because of its high hydrophilicity. The lower surface activity of denatured OA observed in this study may be related to the aggregating process reported by Lechevalier, et al., (2003).
5. Conclusions

The authors conducted removal of dissolved protein by batch foam separation method. The experimental results of batch foam separation and foaming ability test revealed that residual OA molecules were not adsorbed onto bubble surface. Residual fraction of OA was ca. 18 % of the initial OA concentration in the present experimental conditions. These suggest that OA molecules were denatured due to some interactions between the molecules and gas-liquid interface. The corrected model estimating the bulk concentration profile could represent the concentration profile in bulk liquid within the column by taking account of average surface density, $X_d$, converting OA molecule into the denatured one. From the calculations, The maximum value of denatured fraction was ca. 0.15 in the present experimental conditions. The further study of improvement on more effective protein removal by batch foam separation will be needed from the viewpoints of wastewater treatment.
**Notation**

\( A \) = a cross sectional area of bubble column \[ \text{m}^2 \]  
\( C_b \) = concentration of bulk liquid in bubble column \[ \text{kg/m}^3 \]  
\( C_i \) = initial concentration of bulk liquid in bubble column \[ \text{kg/m}^3 \]  
\( d_b \) = the average bubble diameter defined by Eq. [4] \[ \text{m} \]  
\( \varepsilon \) = gas holdup \[ \text{-} \]  
\( g \) = gravitational acceleration \[ \text{m/s}^2 \]  
\( \gamma \) = saturated surface density of protein \[ \text{kg/m}^2 \]  
\( \mu_L \) = liquid viscosity \[ \text{kg/(m s)} \]  
\( \rho_G \) = gas density \[ \text{kg/m}^3 \]  
\( \rho_L \) = liquid density \[ \text{kg/m}^3 \]  
\( \sigma \) = surface tension \[ \text{N/m} \]  
\( S_b \) = the production rate of bubble surface area in the dispersion phase \[ \text{m}^2/\text{s} \]  
\( U_g \) = superficial gas velocity \[ \text{m/s} \]  
\( V \) = liquid volume within bubble column \[ \text{m}^3 \]  
\( W_{f0} \) = the intrinsic volumetric flow rate of the liquid in foam at the liquid – foam interface within bubble column \[ \text{m}^3/\text{s} \]  
\( X \) = surface density of protein \[ \text{kg/m}^2 \]  
\( X_d \) = average surface density which should convert protein molecule into the denatured one \[ \text{kg/m}^2 \]  
\( Z \) = Ohnesorge number \[ \text{-} \]
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Captions of Figures

Fig. 1.  Schematic diagram of experimental setup for batch foam separation. 1. bubble column; 2. gas distributor; 3. pressure measuring tap; 4. gas flow meter; 5. flow control valve; 6. nitrogen gas cylinder; 7. pressure/voltage transducer; 8. amplifier; 9. voltmeter; 10. personal computer.

Fig. 2.  Influence of pH on removal efficiency of ovalbumin (OA) by batch wise foam separation. The initial concentration of OA was 3.68×10^{-2} kg/m^{3} and superficial gas velocity is 5.37×10^{-4} m/s.

Fig. 3.  Typical time course of ovalbumin removal from bulk liquid by batch wise foam separation. Solid lines were model predictions calculated from the present model.

Fig. 4.  (a) Change in gas holdup with superficial gas velocity, \( U_g \). The solid line was calculated by a least square regression. (b) Variation of bubble surface area production rate, \( S_b \), with superficial gas velocity, \( U_g \). The values were calculated from Eq. [2].

Fig. 5.  Relationship between the initial and the residual concentration of OA solution. The solid line is calculated by the least squares regression.

Fig. 6.  (a) Change in foam height within the column for ovalbumin in foaming
ability experiments at pH 4.6. (b) Variation of foam height at 3 min after aeration with the initial concentration of OA solution. The solid line is calculated by the least squares regression.

Fig. 7. Typical results of fitting of the data to Eq. [7].

Fig. 8. Relation between $X_d$ and $S_b$. $X_d$ was estimated by Eq. [7].

Fig. 9. Comparison of the calculated profile (solid line) and the experimental one. The experiment was conducted mixture solution of prepared fresh and the residual solution of OA. The former and the latter concentrations were 0.0471 and 0.0289 g/L, respectively. In this calculation, $X_d = 4.57 \times 10^{-8}$ g/cm² was employed.
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