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Flocculation of diatomite by methylated milk casein in seawater

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Abbreviated title:

Flocculation by methylated casein in seawater

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Abstract

A new biodegradable flocculant was prepared from a common and inexpensive protein. Milk casein was methylated in a 0.05 M HCl methyl alcohol solution at room temperature. The methylated milk casein (MeCS) having a methylation degree of 81% was applied to the separation or flocculation of diatomite in seawater (pH8.1±0.1) at room temperature (18 - 23°C). The flocculating ability of MeCS was evaluated by a sedimentation balance method (cumulative measuring method). The diatomite suspension was effectively flocculated by the addition of a small amount of MeCS (0.25 wt% of the diatomite weight). The results of sedimentation analysis showed that the size-frequency curve had a very sharp and high peak thus the diatomite floc formed by MeCS had a rather uniform size. The settling velocity of diatomite floc at the appropriate MeCS dosages (0.25 - 2 wt% to diatomite) was about 3×10^{-3} m s^{-1}.

Key Words: Flocculation; Methylated protein; Milk casein, Seawater, Diatomite

1. Introduction

Soil spillage due to the recent deforestation and the coastal development has become a serious environmental problem. Sedimentation of soil particles in estuary area has caused serious damages on the coastal ecosystem and the coastal fisheries. The flocculation of suspensions by chemical flocculants is used to improve solid-liquid separation in mineral processing operations, water treatment, and wastewater treatment. The currently accepted chemical flocculants are synthesized high-molecular-weight polymers and alum. Both of these flocculants are environmentally undesirable. Especially, chemically synthesized flocculants remain in natural environments for long periods of time without degradation. Thus, the use of extracellular polymers produced by bacteria (bioflocculants) for the separation of suspended solids has been a topic of intense research in recent years [1-8]. However, for practical application, such bioflocculants must be separated or purified from culture media by intricate treatments.

In our previous study [9], a biodegradable flocculant was prepared from egg albumin by methylation with HCl methyl alcohol solution. The methylated egg albumin, having methylation degree ca. 90%, efficiently flocculated a suspended diatomite in fresh water over a wide pH range from pH 3 to 10. However, the highly methylated egg albumin underwent self-aggregation in a high ionic strength solution and the flocculating ability of methylated egg albumin decreased with increasing ionic strength.

In marine and coastal construction projects, an effective separation system for suspended solids in seawater is necessary to prevent the inflow of soil particles into the sea. In the present study, we prepared another biodegradable flocculant from a common and inexpensive protein (milk casein) by methylation. The methylated milk casein (MeCS), having methylation degree ca. 80%, was employed for the separation of diatomite particles in seawater. The flocculation behavior of diatomite-MeCS-seawater system was examined using a sedimentation balance method (cumulative measuring method). Based on the results of sedimentation analysis (cumulative analysis), the flocculating ability of MeCS will be discussed.

2. Materials and methods

2.1. Materials

Milk casein, methyl alcohol, sodium hydroxide, and hydrochloric acid were purchased from Wako Pure Chemical Industries (Japan). Milk casein was of practical-grade quality and other chemicals were of reagent-grade quality. They were used with no further purification. Distilled water, boiled for 15 min and cooled under a nitrogen atmosphere, was used in all experiments.

Seawater was collected in Funka Bay, Japan and it was filtered with a 0.45 μm membrane filter to remove suspended substances. The density and the relative viscosity were measured by a pycnometer and an Ostwald viscometer and they were determined as 1.025 g cm^{-3} and 1.052, respectively.

Diatomite (Diatomaceous earth, Kanto Chemical Co. Inc., Japan) was used in making the suspensions to be flocculated. This material was selected because of its strong surface-negative character in water; it may remain negative even when the pH is decreased to as low as 3 or so. According to the data supplied by the company, the diatomite is composed of about 90% SiO_2. The diatomite was screened by an 80-mesh (0.177-

mm) sieve and the undersize fraction was washed repeatedly with distilled water. After that it was dried at 80°C for 2 days and stored in a desiccator. The specific surface area and the density measured by an air-permeability method and a pycnometer were 2.84 m^2 g^{-1} and 2.32 g cm^{-3}, respectively.

Milk casein was methylated according to the method reported by Fraenkel-Conrat and Olcott [10]. An aqueous solution of milk casein (10 g dm^{-3}) was prepared. The milk casein was precipitated by the addition of HCl solution (0.1 mol dm^{-3}). It was separated from the liquid phase in a centrifuge at 3000 rpm for 20 min and washed twice with methyl alcohol. Then it was dispersed in a 100-fold amount of methyl alcohol containing HCl (0.05 mol dm^{-3}) and stirred for 24 h at room temperature. The methylated milk casein was collected in a centrifuge at 3000 rpm for 20 min and washed repeatedly with HCl solution (1×10^{-4} mol dm^{-3}). Hereafter, the untreated milk casein and methylated milk casein will be abbreviated as CS and MeCS, respectively. The methylation reaction proceeded rapidly and 20 h or so was enough to attain the methylation degree of 90%. From the results, we determined the reaction time for the preparation of MeCS as 24 h. The degree of methylation was determined from the change in the number of carboxylic groups before and after methylation. The number of carboxylic groups was determined by a potentiometric titration. A detailed method to determine the degree of methylation has been presented in our previous study [9]. The methylation degree of MeCS used in this study was 81%.

2.2. Sedimentation experiments (cumulative measuring method)

To examine the influence of salinity on the sedimentation of diatomite, the following experiment was conducted. Diatomite (2 g) was suspended in a 30-ml of seawater, diluted seawater (2.0 vol%) or distilled water in a 300-ml flask. The suspension was boiled at room temperature under a reduced pressure. An appropriate amount of seawater, diluted seawater (2.0 vol%) or distilled water was added so as to form finally a 200-ml suspension. The pH of the suspension was adjusted to pH8.1±0.1 with a small amount of HCl or NaOH solution and it was stirred at room temperature (18 - 23°C). After 30 min stirring, the suspension was poured into a cylindrical tube (0.038 m diameter, 0.25 m height) in which a sediment trap (0.026 m diameter, 0.03 m height) was hung from an electronic micro balance. The change of the weight of settled particles on the trap was measured with the sedimentation time until it reached a constant weight.

To examine the flocculating ability of MeCS, the following experiment was conducted. A suspension containing 2 g of diatomite in seawater (197 ml, pH8.1±0.1) was prepared in a similar manner as described above. It was stirred at room temperature for 30 min. A solution (3 ml, pH8.1±0.1) containing a certain amount of MeCS was added to the suspension. After 3 min stirring, the suspension was poured into the cylindrical tube and the change of the weight of settled particles on the trap was measured with sedimentation time until it reached a constant weight. We conducted the sedimentation experiments 3 times and the average values were used for sedimentation analysis.

3. Results and discussion

3.1. Effect of salinity on sedimentation of diatomite suspension

Figure 1 shows the results of the sedimentation experiments conducted with the diatomite suspensions containing 0, 2.0, and 100 vol% of seawater. These experiments were carried out in the absence of MeCS. W_t and W_f represent the cumulative weight of diatomite settled on the trap at time t and the weight at final state, respectively. L represents the sedimentation distance between the bottom of trap and the suspension surface. The sedimentation of diatomite was markedly enhanced by the addition of only 2.0 vol% of seawater. The settling velocity of diatomite particles in seawater was not so different from that in the diluted seawater (2.0 vol%).

A conventional and well established sedimentation analysis (cumulative method) was used to analyze the sedimentation behavior of diatomite [11]. In the sedimentation balance method, the cumulative weight of settled particles on the trap at time t, W_t, can be expressed by the sum of two terms as:

$$W_t = W_f \int_x^{x_{\max}} f(x)dx + W_f \int_{x_{\min}}^{x} f(x)\frac{vt}{L}dx \tag{1}$$

where $f(x)$ is the size-frequency (density) distribution function of particles, and x is the diameter of particles. The first term in Eq.(1) denotes the weight of settled particles having the settling velocity $v > L/t$, and the second term denotes the weight of settled particles having the settling velocity $v < L/t$. The differentiation of above equation with respect to time gives

$$\frac{dW_t}{dt} = W_f \int_{x_{\min}}^{x} f(x)\frac{v}{L}dx\,. \tag{2}$$

Combining Eqs. (1) and (2), we obtain the following equation

$$R = \int_x^{x_{\max}} f(x)dx = \frac{W_t}{W_f} - t\frac{d(W_t/W_f)}{dt}\,. \tag{3}$$

R denotes the cumulative oversize fraction of suspended particles or the weight fraction of particles having the settling velocity $v > L/t$.

Thus, we can obtain the R curve as a function of t from the sedimentation data and Eq. (3). In this study, to avoid the differentiation process, we tried to approximate the experimental data in Fig. 1 by the following equation

$$W_t / W_f = \sum_{i=1}^{n} r_i \{1 - \exp(-k_i t)\} . \qquad (4)$$

It should be noted that the fitting parameters k_i and r_i have no theoretical meaning, since Eq. (4) was introduced only for fitting the experimental data. When we set $n = 4$, a very good correlation (correlation coefficient of 1.000) was obtained for all sets of data. The solid lines in Fig. 1 are the values calculated by Eq. (4). Since W_f is constant in each experiment, the differentiation term in Eq. (3) can be expressed as

$$\frac{d(W_t / W_f)}{dt} = \sum_{i=1}^{4} k_i r_i \exp(-k_i t) . \qquad (5)$$

Finally, Eqs. (3), (4) and (5) give the following equation

$$R = \sum_{i=1}^{4} r_i \{1 - \exp(-k_i t)\} - t \sum_{i=1}^{4} k_i r_i \exp(-k_i t) \,. \qquad (6)$$

On the other hand, the abscissa in Fig. 1, t/L, can be converted to the equivalent Stokes' diameter of settling particles, D_e, as

$$D_e = \sqrt{\frac{18\mu L}{(\rho_p - \rho_l) g t}} \qquad (7)$$

where g, L, ρ_p, ρ_l, and μ denote the gravitational acceleration, the sedimentation distance, the density of settling particle or floc, the density of liquid, and the viscosities of liquid, respectively. For dispersed solid particles, the particle diameters can be calculated directly from Eq. (7) because we can easily determine the particle density. For aggregated particles or floc, however, it is difficult to determine the floc density. Therefore, we used the square root of L/t as an index of floc size.

Figure 2a shows the cumulative oversize fraction curves of diatomite particles obtained from the data in Fig. 1. The solid lines represent the calculated results by Eq. (6). Figure 2b shows the size-frequency curves obtained from the calculated lines in Fig. 2a. The results demonstrate that diatomite particles were coagulated by the addition of a small amount of seawater. The apparent mode size (the square root of L/t at the peak of size-frequency curve) of the diatomite particles in the diluted seawater (2.0 vol%) was about two times larger than that in fresh water. However, the apparent mode size of the diatomite particles in seawater was not so different from that in the diluted seawater.

3.2. Flocculation of diatomite suspension by MeCS in seawater

Figure 3 shows the results of the sedimentation experiments conducted with the diatomite suspensions in seawater containing 0, 0.1, 0.15, 0.25, 0.5, 2.0, and 4.0 wt% of MeCS to diatomite. For the comparison, the results of the sedimentation experiments without MeCS (solid circles) and with CS (open circles) were also presented in the figure. C_{CS}, C_{MS}, and C_D represent the concentration of CS, MeCS, and diatomite in suspension, respectively. The diatomite suspension was effectively flocculated by the addition of small amounts of MeCS and the sedimentation curve followed a straight line at $W_t/W_f < 0.7$.

To describe the experimental data in Fig. 3, the following equations were used

$$W_t / W_f = at \quad \text{(at } t < t_a\text{)} \tag{8}$$

$$W_t / W_f = at_a + \sum_{i=1}^{4} r_i \{1 - \exp\{-k_i (t - t_a)\}\} \quad \text{(at } t > t_a\text{)}. \tag{9}$$

Equations (8) and (9) were also used only for fitting the experimental data, thus the fitting parameters a, k_i, r_i, and t_a had no theoretical meaning. A very good correlation (correlation coefficient of 1.000) was obtained for all sets of data. The solid lines in Fig. 3 represent the calculated results by Eqs. (8) and (9). Since the differentiation of Eq. (8) gives $d(W_t/W_f)/dt = a$, a combination of Eqs. (3) and (8) yields the result of $R = 0$ at $t < t_a$. On the other hand, the differentiation of Eq. (9) gives the following equation

$$\frac{d(W_t / W_f)}{dt} = \sum_{i=1}^{4} k_i r_i \exp\{-k_i (t - t_a)\}\,. \tag{10}$$

R at $t > t_a$ can be obtained by combining Eqs. (3), (9), and (10) as follows

$$R = at_a + \sum_{i=1}^{4} r_i \{1 - \exp\{-k_i (t - t_a)\}\} - t \sum_{i=1}^{4} k_i r_i \exp\{-k_i (t - t_a)\}\,.$$

(11)

Figure 4a shows the cumulative oversize fraction curves of diatomite flocs obtained from the data in Fig. 3. The solid lines represent the calculated results by Eq. (11). Figure 4b is the size-frequency curves obtained from the calculated lines in Fig. 4a. In the presence of MeCS, each size-frequency curve showed a very sharp and high peak. The result showed that the diatomite flocs formed by MeCS had a rather uniform size distribution. The apparent mode size of the diatomite flocs markedly increased by the addition of only a small amount of MeCS (C_{MS}/C_D = 0.1 wt%) and it increased with increasing MeCS dosage up to C_{MS}/C_D = 2.0 wt%.

Since the cumulative curve in Fig. 3 showed a good linearity at $t < t_a$ or at $W_t/W_f < 0.7$, we determined the settling velocity of the diatomite floc from the slope of the linear part of the curve. Figure 5 shows the settling velocity of the flocs as a function of MeCS dosage. For the comparison, the result of the CS-diatomite system was also presented in the figure. In the MeCS-diatomite system, the settling velocity sharply increased with increasing MeCS dosage up to C_{MS}/C_D = 0.25 wt% and MeCS showed a good flocculating ability from C_{MS}/C_D = 0.25 to 4 wt%.

4. Conclusion

A biodegradable flocculant was prepared from a common and inexpensive protein. Milk casein was methylated according to the method reported by Fraenkel-Conrat and Olcott [10]. The methylated milk casein having a methylation degree of 81% (MeCS) was employed for the flocculation of diatomite in seawater. A sedimentation balance method was applied to investigate the effect of seawater concentration on the sedimentation behavior of diatomite and the flocculating ability of MeCS in seawater.

The effect of salinity on the sedimentation of diatomite was examined with the suspensions containing 0, 2, and 100 vol% of seawater at pH 8.1±0.1 and at room temperature (18 - 23°C). The apparent mode size of the settling particles in a diluted seawater (2.0 vol%) was about two times larger than that in fresh water. However, the apparent mode size of the settling particles in seawater was not so different from that in the diluted seawater.

The flocculating ability of MeCS having the methylation degree of 81% was examined with a diatomite suspension in seawater (pH 8.1±0.1) at room temperature (18 - 23°C). The diatomite suspension was effectively flocculated by the addition of a small amount of MeCS (0.25 wt% to diatomite). The results of sedimentation analysis showed that the size-frequency curve had a very sharp and high peak thus the diatomite floc formed by MeCS had a rather uniform size. The settling velocity of diatomite floc at the appropriate MeCS dosages (0.25 - 2 wt% to diatomite) was about 3×10^{-3} m s^{-1}.

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Figure captions

Fig. 1. Cumulative sedimentation curves of diatomite suspensions in fresh water (open circles), 2.0 vol% diluted seawater (open triangles), and seawater (open squares) at pH8.1±0.1 and at room temperature (18 - 23°C). W_t and W_f respectively represent the weight of the diatomite settled on the trap at time t and the weight at final state, and L represents the sedimentation distance. The concentration of diatomite was 10 g dm^{-3}. The solid lines represent the values calculated by Eq. (4).

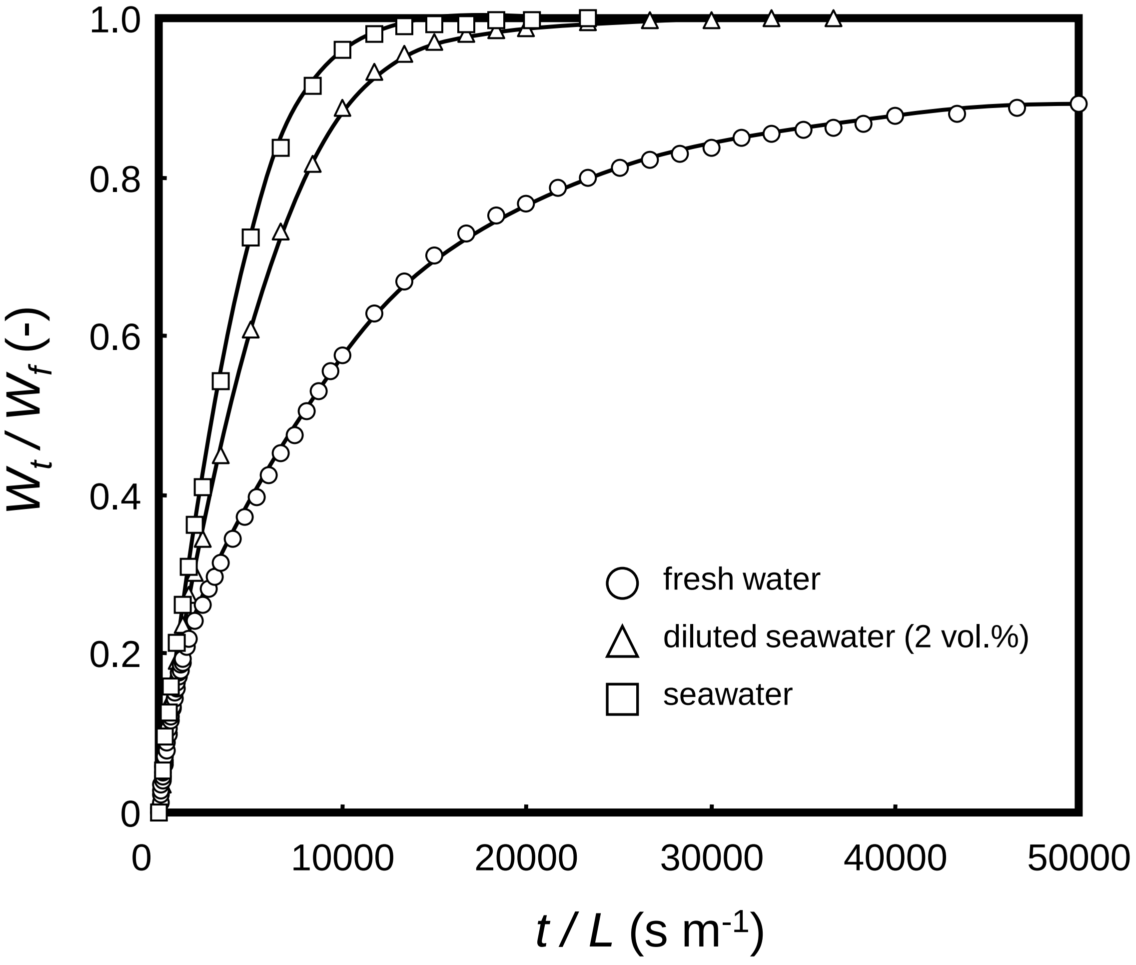
Fig. 2. (a) Cumulative oversize fraction curves obtained from the data in Fig. 1 and (b) size-frequency curves obtained from the solid lines in (a). The solid lines in (a) represent the values calculated by Eq. (6). The square root of the settling velocity, $\sqrt{L/t}$, was used as an index of floc size.

Fig. 3. Cumulative sedimentation curves of diatomite suspensions in seawater containing 0.1, 0.15, 0.25, 0.5, 2.0, and 4.0 wt% of MeCS to diatomite at pH8.1±0.1 and at room temperature (18 - 23°C). The concentration of diatomite was 10 g dm^{-3}. The results without MeCS (solid circles) and with CS (open circles) were presented for the comparison. C_{CS},

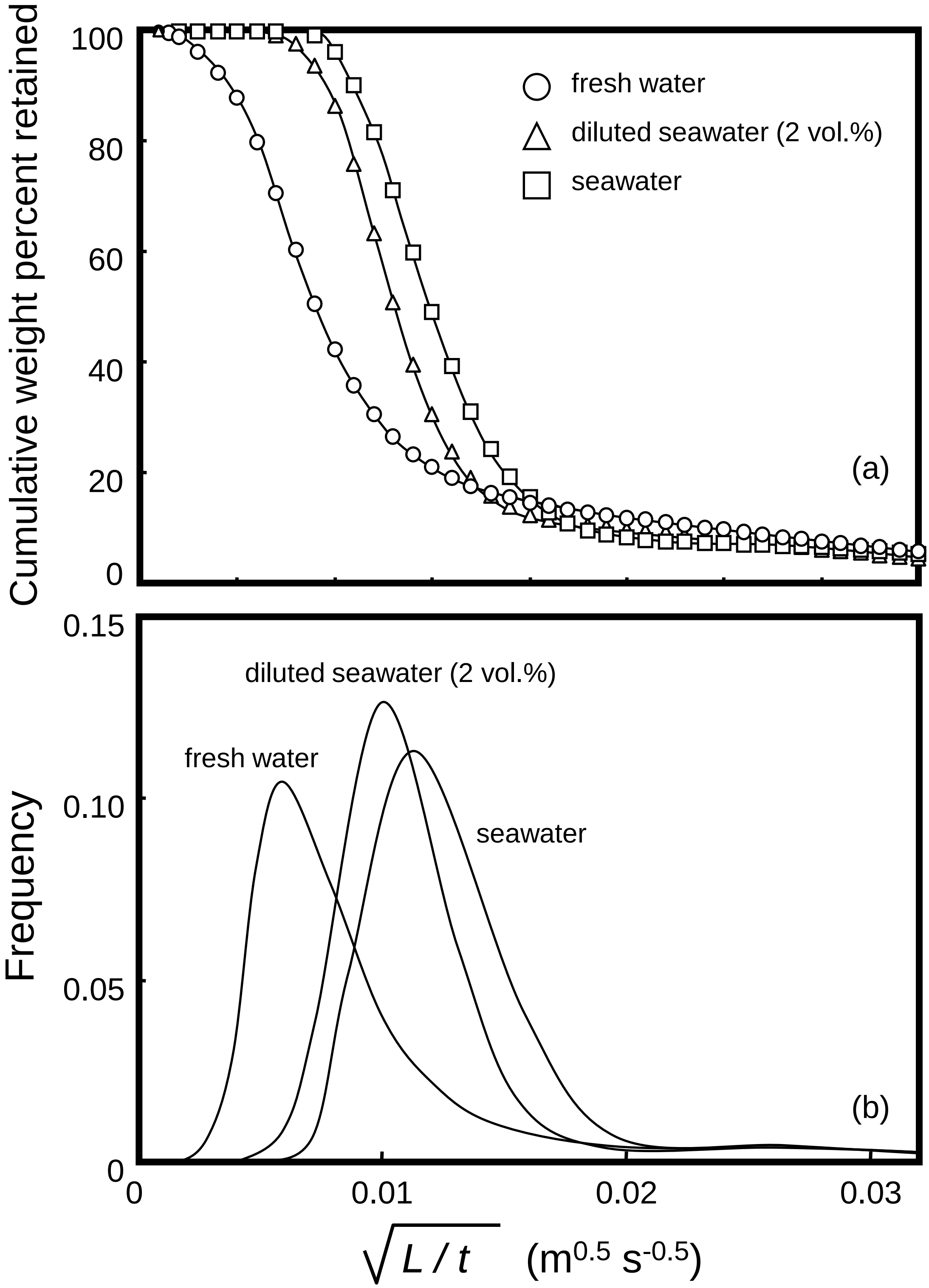
C_{MS}, and C_D are the concentrations of CS, MeCS, and diatomite in the suspension, respectively. The solid lines represent the values calculated by Eqs. (8) and (9).

Fig. 4. (a) Cumulative oversize fraction curves obtained from the data in Fig. 3 and (b) size-frequency curves obtained from the solid lines in (a). The solid lines in (a) represent the values calculated by Eq. (11). The square root of the settling velocity, $\sqrt{L/t}$, was used as an index of floc size.

Fig. 5. Settling velocity of diatomite floc as a function of the dosage of MeCS (open circles) and CS (solid circles). The settling velocity was determined from the slope of the linear part of the solid lines in Fig. 3.



Seki et al. Fig. 1



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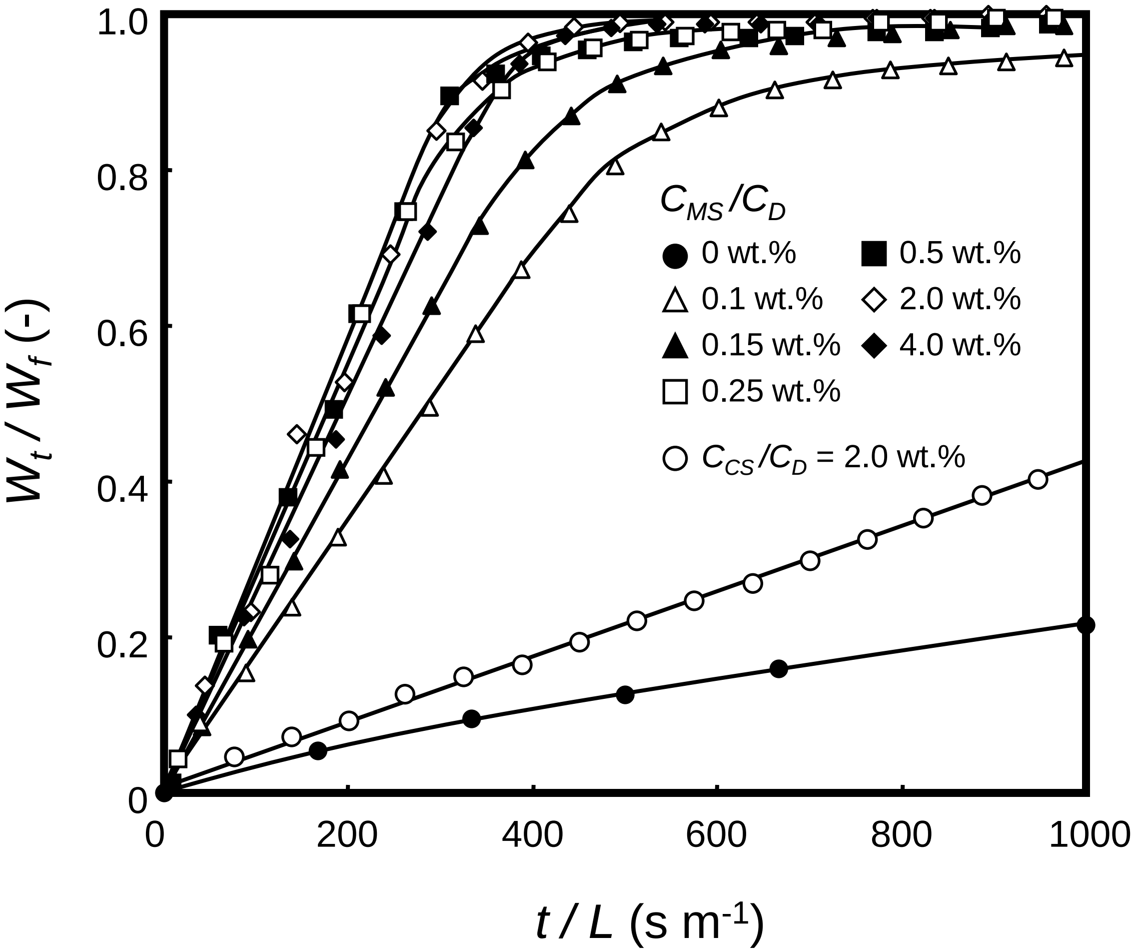
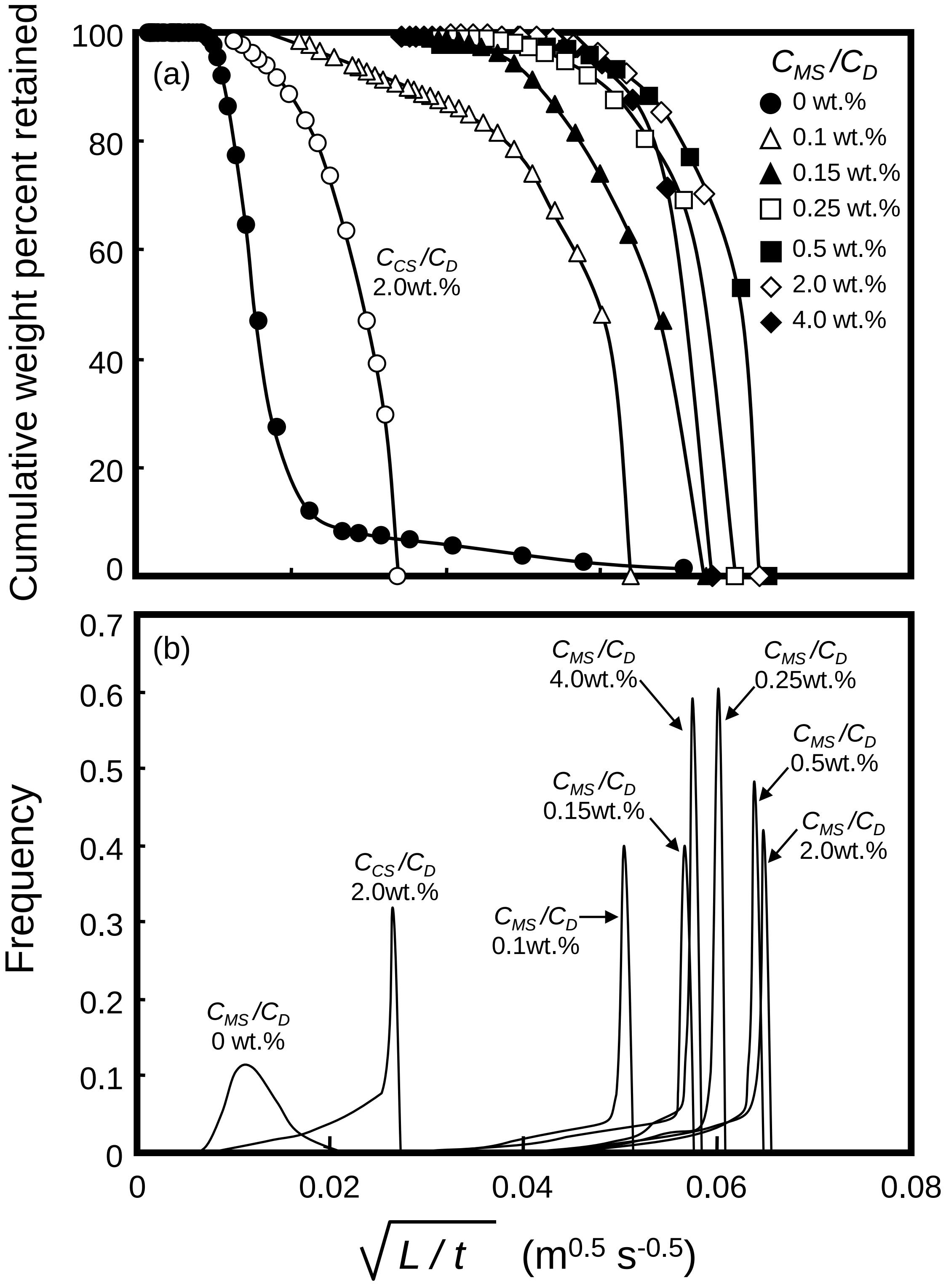
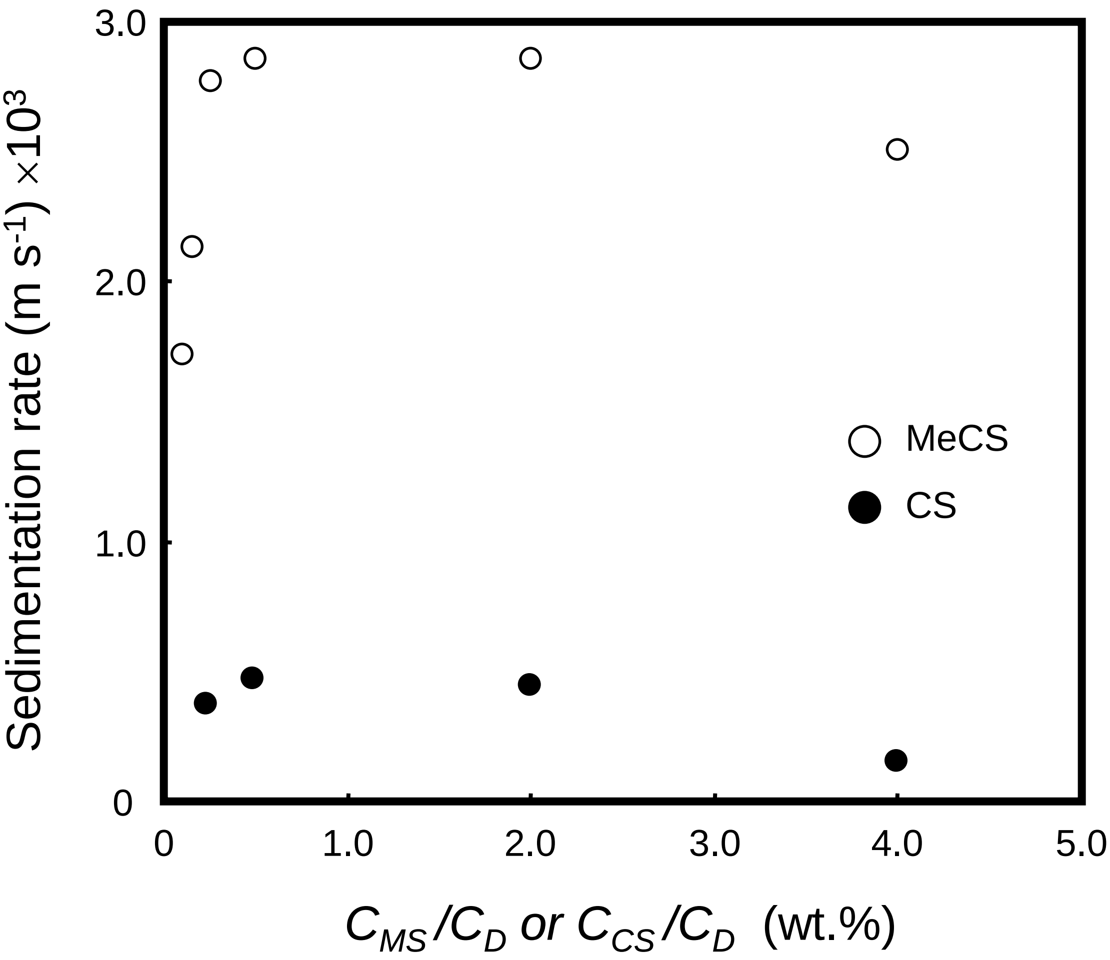


Seki et al. Fig. 3



Seki et al. Fig. 4



Seki et al. Fig. 5