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Flocculation of diatomite by a soy protein-based bioflocculant

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Abbreviated title:

Soy protein-based bioflocculant

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ABSTRACT

A novel bioflocculant, methylated soy protein (MeSP), has been developed and its flocculation performance was tested with diatomite suspensions in freshwater (pH 2-11) and in seawater. The flocculation performance of MeSP was much higher than that of commercial polyaluminum chloride (PAC) in terms of supernatant clarity and floc settling velocity. In freshwater and at a fixed flocculant dosage of 60 ppm, MeSP could reduce the relative absorbance to 0.1 within 1 min over a wide pH range (pH 3-10), while PAC was effective only at around pH 5. In seawater (pH 8), there was a striking difference in flocculation performance between MeSP and PAC. MeSP (20-50 ppm) could reduce the relative absorbance to less than 0.1, while PAC was ineffective. The settling velocity of the floc formed by MeSP in freshwater at pH 7 and in seawater was about 4 mm/s. MeSP was applied to the flocculation of a real wastewater generated from an andesite quarry. The relative absorbance could be reduced to less than 0.05 by the addition of 40 ppm of MeSP. The floc settling velocity was about 5 mm/s.

1. Introduction

Flocculation is a long-established and well-known operation in the purification of drinking water as well as sewage treatment, stormwater treatment and treatment of other industrial wastewater streams. The wastewaters from meat, poultry and fish processing industries contain a high concentration of protein, fat and other organic matter of animal and fish origin. For example, in the surimi or wash minced fish muscle processing industry, the protein lost in the wastewater account for 15-30% of the total protein of minced fish. Thus the flocculated sludge from these wastewaters is considered to be a potential source of animal feed, fish feed and compost [1, 2]. Ferric salts, aluminum sulfate, polyaluminum chloride and synthetic high-molar mass polyelectrolytes have been used as flocculating agents or flocculants. However, the harmfulness of aluminum ion and synthetic polyelectrolytes to plants and aquatic organisms has been pointed by many researchers.

Aluminum toxicity in plants is one of the major limitations to crops grown on acid soils. Aluminum exposure rapidly inhibits root elongation [3-10]. This rapid inhibition of root growth, of which the visual symptoms are swollen root apices, is due to primarily to block in cell elongation and not in cell division, whereas over longer time periods both cell division and elongation are inhibited [4]. Compared with inorganic flocculants, synthetic polyelectrolytes are used in very low quantities to produce large shear-stable flocs. Because of the overall negative charge of natural colloids, cationic polyelectrolytes are of particular interest for application as flocculants. The normally used anionic and non-ionic polymers are of low toxicity generally, but cationic types such as polyacrylamides are more toxic, especially to aquatic organisms [11-16]. Concerns about contaminants have led Japan and Switzerland not to permit the use of polyelectrolytes in drinking water treatment, while Germany and France have set stringent limits [11]. In Germany, sludges treated with polyacrylamides will be excluded from disposal on areas under cultivation by the end of 2013 [17].

As a result, there has been increasing interest recently in developing new biobased, biodegradable flocculants such as modified starches [17-20], celluloses [21], chitosans [22-26] and bacterial polysaccharides [27-32]. Bioflocculants are environmentally safe, and the sludges treated with bioflocculants can be reused on agricultural land. Moreover, the flocculated sludge from meat, poultry and fish processing industries by using safe bioflocculants has a potential for application to animal and fish feed.

We have reported that hen egg white albumin and cow’s milk casein esterified with methyl and ethyl alcohols are effective flocculants for diatomite in freshwater and seawater [33-35]. Native albumin and casein were ineffective as flocculants for diatomite at pH > 4.5 because they had the same negative charge as diatomite particles in that pH range. However, the proteins esterified with methyl and ethyl alcohols had positive charge in the wide pH range and could be effective flocculants for diatomite from pH 3 to 9.

In this work, another protein-based flocculant, methylated soy protein (MeSP), was prepared since soy protein can easily be obtained as a by-product from the soybean oil industry. MeSP was applied to the flocculation of diatomite in freshwater and seawater. Our main purpose is to apply MeSP to the treatment of turbid water generated from construction activities. The continental crust comprises about 60 wt% of SiO_2, therefore it is considered that the treatment of silica-rich soil suspension is important for practical use. Thus, we chose diatomite which comprises about 90 wt% of SiO_2 as a model suspension. Based on the results of a jar test method and a sedimentation balance method, the flocculation performance of MeSP was compared to that of widely and generally used flocculant, polyaluminum chloride (PAC). Then, we applied MeSP to the flocculation of a real wastewater generated from an andesite quarry.

2. Experimental

2.1. Materials

Protein powder (soy bean), methyl alcohol, ammonia solution, HCl solution, $NaHNO_3$ and NaOH were purchased from Wako Pure Chemical Industries, JPN. Protein powder was of practical grade and other chemicals were of reagent grade. Polyaluminum chloride (PAC) solution (10.2 ± 0.2 wt% as Al_2O_3) was purchased from Taki Chemical Co., JPN. They were used with no further purification. Seawater was collected in Funka Bay (JPN) and it was filtered with a 0.45-μm membrane filter to remove suspended substances.

Diatomite (diatomaceous earth, Kanto Chemical Co. Inc., JPN) 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. The diatomite was screened by an 80-mesh (0.177-mm) sieve and stored in a desiccator. According to the data supplied by the company, the diatomite is composed of about 90% SiO_2 and the density measured by a pycnometer was 2.32 kg/L. The size distribution of diatomite particles was measured using a laser diffraction particle size distribution analyzer (LA-300, Horiba Ltd., JPN). The median diameter and mode diameter were 9.2 and 14.2 μm, respectively.

A real wastewater discharged from an andesite quarry was obtained from Okamoto Kogyo K. K., Sapporo, JPN. The wastewater was centrifuged at 3000 rpm for 10 min and the supernatant solution and sediment were stored separately to prepare a desired concentration of suspension. The supernatant solution was stored in a refrigerator at 2 °C. The sediment was dried at 80 °C and stored in a desiccator. The density, median diameter and mode diameter of the dried sediment were 2.46 kg/L, 8.7 μm and 8.2 μm, respectively. Hereafter, the dried sediment is called as andesite.

2.2. Methyated soy protein

Soy protein (SP) was methylated according to the method reported by Fraenkel-Conrat and Olcott [36]. SP powder (20 g) was dissolved in 0.001 M NaOH solution (1 L) and then precipitated by the addition of 0.1 M HCl solution at about pH 4.5. The precipitated SP was separated by centrifugation (3000 rpm, 10 min) and washed twice with methyl alcohol. It was suspended in 1 L of methyl alcohol containing HCl (0.05 M). After 24 h stirring at room temperature, the suspension was neutralized to about pH 5.5 with ammonia solution. The methylated SP (MeSP) was separated by centrifugation (3000 rpm, 10 min) and air-dried at room temperature with occasional grinding for 1 day. The dried MeSP was pulverized in a high-speed mixer and stored in a desiccator. SP and MeSP were dissolved in distilled water under ultrasonication (20 kHz, 30 W, 2 min) before use.

The degree of esterification was determined from the change in the number of carboxylic groups before and after esterification. The number of carboxylic groups was determined by a potentiometric titration [33]. The degree of esterification of MeSP in this study was 78 %.

2.3. Jar test method

All the flocculation experiments were conducted at a constant room temperature of around 22 ºC and at a constant solid concentration of 3.0 g/L. Diatomite (0.6 g) was suspended in 40 mL of distilled water or seawater in a 300 mL flask. In the case of andesite, 0.6 g of andesite was dispersed in 40 mL of the supernatant solution in a 100 mL flask under ultrasonication (20 kHz, 30 W, 2 min) and then the suspension was transferred into a 300 mL flask. The suspensions were boiled under reduced pressure at room temperature for 1 min. Distilled water or seawater (150 mL) was added and the suspension was stirred using a 4.0 cm long magnetic stir bar at 600 rpm. The pH of the suspension was adjusted to the desired value with a small amount of HCl, NaOH or $NaHCO_3$ solution (0.1

M) and then distilled water or seawater was added to bring the total volume to 197 mL. After 30 min stirring, 3 mL of the required concentration of SP, MeSP or PAC solution was added to the suspension and stirred for 3 min at 400 rpm. Then the prepared suspension was transferred to a cylindrical tube (3.8 cm in diameter, 25 cm in height) and the flocs were then allowed to settle for 1 min without stirring. After the settling period, a 0.5 mL sample was taken from a height of 10 cm below the surface for measurement of absorbance at 700 nm. The sample was 5-fold diluted with 0.001 M NaOH solution to redisperse the flocculated diatomite particles and the absorbance was measured using a spectrophotometer (U-2900, Hitachi High-Technologies Co., JPN). The pH of the suspension was measured using a pH meter (Model 520-A, Orion Research, USA) after the settling period. The relative absorbance, A/A_0, was used as an index of flocculation performance. A and A_0 are the absorbance of diatomite suspension after 1 min settling in the presence and absence of flocculant, respectively.

2.4. Sedimentation balance method

Diatomite and andesite suspensions were prepared as the same manner as for the jar test method. The suspension was stirred for 3 min at 400 rpm and then it was transferred to a cylindrical tube in which a sediment trap (2.6 cm in diameter, 3 cm in height) was hung from an electronic micro balance (PB303-S, Mettler Toledo International Inc., USA). The sediment trap was set a height of 10 cm below the surface. The change of the weight of settled solid on the trap was recorded with settling time until it reached a constant weight. After then, the pH of the suspension was measured.

The initial settling velocity of flocs v was determined from the slope of the linear portion of cumulative sedimentation curve by using the following equation.

$$v = L\frac{d(W_t / W_f)}{dt}$$

where W_t and W_f represent the cumulative weight of diatomite settled in the trap at time t and the weight in the final state, respectively. L is the sedimentation distance between the bottom of the trap and the suspension surface (L = 10 cm).

3. Results and discussions

3.1. Flocculation of diatomite particles in freshwater

Figs. 1 and **2** show the extent of flocculation of diatomite suspension in freshwater as a function of dosage of MeSP, SP and PAC at a fixed pH of 7 ± 0.2. Generally, the settling time in jar test is set to a few to tens of minutes. In this study, we set the settling time to 1 min because the floc formed by MeSP had a rather high settling velocity. The two methods used to characterize the flocculation behavior of diatomite, jar test method (**Fig. 1**) and sedimentation balance method (**Fig. 2**), gave similar results; the maximum settling velocity were found to correspond to minimum relative absorbance. As shown in **Figs. 1** and **2**, MeSP could efficiently flocculated diatomite suspension at dosages of 30 to 200 ppm (1 to 7 wt% of diatomite). In the case of SP, flocculation was observed at dosages of 60 to 200 ppm, however, the flocculation efficiency was lower than that of MeSP. Obviously, the flocculation efficiency of PAC was much lower than that of

MeSP. The settling velocity of diatomite-MeSP flocs was almost constant (4 mm/s) over a wide dosage range (30-200 ppm), while that of diatomite-PAC flocs was 3 mm/s at dosages of 30-90 ppm (**Fig. 2**). However, as shown in **Fig. 1**, the clarity of supernatant treated with MeSP was much higher than that treated with PAC.

Figs. 3 and **4** show the flocculation behavior of diatomite suspension in freshwater with MeSP, SP and PAC as a function of pH at a constant dosage of 60 ppm. MeSP exhibited high flocculation performance over a wide pH range, however, the flocculation efficiency decreased at pH < 3 and pH >10. As the point of zero charge of diatomite is between 2 and 3 [37-40], the negative charge density of diatomite surface is almost constant at pH > 4 and decreases with decreasing pH at pH < 3. On the other hand, it is well-known that the number of positively charged amino groups of proteins is almost constant at pH < 8 and decreases with increasing pH at pH > 9. Therefore, the decrease in flocculation power of MeSP at pH < 3 and pH > 10 can be attributed to the decrease in the electrostatic interaction between diatomite surface and MeSP. Unmethylated soy protein (SP) was effective as flocculant for diatomite at a pH around its isoelectric point of pH 4.5-5.5 [41, 42].

In the case of PAC, it was effective at pH 5-6, however, the flocculation efficiency dramatically decreased at pH < 5. Since the point of zero charge of amorphous $Al(OH)_3$ is 8-9 [43-46], the positive charge density of $Al(OH)_3$ increases with decreasing pH at pH < 9. On the other hand, amorphous $Al(OH)_3$ which has a solubility product constant of $pK_{SP} = 30.5-31.5$ [46-48] begins to dissolve in water at pH < 5 [49]. Therefore, the flocculation efficiency of PAC increased with decreasing pH at pH 5-9, and then dramatically decreased at pH < 5.

3.2. Flocculation of diatomite particles in seawater

In the construction activities in coastal and estuarine area, the treatment of muddy salt water is required. **Fig. 5** shows the flocculation behavior of diatomite in seawater (pH 8.1 ± 0.1) as a function of MeSP and PAC dosage. MeSP showed a good flocculation performance for diatomite suspension in seawater at dosages of 6-50 ppm. The settling velocity of diatomite floc formed by MeSP in seawater was slightly lower than that in freshwater at pH 8 (**Fig. 2**). On the other hand, PAC was found to be much less effective than MeSP in seawater.

3.3. Flocculation of wastewater from andesite quarry

Fig. 6 shows the results of the flocculation experiments using a real wastewater from andesite quarry as a function of MeSP dosage. The pH of the suspension was about pH 7. The solid concentration of the suspension was adjusted to 3 g/L with the supernatant solution of the wastewater to obtain the same solution condition as the real wastewater. The wastewater could be sufficiently clarified by the addition of 40 ppm of MeSP. The flocculation performance was almost the same or higher than that of diatomite-MeSP system at about pH 7 (Fig. 2).

4. Conclusions

A novel bioflocculant, methylated soy protein (MeSP), has been developed and its flocculation performance was tested with diatomite suspensions in freshwater and seawater. The flocculation performance of

MeSP was compared with a widely used commercial flocculant, polyaluminum chloride (PAC) in terms of supernatant clarity and floc settling velocity. MeSP showed a much higher flocculation performance than PAC both in freshwater and seawater. MeSP was effective over a wide range of pH from 3 to 10 in freshwater, while PAC was effective only at around pH 5.

The settling velocity of the floc formed by MeSP was about 4 mm/s in freshwater (pH 7) and in seawater (pH 8). The settling velocity of kaolin flocs formed by polyacrylamide in a suspension of 50 g/L [50] and formed by $Al(OH)_3$-polyacrylamide ionic hybrid in a suspension of 2.5 g/L [51] were about 1 and 0.2 mm/s, respectively. Compared with these values, it is obvious that the diatomite floc formed by MeSP had a very high settling velocity.

The relative absorbance could be reduced to less than 0.1 within 1 min by the addition of 60 ppm (in freshwater) and 20 ppm (in seawater) of MeSP. At the settling time of 3 min, the relative absorbance was reduced to less than 0.05 by the addition of 20 ppm (0.7 % of suspended solid) of MeSP both in freshwater and seawater (data were not shown). Divakaran and Pillai reported that the relative turbidity of river silt suspension [23] and kaolinite suspension [24] could be reduced to less than 0.1 within 30 min by the addition of chitosan (1 % of suspended solids). Krentz *et al*. [17] investigated the flocculation efficiency of cationic derivatives of potato, wheat and maize starch for kaolin suspension (1 g/L). The flocculation efficiency of cationic potato starch was almost comparable to polyacrylamide. The residual turbidity was reduced to 1-2 % within 20 min by the addition of cationic potato starch (0.5 % of suspended solid). Shogren [19] used waxy maize starch phosphates as flocculants for kaolin suspension (10 g/L). In the presence of small amounts of Ca^{2+} (1-4 mM), almost all kaolin was settled within 2 min by the addition of 4 ppm of starch phosphate. It is difficult to compare our results directly with above studies as there were differences in type of solid, concentration of suspended solid and settling time. However, at least, MeSP showed a much higher flocculation performance than that of PAC.

Finally, we applied MeSP to the flocculation of a real wastewater generated from an andesite quarry (3 g/L). The wastewater could be sufficiently clarified by the addition of 40 ppm of MeSP. The relative absorbance could be reduced to less than 0.05 within 1 min, and the floc settling velocity was as high as 5 mm/s.

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Figure captions

Fig. 1. Clarification efficiencies of methylated soybean protein (MeSP), soybean protein (SP) and polyaluminum chloride (PAC) as a function of dosage. Diatomite concentration, 3.0 g/L; pH 7; settling time, 1 min; sampling depth, 0.1 m. Lines are drawn as a guide for the eye.

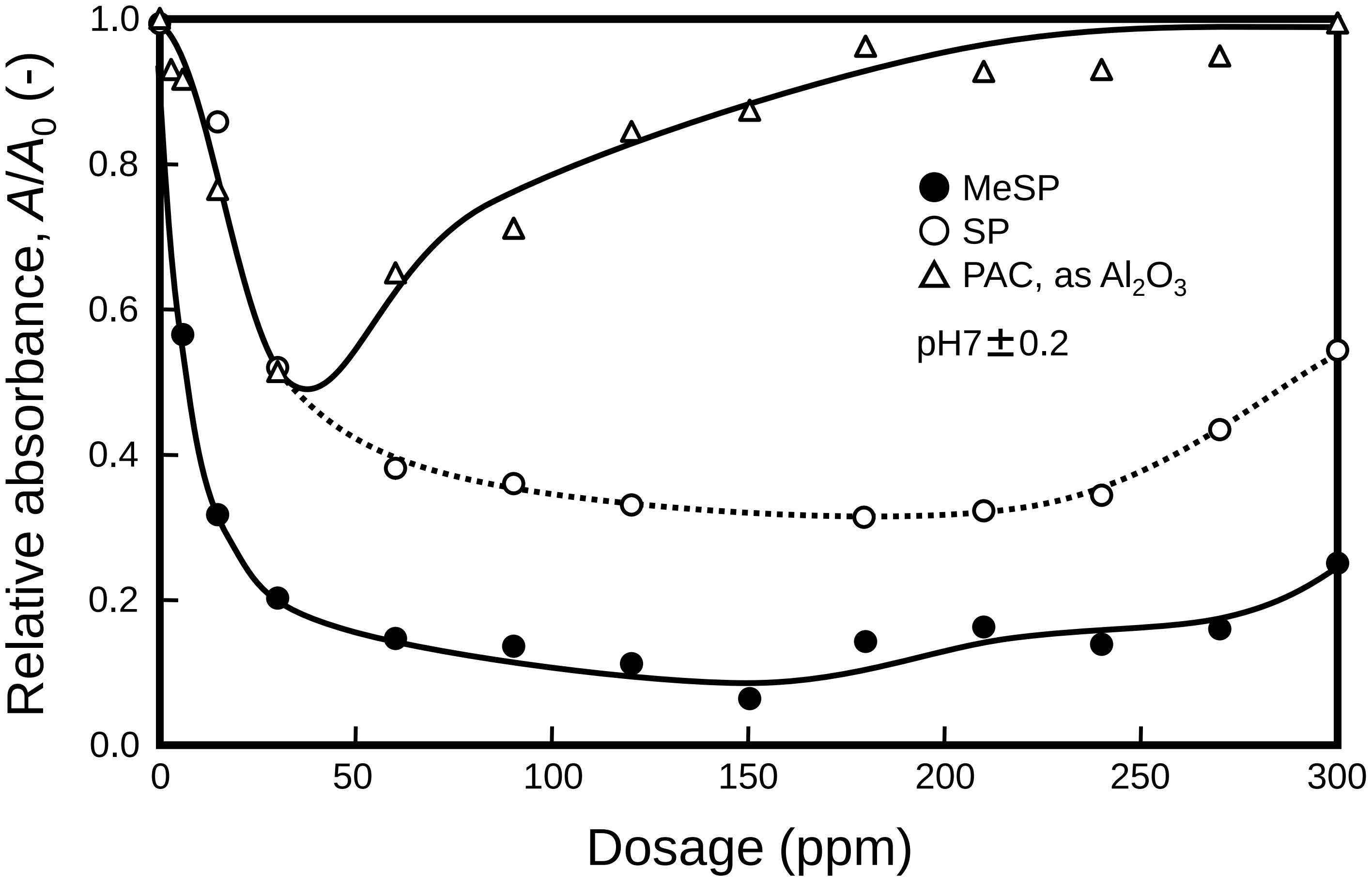
Fig. 2. Settling velocity of flocs formed by MeSP, SP and PAC as a function of dosage. Diatomite concentration, 3.0 g/L; pH 7; sampling depth, 0.1 m. Lines are drawn as a guide for the eye.

Fig. 3. Clarification efficiencies of MeSP, SP and PAC as a function of pH. Diatomite concentration, 3.0 g/L; flocculant dosage, 60 ppm; settling time, 1 min; sampling depth, 0.1 m. Lines are drawn as a guide for the eye.

Fig. 4. Settling velocity of flocs formed by MeSP, SP and PAC as a function of pH. Diatomite concentration, 3.0 g/L; flocculant dosage, 60 ppm; sampling depth, 0.1 m. Lines are drawn as a guide for the eye.

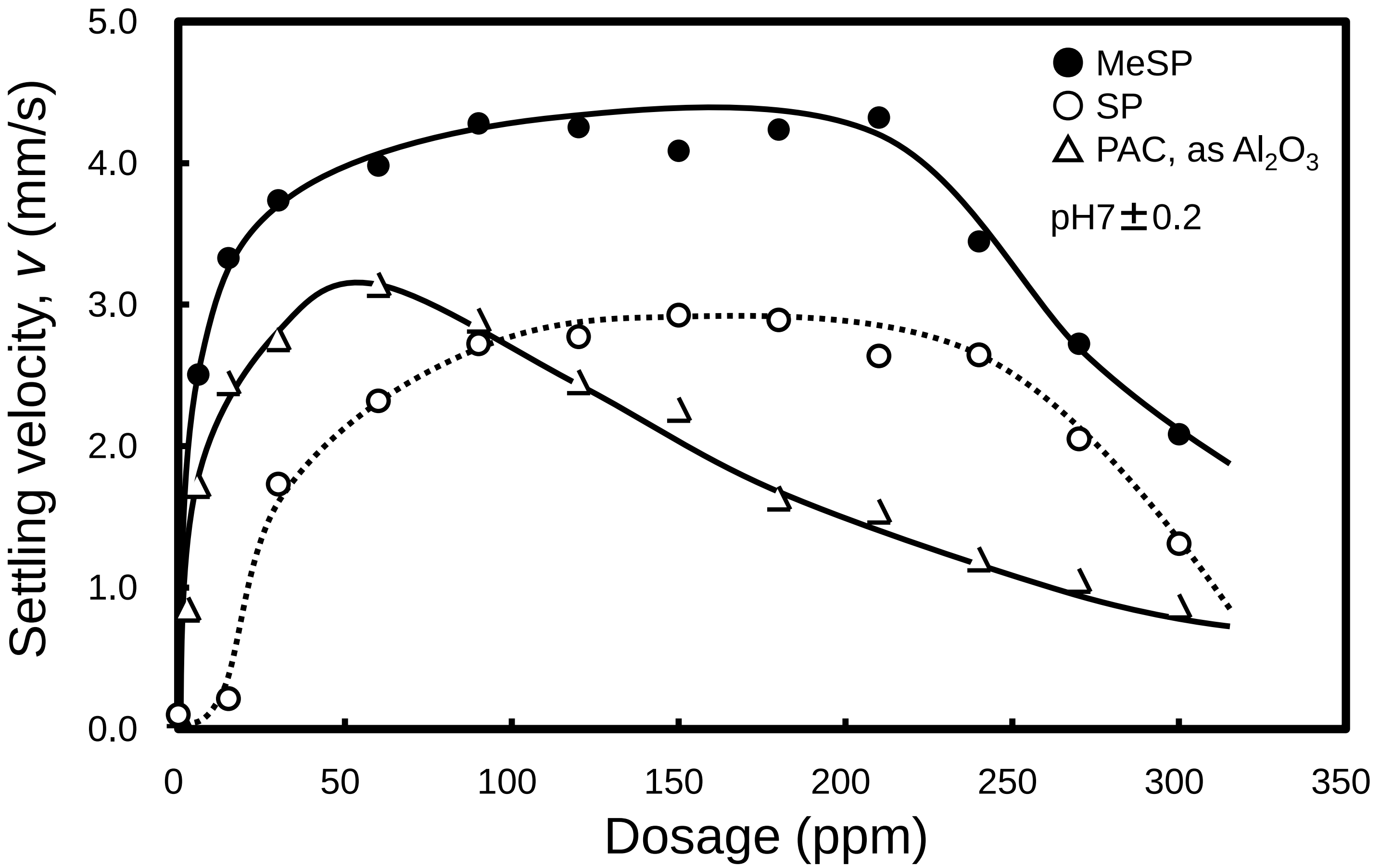
Fig. 5. Clarification efficiencies of MeSP and PAC (open symbols), and settling velocity of flocs formed by MeSP and PAC (solid symbols) in seawater as a function of dosage. Diatomite concentration, 3.0 g/L; pH 8.1; sampling depth, 0.1 m; settling time, 1 min (jar test). Lines are drawn as a guide for the eye.

Fig. 6. Clarification efficiency of MeSP and settling velocity of floc formed by MeSP in a real wastewater from andesite quarry as a function of dosage. Andesite concentration, 3.0 g/L; pH 7; sampling depth, 0.1 m; settling time, 1 min (jar test); Lines are drawn as a guide for the eye.



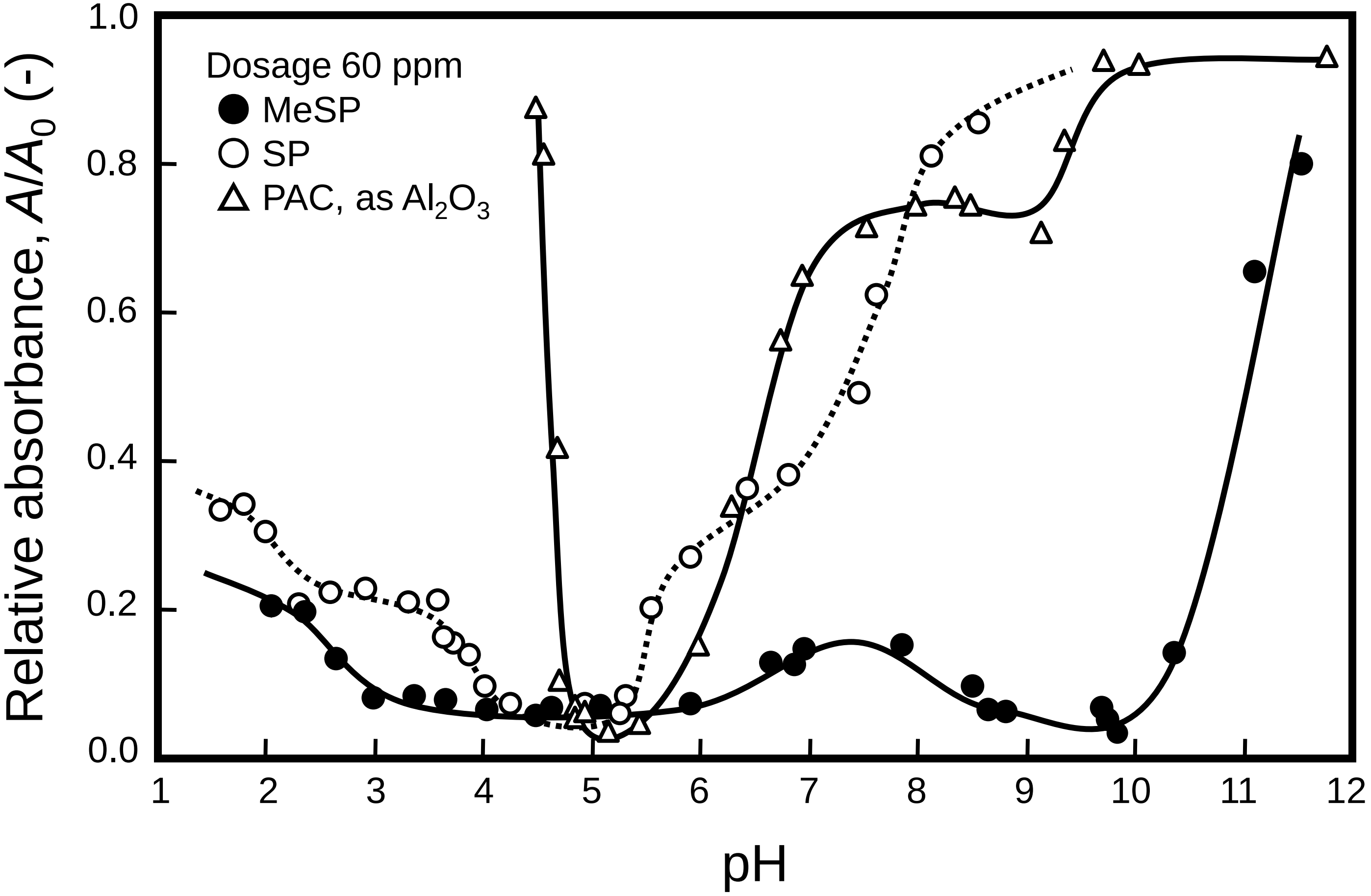
1.0
0.8
0.6
0.4
0.2
0.0
Relative absorbance, A/A_0 (-)
0
50
100
150
200
250
300
Dosage (ppm)
● MeSP
○ SP
△ PAC, as Al_2O_3
pH7 ± 0.2

Fig. 1.



Settling velocity, v (mm/s)
Dosage (ppm)
5.0
4.0
3.0
2.0
1.0
0.0
0
50
100
150
200
250
300
350
● MeSP
○ SP
△ PAC, as Al_2O_3
pH7 ± 0.2

Fig. 2.



Dosage 60 ppm
● MeSP
○ SP
△ PAC, as Al_2O_3
Relative absorbance, A/A_0 (-)
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
5
6
7
8
9
10
11
12
pH

Fig. 3.

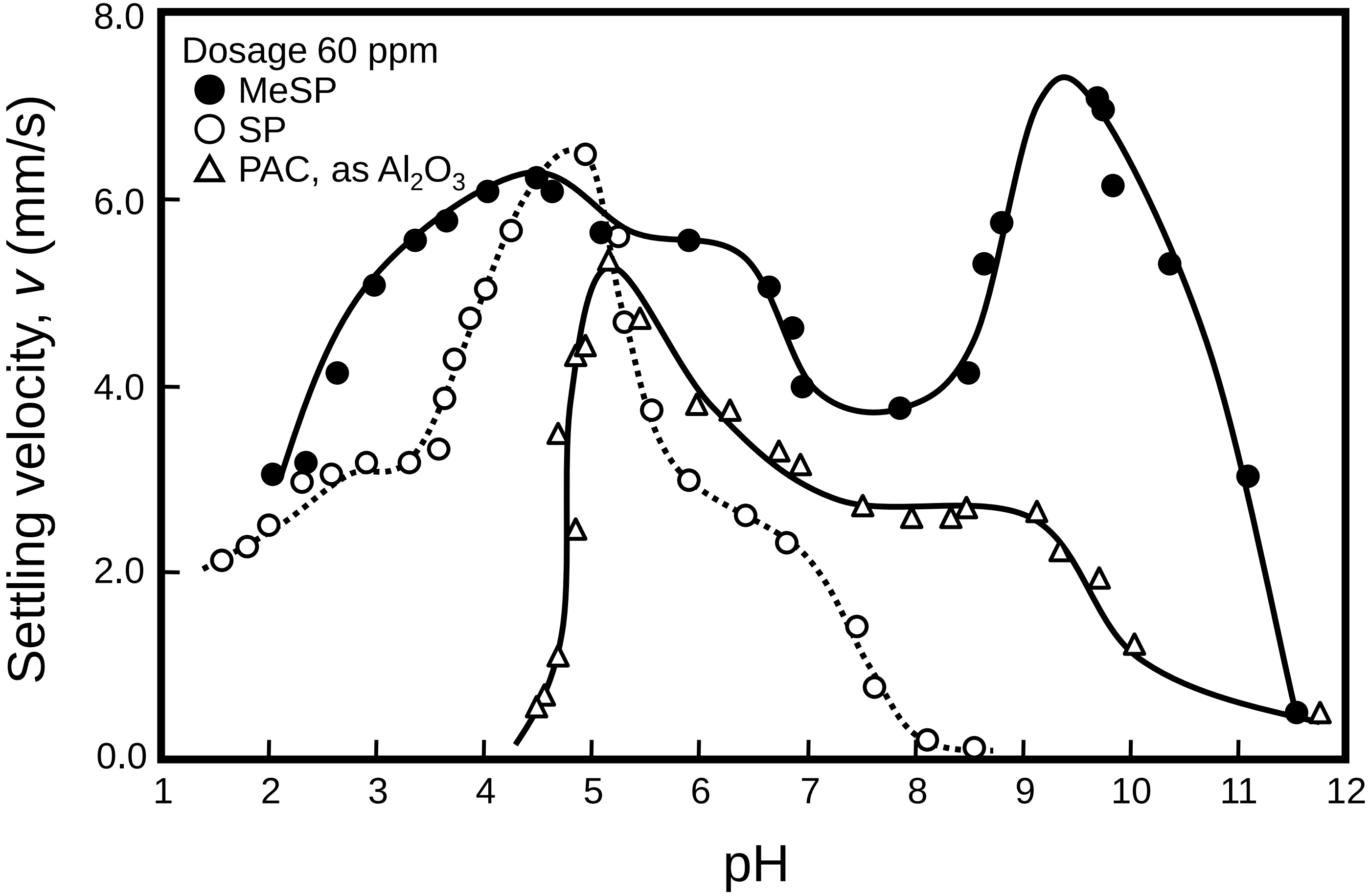

Dosage 60 ppm
● MeSP
○ SP
△ PAC, as Al_2O_3
Settling velocity, *v* (mm/s)
8.0
6.0
4.0
2.0
0.0
1
2
3
4
5
6
7
8
9
10
11
12
pH

Fig. 4.

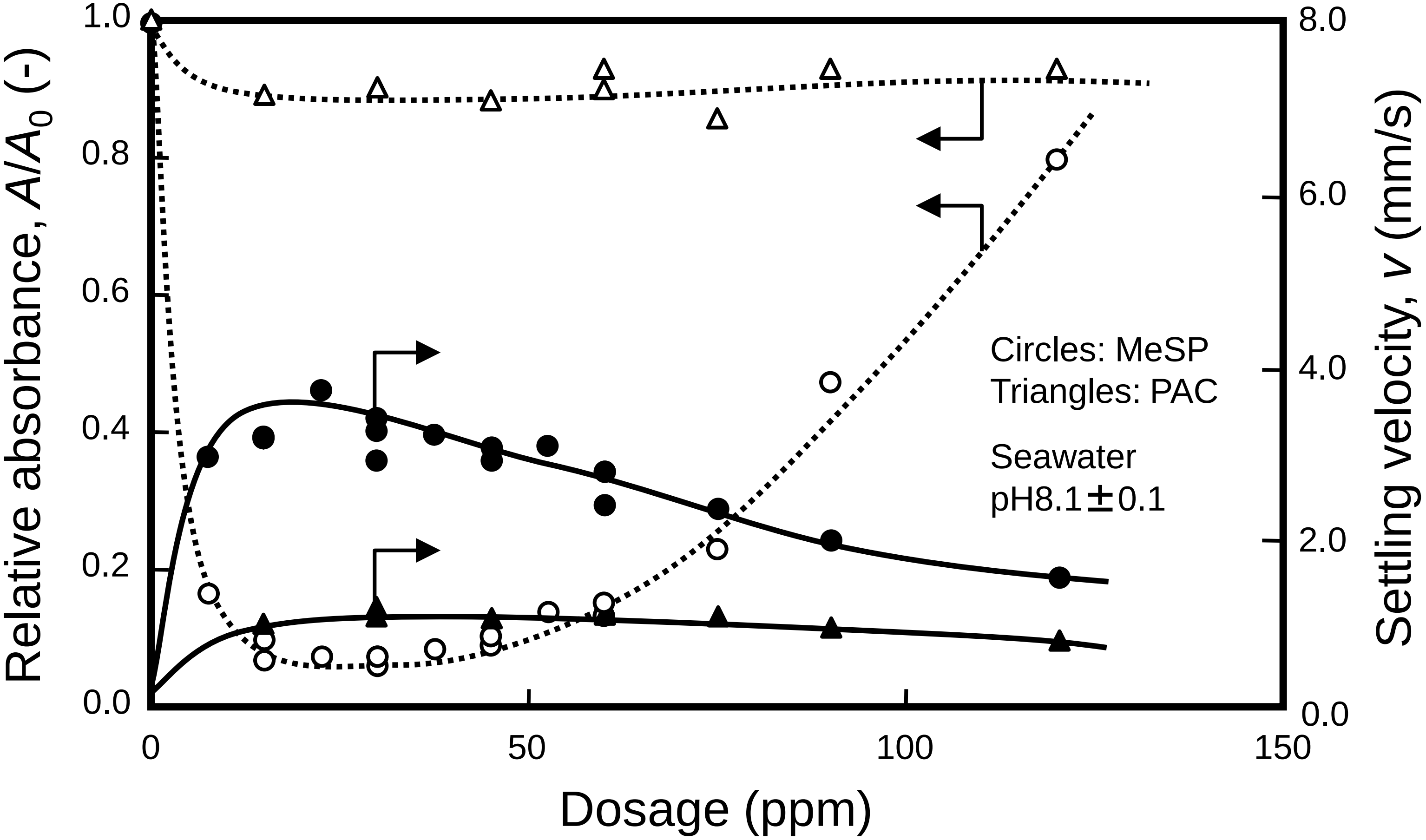
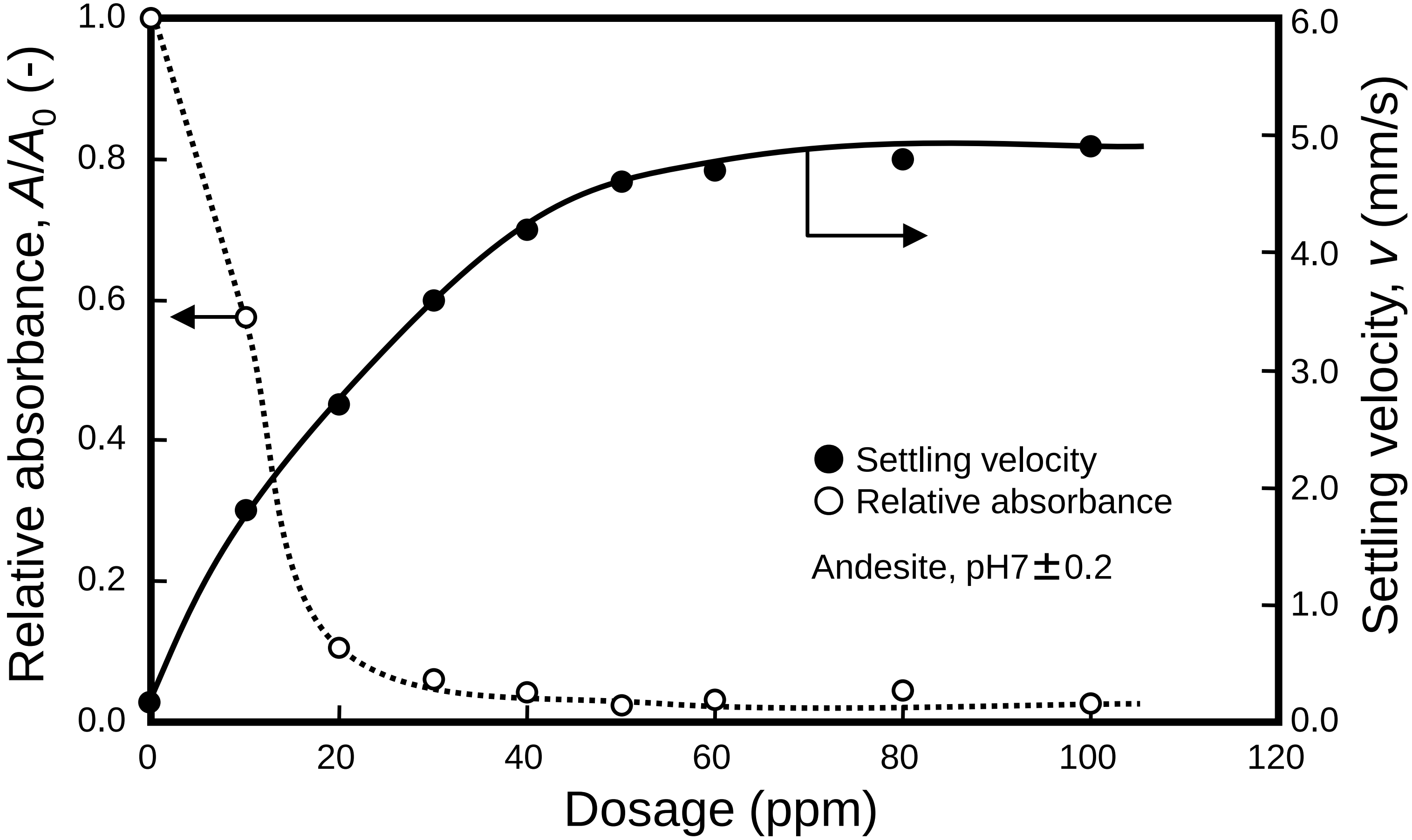

1.0
0.8
0.6
0.4
0.2
0.0
Relative absorbance, A/A0 (-)
8.0
6.0
4.0
2.0
0.0
Settling velocity, v (mm/s)
0
50
100
150
Dosage (ppm)
Circles: MeSP
Triangles: PAC
Seawater
pH8.1±0.1

Fig. 5.



1.0
0.8
0.6
0.4
0.2
0.0
Relative absorbance, A/A0 (-)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Settling velocity, v (mm/s)
0
20
40
60
80
100
120
Dosage (ppm)
● Settling velocity
○ Relative absorbance
Andesite, pH7±0.2

Fig. 6.