Flocculation of kaolin and kanto loam by methylated soy protein

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ABSTRACT

In this study, methylated soy protein (MeSP) was applied to the solid-liquid separation or flocculation of kaolin and kanto loam (deposits of volcanic ash) suspensions (3.0 g/L). The flocculation performance of MeSP was evaluated in terms of supernatant clarity (jar test method) and floc settling rate (sedimentation balance method), and compared with those of chitosan and polyaluminum chloride (PAC). At pH 7, the optimum dosage of MeSP, chitosan and PAC were almost the same (about 1.5 wt.% of the suspended solids), but there was a remarkable difference in the floc settling rate. At the optimum dosage, the settling rate of the floc formed by MeSP was 6-7 mm/s, while those formed by chitosan and PAC were about 2 mm/s. MeSP showed the highest flocculation performance at pH 3-7 among these flocculants. At pH 8-12, the flocculation performance of MeSP was much higher than that of PAC, but lower than that of chitosan. The flocculation performance of MeSP for kanto loam suspension was strongly affected by the addition of seawater, while the effect of seawater concentration on the flocculation performance of MeSP for kaolin was moderate.

1. Introduction

Flocculation is an easy and effective method to improve solid–liquid separation in wastewater treatment, tap water production, dredging/downstream processes and other industrial fields. Various chemical flocculating agents or flocculants have been developed, but the harmfulness has also been pointed out by many researchers. Synthetic high-molecular-weight polymers, such as polyacrylamide, are proved to be toxic especially to aquatic organisms [1–6], while others, such as aluminum compounds are known to inhibit the elongation of plant root [7-9]. Therefore, effective and eco-friendly bioflocculants that are easily biodegraded and produce no secondary pollution have been a topic of intense research and gain much wider attention in recent years [10-23]. However, for practical applications, high production costs have been a major problem in bioflocculant production by using microorganisms due to the relatively expensive conventional substrates such as glucose, fructose, sucrose and so on [10-12, 14-16, 21].

Based on the strong demand for safe, eco-friendly and low cost bioflocculants, we have investigated the use of general proteins, such as

hen egg white albumin, cow's milk casein and soy protein as raw materials for bioflocculant. We have reported that the bioflocculants prepared by methylation of the proteins exhibited much higher flocculation performance for diatomite suspension than one of the most commonly used flocculant, polyaluminum chloride (PAC) [17-20]. These proteins could not work as flocculants for diatomite at pH > 5 because they had the same negative charge as diatomite at pH above their isoelectric points (pH > 5). The negative charge of protein is mainly attributed to the dissociated carboxyl groups, so we esterified the proteins with methyl alcohol in order to nonionize the carboxyl groups. As a result, the methylated proteins worked as effective flocculants for diatomite at pH 3-9. Our main purpose is to apply the methylated proteins to the treatment of turbid water generated from construction activities. For this purpose, the treatment of silica-rich soil suspension is important because the continental crust comprises about 60 wt.% of SiO_2. Therefore, in our previous studies, we used diatomite which contains about 90 wt.% of SiO_2 as a model suspension.

In this study, we examined the applicability of methylated soy

protein (MeSP) to the flocculation of suspended solids containing metal oxides other than SiO_2. For this purpose, we chose kaolin which contains 40 wt.% of Al_2O_3 and kanto loam (deposits of volcanic ash in Kanto area in Japan) which contains 30 wt.% of Al_2O_3 and 20 wt.% of Fe_2O_3 as model suspensions. We chose methylated soy protein as a flocculant because we consider a residue of soybean-oil production is a potential raw material for soy protein-based bioflocculant. The flocculation ability of MeSP for kaolin and kanto loam suspensions were compared with those of chitosan and PAC in terms of supernatant clarity (jar test method) and floc settling rate (sedimentation balance method) in this study.

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, Japan. 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., Japan.

Chitosan which has an average molecular weight of about 1000 kDa was purchased from Hokkaido Soda, Japan. The chitosan powder was dissolved in the same weight of acetic acid and diluted to 5.0 g/L with distilled water. They were used without further purification. Seawater was collected in Funka Bay (Japan) and it was filtered with a 0.45μm membrane filter to remove suspended substances.

Kaolin and kanto loam (deposits of volcanic ash in Kanto area of Japan) were used in the evaluation of flocculation performance of the flocculants. Kaolin was purchased from Wako Pure Chemical Industries, Japan. Kaolin has been used in many studies on flocculation thus the flocculation performance of our protein-based flocculant can be compared with those of flocculants used in other studies [13, 22, 24, 25]. According to the data supplied by the company, the major components of kaolin are 45 % SiO_2 and 40 % Al_2O_3. The size distribution of kaolin particles was measured using a laser diffraction particle size distribution analyzer (LA-300, Horiba Ltd., Japan). The median diameter and mode diameter were 4.9 and 4.8 μm, respectively. The density measured by a pycnometer was 2.61 kg/L.

Kanto loam (dust for industrial testing No. 11) was purchased from Association of Powder Industry and Engineering, Japan. It was selected because of the difference in the chemical composition from kaolin. The major components of kanto loam supplied by the company are 40 % SiO_2, 30 % Al_2O_3 and 20% Fe_2O_3. It has a median diameter of 2.7 μm, mode diameter of 2.8 μm and a density of 2.96 kg/L.

2.2. Preparation of bioflocculant

Soy protein was methylated according to the method reported by Fraenkel-Conrat and Olcott [26]. A 20 g soy protein powder was dissolved in 1.0 L of 0.001M NaOH and then precipitated by the addition of 0.1M HCl solution at about pH 4.5. It was separated from the liquid phase by filtration and washed twice with methyl alcohol. It was suspended in 1.0 L of methyl alcohol containing HCl (0.1 M) and stirred for 24 h at room temperature. After neutralization with ammonia solution, the methylated soy protein (MeSP) was separated by centrifugation at 3000 rpm for 10min 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.

MeSP were dissolved in 5% ethanol solution under ultrasonication (20 kHz, 30 W, 2min) before use.

2.3. Jar test method

The flocculation performance of the flocculants in terms of the clarity of the supernatant was evaluated by a jar test method. A 0.6 g of kaolin or kanto loam was suspended in 200 ml of distilled water and the pH of the suspension was adjusted to the desired value with a small volume of 0.1 M HCl, NaOH or $NaHNO_3$ solution. A certain amount flocculant solution was added to the suspension, and it was stirred rapidly at 450 rpm for 3 min, followed by slow stirring at 150 rpm for 1 min. After the settling for 1 min, a 3 ml of suspension was withdrawn at 10 cm below the surface. The absorbance of the sample T was measured at 700 nm by using a spectrophotometer (U-2900, Hitachi High-Technologies Co., Japan). The clarification efficiency was defined and calculated by the following equation.

$$\text{Clarification efficiency} = 1 - T/T_0 \qquad (1)$$

where T_0 represents the absorbance obtained from the control experiments. There were good correlations (correlation factor, $R^2 > 0.99$) between the weight concentration of suspensions and their absorbance at 700 nm. Therefore, the clarification efficiency indicates not only the clarity of the supernatant but the weight fraction of the flocs that have the settling rate higher than 1.67 mm/s (100 mm/60 s).

2.4. Sedimentation balance method

The flocculation performance of the flocculants in terms of the settling rate of the floc was evaluated by a sedimentation balance method. A certain amount of flocculant solution was added to a kaolin or kanto loam suspension (3.0 g/L, 200 ml) and stirred at 500 rpm for 3 min. The suspension was transferred to a cylindrical tube in which a sediment trap was hung from an electronic micro-balance at a depth of 15.0 cm below the surface. The weight of solid/floc settled on the trap at time t, W_t, was recorded with time until it reached to a constant weight, W_f. The measurement was started five seconds after the suspension was poured into

the cylindrical tube to reduce the measurement error caused by turbulence of the suspension.

3. Results and discussions

3.1. Electrostatic property of MeSP

Fig. 1 shows the self-aggregation behavior of soy protein and MeSP as a function of pH. It is well-known that proteins undergo self-aggregation at their isoelectric point. Thus, we measured the absorbance of soy protein and MeSP solutions (pH 3-11) as an index of the extent of self-aggregation. Soy protein underwent self-aggregation at pH < 6 and the absorbance reached to the maximum value at about pH 4. The pH at which the absorbance reached to the maximum value agreed with the isoelectric point of a soy protein reported by Chen and Soucie [27]. They reported that the electrophoretic mobility of a soy protein (obtained from Kraft, Inc., Glenview, Illinois) was $+2.2 \times 10^{-8}$ m/s·V·m at pH 3 and -3.4×10^{-8} m/s·V·m at pH 9, and the protein had an isoelectric point at around pH 4.5. On the other hand, the absorbance of MeSP solution increased at pH > 6 and reached to the maximum value at about pH 10. In the both cases, the

formation of aggregates could be observed visually. The results suggest that the isoelectric point of soy protein shifted from about pH 4 to pH 10 by the methylation or nonionization of carboxyl groups.

3.2. Flocculation dynamics and reversibility of flocs

Fig. 2 shows the photographs of jar test of kaolin-MeSP and kaolin-chitosan systems. The concentration and pH of the suspension were 3.0 g/L and about pH 7, respectively. The dosage of flocculant was 1.0 wt.% of kaolin. In the case of kaolin-MeSP system, the formation of large floc was observed and high clarity of the supernatant was obtained within 30 seconds. On the other hand, in the case of kaolin-chitosan system, the size of floc was so small that the clarity of supernatant was very low.

Fig. 3 shows the results of sedimentation balance measurement of kaolin, kanto loam and diatomite at the same MeSP dosage and pH. The concentration and pH of the suspension were 3.0 g/L and about pH 7, respectively. The dosage of MeSP was 1.0 wt% of suspended solids. The ordinate, $1 - W_t/W_f$, represents the weight ratio of suspended solid remaining above the sediment trap. There was no significant difference in

the flocculation dynamics among kaolin, kanto loam and diatomite. The value of $1 - W_t/W_f$ decreased lineally from the beginning of the measurement (5 seconds after the suspension was poured into a cylindrical tube for the measurement). It indicates that the process of floc formation was rapid and the formed flocs had rather uniform in size.

In order to examine the reversibility of flocs formed by MeSP, the sedimentation balance measurement was repeated four times using the same suspension. After the first measurement (circles), the suspension was transferred into an Erlenmeyer flask and stirred again at 500 rpm for 3 min using a magnetic stirrer, and then the suspension was used for the second measurement (triangles). In the same manner, the third (squares) and the fourth (rhombuses) measurements were carried out. Figs. 4 and 5 show the results of kaolin-MeSP (Fig. 4) and kanto loam-MeSP (Fig. 5) systems at about pH 7. The dosages of MeSP were 0.2 wt.% (solid gray symbols), 0.5 wt.% (solid black symbols) and 1.0 wt.% (open symbols) of suspended solids. The results show that the flocs formed by MeSP have high reversibility.

3.3. Determination of settling rate of flocs

According to the conventional and well-established sedimentation analysis (cumulative method), the value of W_t/W_f can be expressed by the sum of two terms as

$$\frac{W_t}{W_f} = \int_{D}^{D\max} f(D)dD + \frac{vt}{L}\int_{D\min}^{D} f(D)dD \qquad (2)$$

where $f(D)$ is the size–frequency distribution function of particles, D is the diameter of particles, and L is the sedimentation distance between the bottom of the sediment trap and the suspension surface. The first term on the right-hand side of Eq. (2) denotes the weight fraction of settled particles having the settling rate $v > L/t$, and the second term denotes the weight fraction of settled particles having the settling rate $v < L/t$. The differentiation of the above equation with respect to time t gives

$$\frac{d(W_t/W_f)}{dt} = \frac{v}{L}\int_{D\min}^{D} f(D)dD = \frac{v}{L}(1-R) \qquad (3)$$

R denotes the cumulative oversize fraction of suspended particles or the weight fraction of particles having settling rate $v > L/t$. Combining Eqs. (2) and (3), we obtain the equation

$$\frac{W_t}{W_f} = \frac{d(Wt/Wf)}{dt} t + R \tag{4}$$

Eq. (4) indicates that the value of R at time t can be obtained from the intercept of tangential line of the sedimentation curve. In the linear part of the sedimentation curve, the value of R should be zero and the following equation is obtained by substituting $R = 0$ to Eq. (3).

$$v = L \frac{d(W_t / W_f)}{dt} \tag{5}$$

Thus the settling rate of flocs v in the linear part of the sedimentation curve can be determined from the slope of the linear part of sedimentation curve and the sedimentation distance (L = 150 mm) using Eq. (5).

3.4. Effect of flocculant dosage on flocculation efficiency

Fig. 6 shows the comparison of the flocculation performance of MeSP (triangles), chitosan (rhombuses) and PAC (circles) for kaolin suspension as a function of flocculant dosage. The dosage was expressed as the percentage of the flocculants relative to kaolin on a dry weight basis. The concentration and pH of the suspension were 3.0 g/L and about pH 7, respectively. The solid and open symbols represent the floc settling rate and the clarification efficiency, respectively.

The results showed that the flocculation performance of MeSP was obviously higher than those of chitosan and PAC. The clarification efficiency reached almost 1.0 at the MeSP dosage of 1.0-4.0 wt.%. The clarification efficiency with chitosan reached almost 1.0 at the dosage of 1.0 wt.%, but it decreased to 0.6 with the increase of dosage. In the case of PAC, the clarification efficiency did not reach 1.0 and decreased to 0.4 with the increase of dosage. It should be noted that there was a significant difference among the settling rates of the flocs formed by MeSP, chitosan and PAC. The settling rate of the floc formed by MeSP was about three times higher than those formed by chitosan and PAC. Fig. 7 shows the

comparison of the flocculation performance of MeSP, chitosan and PAC for kanto loam suspension as a function of flocculant dosage. The concentration and pH of the suspension were 3.0 g/L and about pH 7, respectively. The meanings of the symbols are the same as those in Fig. 6. There was no significant difference between the results shown in Figs. 6 and 7. In this case also the settling rate of the floc formed by MeSP was about three times higher than those formed by chitosan and PAC.

3.5. Effect of pH on flocculation efficiency

Figs. 8 and 9 show the effect of pH on the flocculation performance of MeSP (triangles), chitosan (rhombuses) and PAC (circles) at a fixed flocculant dosage of 1.5 wt.% for kaolin (Fig. 8) and kanto loam (Fig. 9) suspensions, respectively. The concentration of the suspension was 3.0 g/L. The solid and open symbols represent the floc settling rate and the clarification efficiency, respectively.

MeSP showed high clarification efficiency of almost 1.0 at pH 3-10 for both kaolin and kanto loam suspensions but it decreased at pH > 10. The settling rate of kaolin-MeSP floc was about 8 mm/s at pH 3-6 and

decreased gradually to 2 mm/s with the increase of pH. In our previous study [20], MeSP showed a similar pH dependence of flocculation performance for diatomite suspension. The negative charge density of kaolin and diatomite surfaces are almost constant at pH > 4 because the point of zero charge of kaolin is pH 3-4 [28, 29] and that of diatomite is pH 2-3 [30, 31]. 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 the increase of pH at pH > 8. Therefore, the decrease in the settling rate of kaolin-MeSP floc at pH > 8 can be attributed to the decrease in the electrostatic interaction between kaolin surface and MeSP.

In the case of kanto loam suspension, the settling rate of floc formed by MeSP was about 5 mm/s at pH 5-7 and it decreased at pH > 7 and at pH < 5. Diatomite comprises about 90 % of SiO_2, and the major components of kaolin are 45 % SiO_2 and 40 % Al_2O_3. On the other hand, kanto loam is composed of 40 % SiO_2, 30 % Al_2O_3 and 20% Fe_2O_3. The point of zero charge of kanto loam should be higher than that of kaolin and diatomite, because the point of zero charge of SiO_2, Al_2O_3 and Fe_2O_3 are at pH 2-3, pH 8-10 and pH 6-8, respectively [32]. Thus, it can be considered

that the negative charge density of kanto loam surface decreased with the decrease of pH from 5 to 3, and the electrostatic interaction between kanto loam surface and MeSP decreased with the decrease of pH.

Chitosan showed high clarification efficiency at pH 7-12 for the suspensions of kaolin and kanto loam, but it declined sharply at pH 6-7. The settling rate of the flocs formed by chitosan at pH < 7 was much lower than those formed by MeSP. However, the settling rate increased sharply from 1.5 to 11 mm/s at pH 7-8 and surpassed the settling rate of the floc formed by MeSP. Although the data were not shown, we measured the absorbance of chitosan solution in the absence of solid particles at 270 nm as a function of pH. The absorbance increased sharply at pH 7-8 with the increase of pH. The result suggests that the self-aggregation of chitosan occurred at pH > 7 and the formed large aggregates enhanced the flocculation of suspended solids. In the case of PAC, the clarification efficiency increased sharply at pH 5-6, but it decreased gradually at pH 6-9 and sharply at pH 9-10. The settling rate was much lower than those formed by MeSP and chitosan over the pH range studied.

3.6. Effect of seawater concentration on flocculation efficiency

In order to evaluate the applicability of MeSP to the turbid water treatment at brackish water wellfield, the effect of seawater concentration on the flocculation performance of MeSP was examined (Fig. 10). The clarification efficiency of kaolin-MeSP system (open circles) was kept at 0.96 even in seawater, while that of kanto loam-MeSP system (open triangles) was decreased to 0.8 by the addition of 50 % of seawater. The difference in the effect on floc settling rate between kaolin-MeSP (solid circles) and kanto loam-MeSP (solid triangles) systems was more remarkable. In the case of kaolin, the floc settling rate gradually decreased from 6 to 4 mm/s with the increase of seawater concentration. On the other hand, in the case of kanto loam, the floc settling rate sharply decreased from 6 to 2 mm/s by the addition of 25 % of seawater. The result of kaolin-MeSP system was similar to the result of diatomite-MeSP system reported in our previous study [20]. In the case of diatomite-MeSP system, the clarification efficiency and floc settling rate in seawater were kept at about 0.95 and 4 mm/s, respectively.

It is generally accepted that flocculation mechanism can be divided into three categories: charge neutralization mechanism, electrostatic patch mechanism and bridging mechanism [33, 34]. Flocculation induced by charge neutralization is effective through an increase in solution ionic strength. In the case of MeSP, the flocculation performance was lowered by the addition of seawater or the increase of ionic strength (Fig. 10). Flocculation of colloidal particles with long chain polymers often belong to the category of bridging mechanism. In this case, the long chain polymers generally have molecular weight in the range of several millions to ten million [34]. Soy protein is composed of 30 % of glycinin and 40 % of β-conglycinin, and the molecular weight of glycinin (320-380 kDa) and β-conglycinin (150-180 kDa) were much smaller than that of the polymers which can induce bridging flocculation [35]. On average, bridging flocculation gives flocs which are much stronger than those produced by addition of salt. However, such stronger flocs produced by the bridging mechanism may not reform once broken at high shear rates [34]. In the case of MeSP, the formed flocs had high reversibility as shown in Figs 4 and 5. Thus, we considered that the flocculation mechanism of MeSP

belong to the category of electrostatic patch mechanism.

4. Conclusions

The flocculation performance of soy protein-based bioflocculant, methylated soy protein (MeSP), was tested with suspensions of kaolin and kanto loam (deposits of volcanic ash). The flocculation performance of MeSP was compared with a well-known bioflocculant chitosan and a widely used commercial flocculant, polyaluminum chloride (PAC) in terms of the clarification efficiency and the floc settling rate.

At pH 7, MeSP showed a much higher flocculation performance than chitosan and PAC for both kaolin and kanto loam suspensions, and the flocs formed by MeSP had high reversibility. A high clarification efficiency of almost 1.0 or relative turbidity of almost 0 was obtained at the dosage of 1.0-4.0 wt.% of suspended solids within a settling time of 1 min. At the dosage of 1.5-4.0 wt.%, the settling rate of the flocs formed by MeSP was about 7 mm/s for kaolin and about 6 mm/s for kanto loam. The settling rate was about three times higher than those of chitosan and PAC.

Krentz et al. [13] tested the flocculation performance of cationic

derivatives of potato, wheat and maize starch with a kaolin suspension (1 g/L). The flocculation efficiency of cationic potato starch was almost comparable to that of polyacrylamide. The relative turbidity decreased to almost 0 by the addition of cationic potato starch (0.5 wt.% of suspended solids) within a settling time of 20 min. Shogren [22] tested the flocculation efficiency of waxy maize starch phosphates with a kaolin suspension (10 g/L). The waxy maize starch was ineffective as flocculant for kaolin and it required the addition of $CaCl_2$ as a flocculant aid. By the addition of 2-4 mM of $CaCl_2$, almost all kaolin could be settled by the addition of 5 ppm of starch phosphate within a settling time of 2 min. In terms of the floc settling rate, the kaolin floc formed by polyacrylamide in a suspension of 50 g/L [24] and formed by $Al(OH)_3$–polyacrylamide ionic hybrid in a suspension of 2.5 g/L [25] were about 1 and 0.2 mm/s, respectively. Compared with these values, it is obvious that the kaolin floc formed by MeSP had a very high settling rate.

The effect of pH on the flocculation performance of MeSP, chitosan and PAC was also examined at an optimum dosage of 1.5 wt.%. The flocculation performance of MeSP was much higher than that of PAC at pH

3-12, in terms of both the clarification efficiency and the floc settling rate. MeSP showed much higher flocculation performance than chitosan at pH 3-7, in terms of both the clarification efficiency and the settling rate. The clarification efficiency of MeSP was almost the same as that of chitosan at pH 7-10, however the settling rate of floc formed by chitosan increased sharply at pH 7-8 and surpassed that formed by MeSP.

The flocculation performance of MeSP for kanto loam suspension was strongly affected by the addition of seawater, while the effect of seawater concentration on the flocculation performance of MeSP for kaolin was moderate.

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Figure captions

Fig. 1. Self-aggregation behavior of soy protein and methylated soy protein (MeSP) as a function of pH. Concentration of protein was 0.1 g/L.

Fig. 2. Photographs of jar test of kaolin-MeSP and kaolin-chitosan systems. Concentration and pH of the suspensions were 3.0 g/L and about pH 7, respectively. Flocculant dosage was 1.0 wt.% of kaolin.

Fig. 3. Results of sedimentation balance measurement of kaolin (triangles), kanto loam (circles) and diatomite (squares) with MeSP. The ordinate, $1\text{-}W_t/W_f$, represents the weight ratio of suspended solids remaining above the sediment trap set at the depth of 15 cm. Concentration and pH of suspensions were 3.0 g/L and about pH 7, respectively. Dosage of MeSP was 1.0 wt.% of suspended solid.

Fig. 4. Reversibility of kaolin-MeSP floc at the MeSP dosages of 0.2 (solid gray symbols), 0.5 (solid black symbols) and 1.5 wt.% (open symboles) of kaolin. The sedimentation balance measurement was repeated

four times using the same suspension; first time (circles), second time (triangles), third time (squares) and fourth time (rhombuses). Concentration and pH of kaolin suspension were 3.0 g/L and about pH 7, respectively.

Fig. 5. Reversibility of kanto loam-MeSP floc at the MeSP dosages of 0.2 (solid gray symbols), 0.5 (solid black symbols) and 1.5 wt.% (open symboles) of kaolin. The sedimentation balance measurement was repeated four times using the same suspension; first time (circles), second time (triangles), third time (squares) and fourth time (rhombuses). Concentration and pH of kanto loam suspension were 3.0 g/L and about pH 7, respectively.

Fig. 6. Floc settling rate (solid symbols), and clarification efficiency (open symbols) as a function of dosage of MeSP (triangles), chitosan (rhombuses) and PAC (circles). Concentration and pH of kaolin suspension were 3.0 g/L and pH 7, respectively. Lines are drawn as a guide for the eye.

Fig. 7. Floc settling rate (solid symbols), and clarification efficiency (open symbols) as a function of dosage of MeSP (triangles), chitosan (rhombuses) and PAC (circles). Concentration and pH of kanto loam suspension were 3.0 g/L and pH 7, respectively. Lines are drawn as a guide for the eye.

Fig. 8. Floc settling rate (solid symbols), and clarification efficiency (open symbols) as a function of pH. Dosage of MeSP (triangles), chitosan (rhombuses) and PAC (circles) were 1.5 wt.% of kaolin. Concentration of kaolin was 3.0 g/L. Lines are drawn as a guide for the eye.

Fig. 9. Floc settling rate (solid symbols), and clarification efficiency (open symbols) as a function of pH. Dosage of MeSP (triangles), chitosan (rhombuses) and PAC (circles) were 1.5 wt.% of kanto loam. Concentration of kanto loam was 3.0 g/L. Lines are drawn as a guide for the eye.

Fig. 10. Effect of seawater concentration on the floc settling rate (solid symbols) and clarification efficiency (open symbols) in kaolin-MeSP

(circles) and kanto loam (triangles) systems. Dosage of MeSP was 1.0 wt.% of suspended solids.

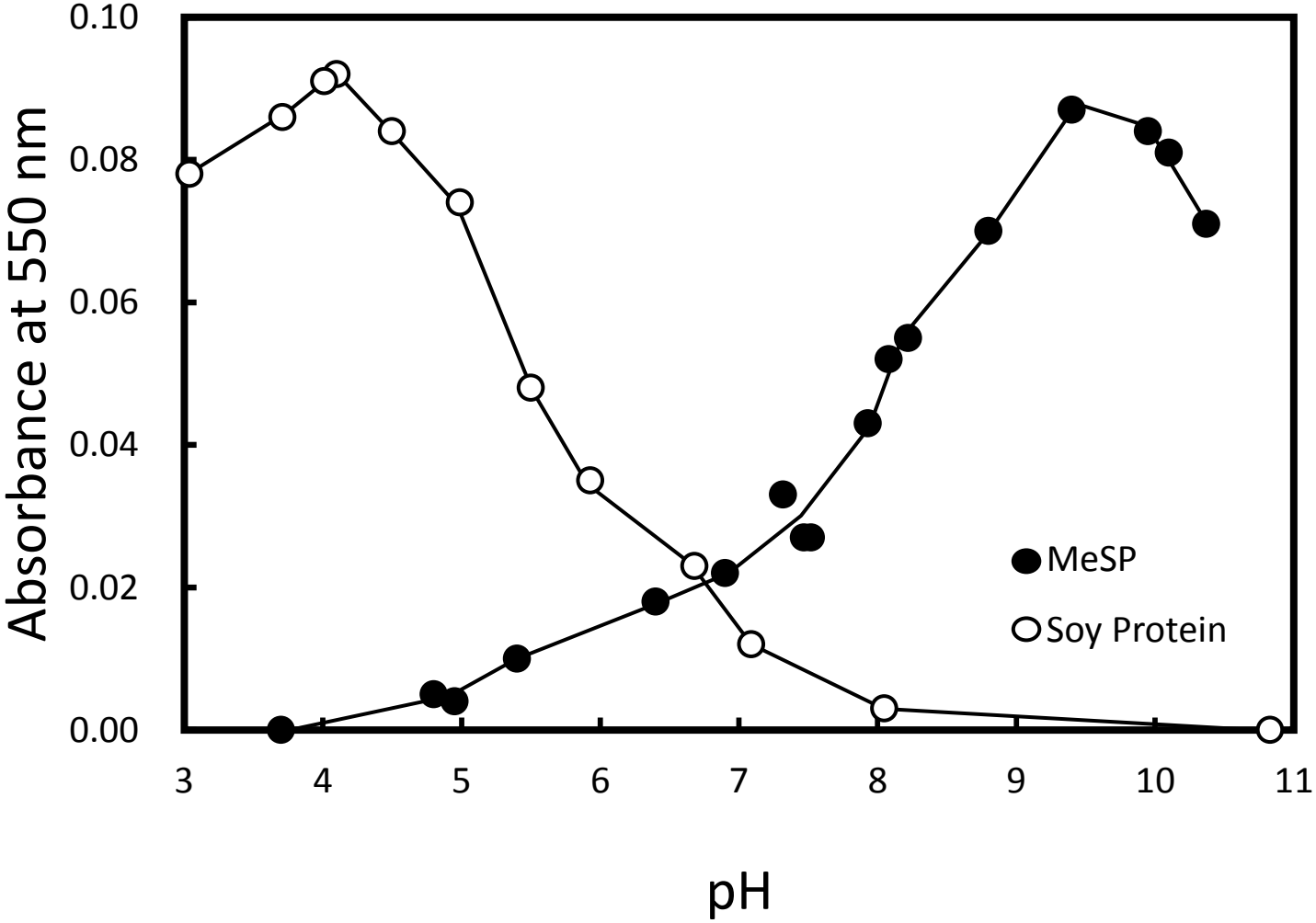
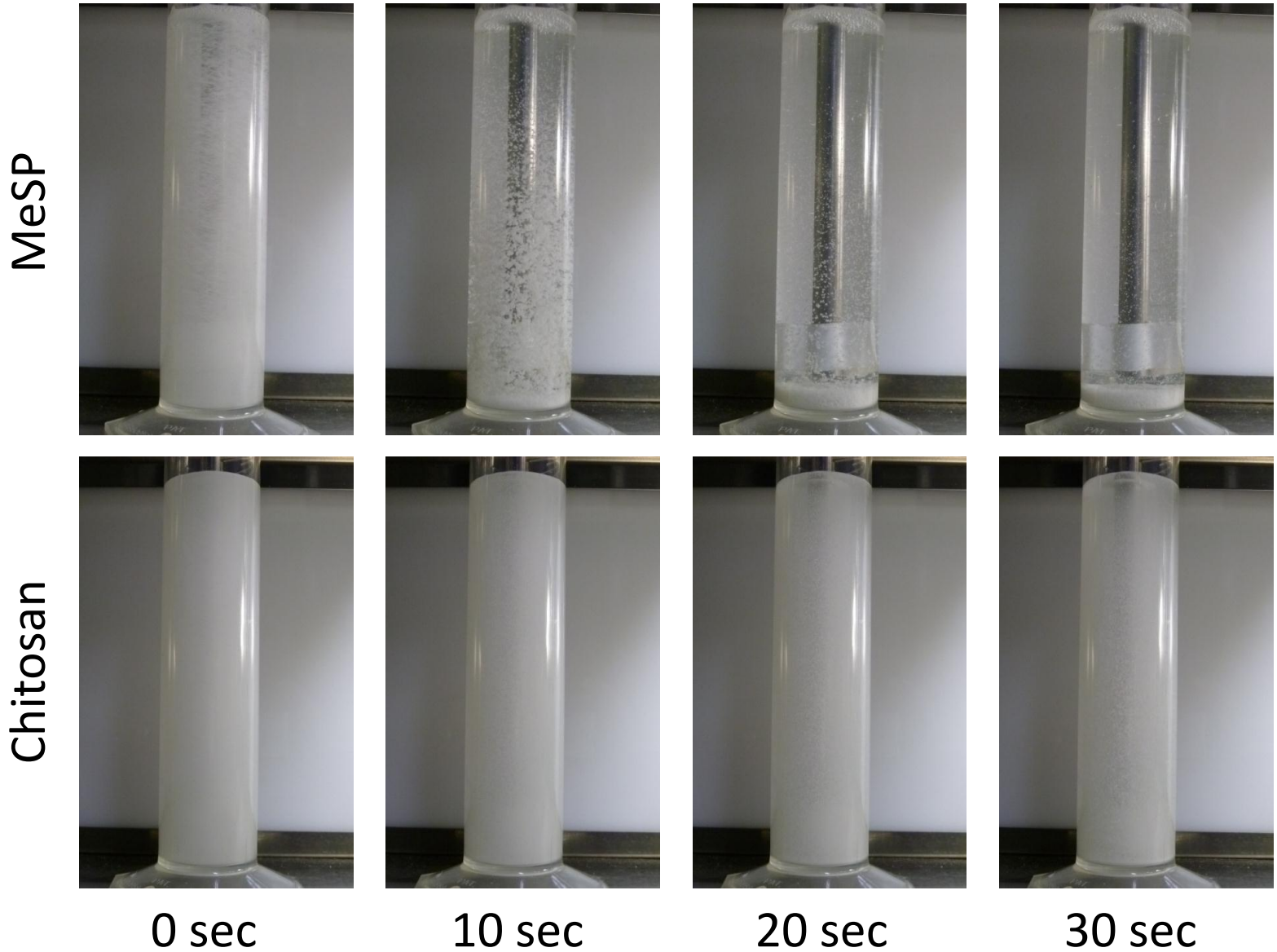


Fig. 1. Liu *et. al.*



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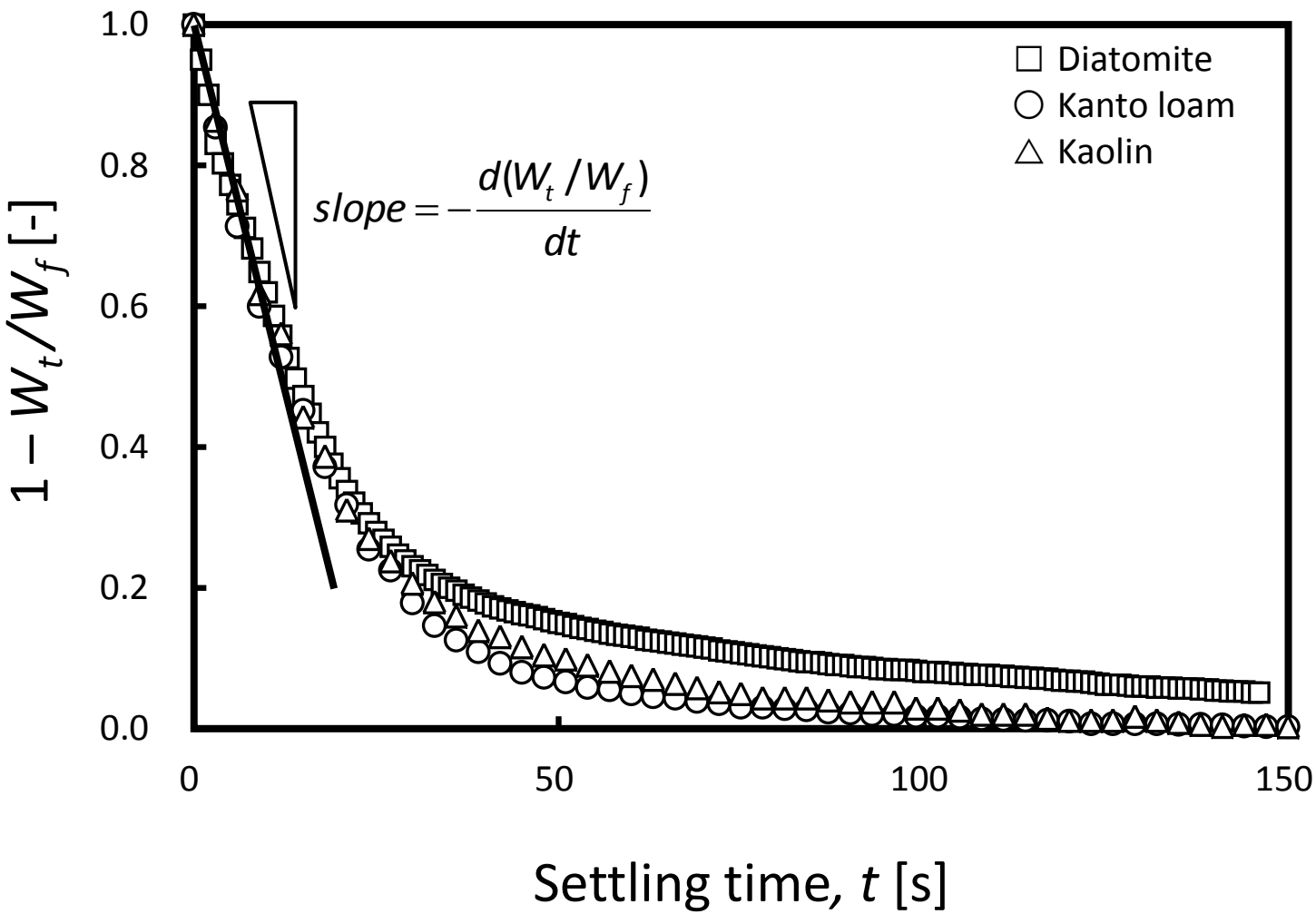

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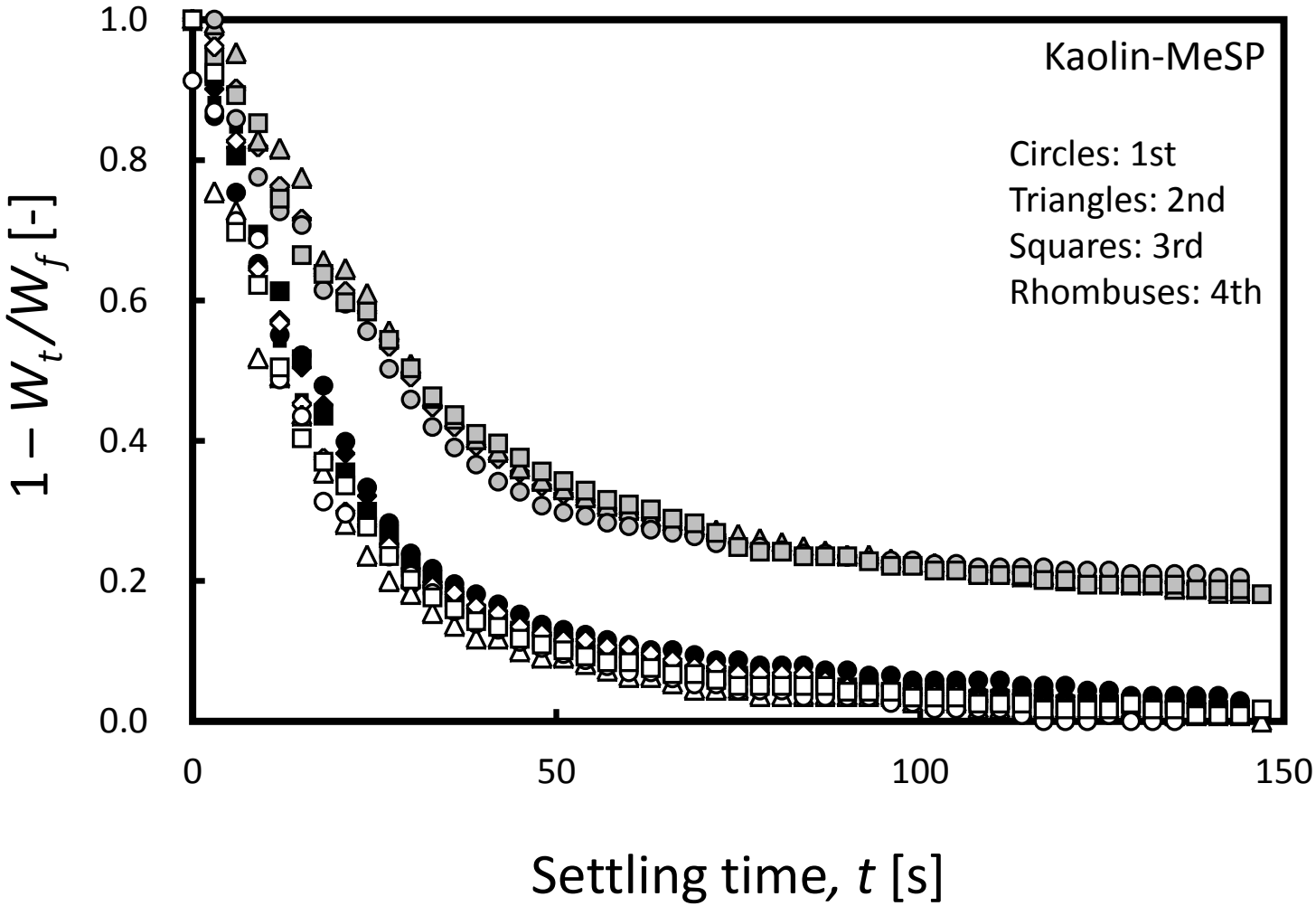


Fig. 4. Liu *et. al.*

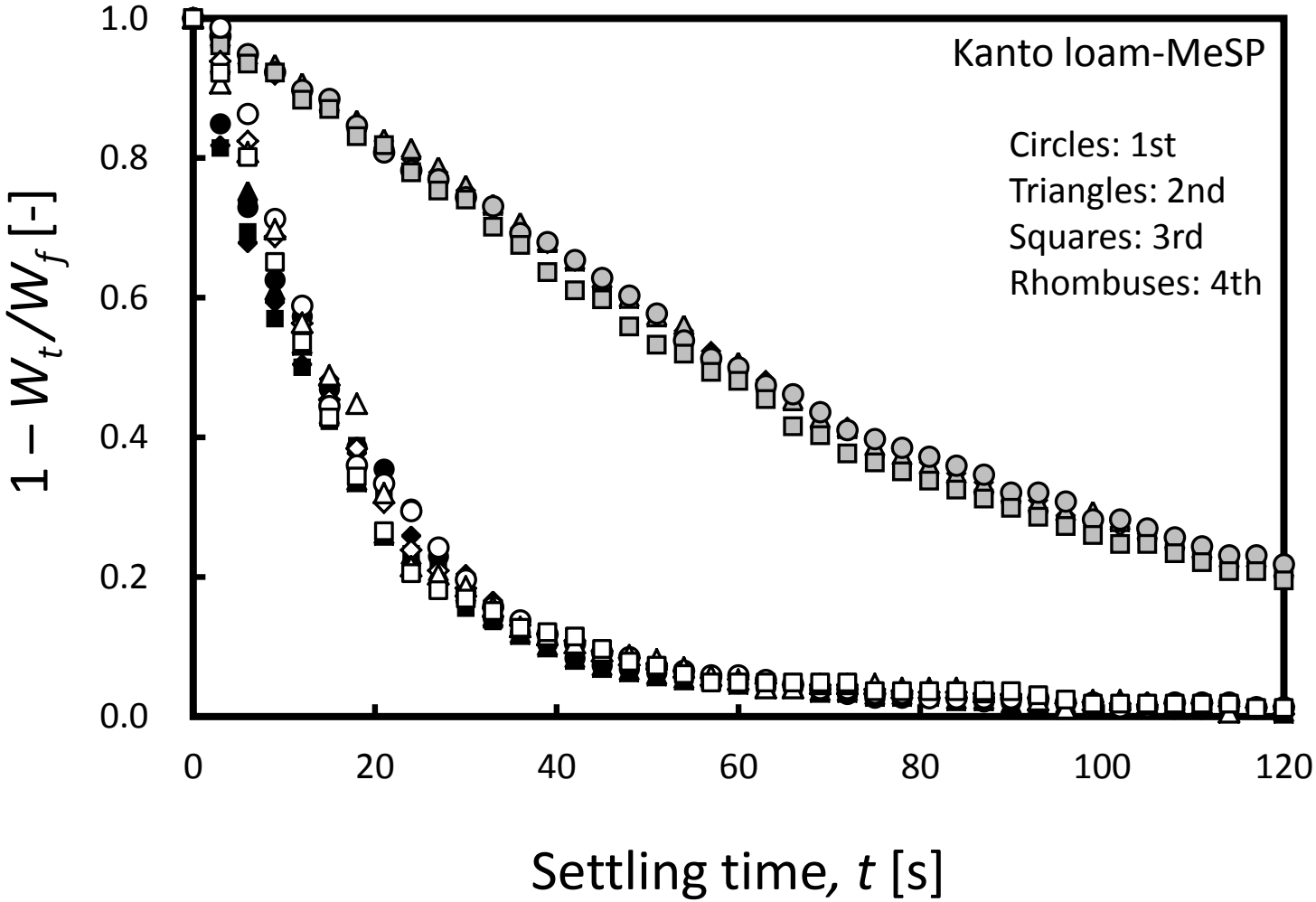


Fig. 5. Liu *et. al.*

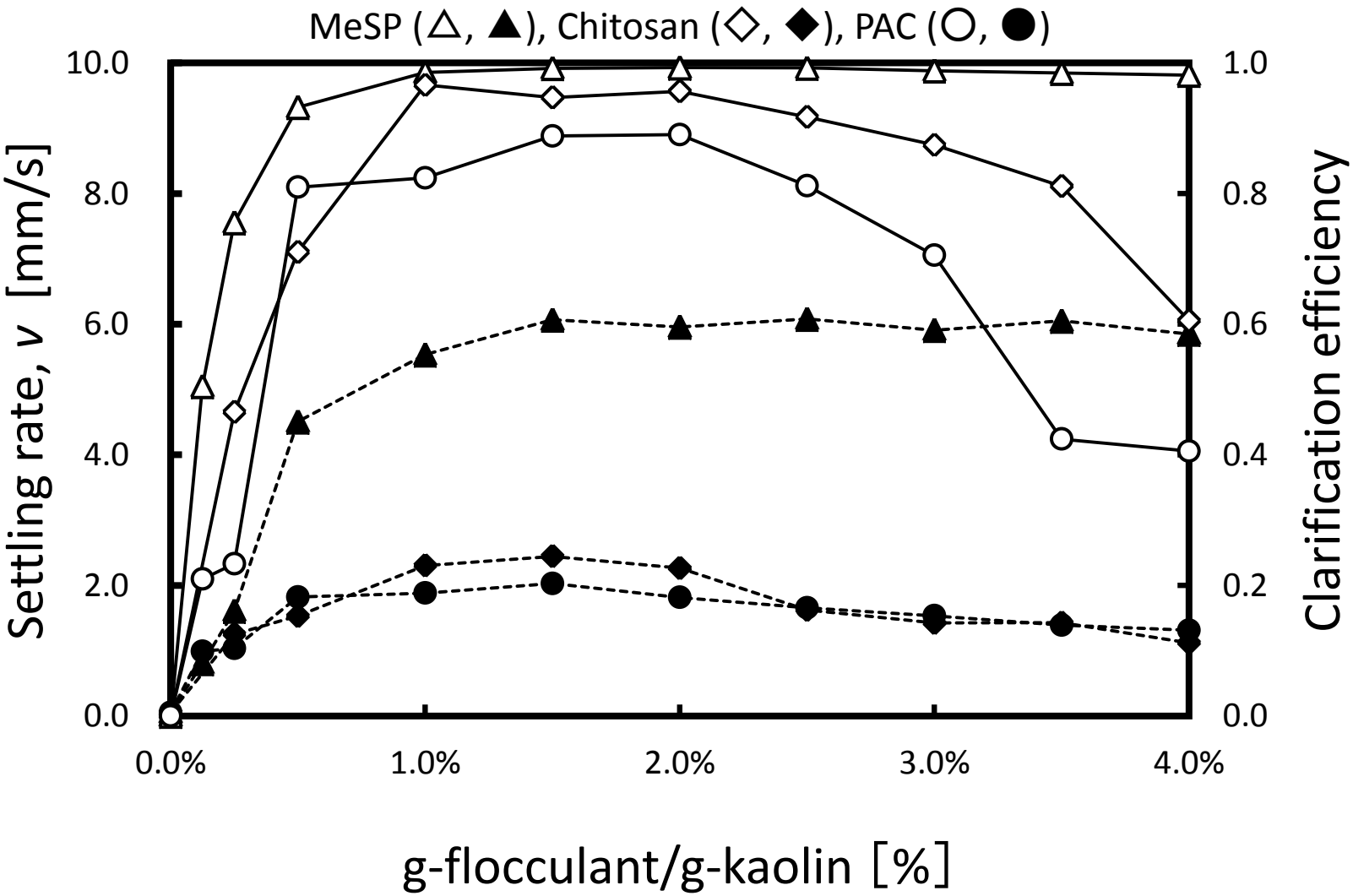


Fig. 6. Liu *et. al.*

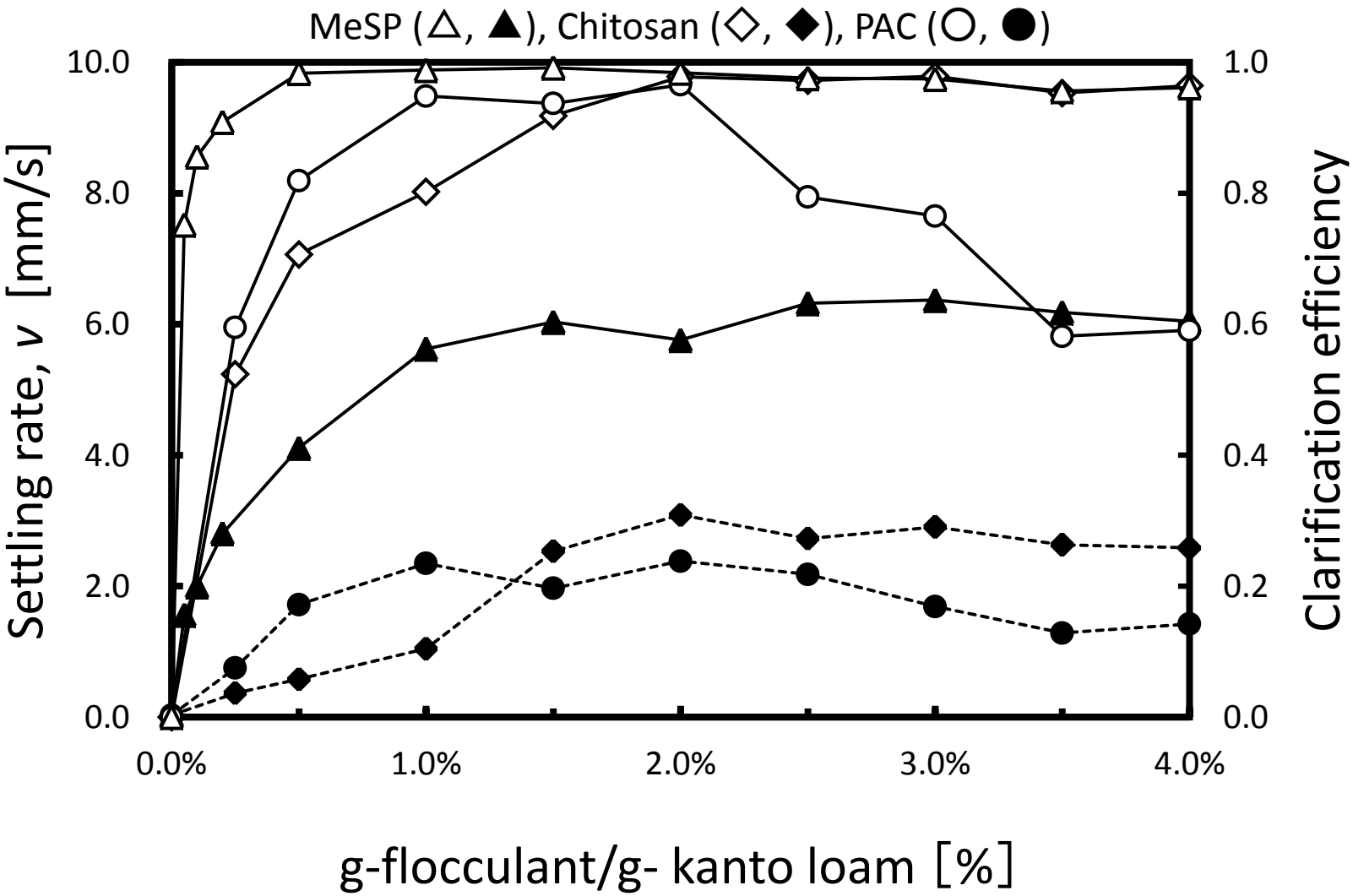


Fig. 7. Liu *et. al.*

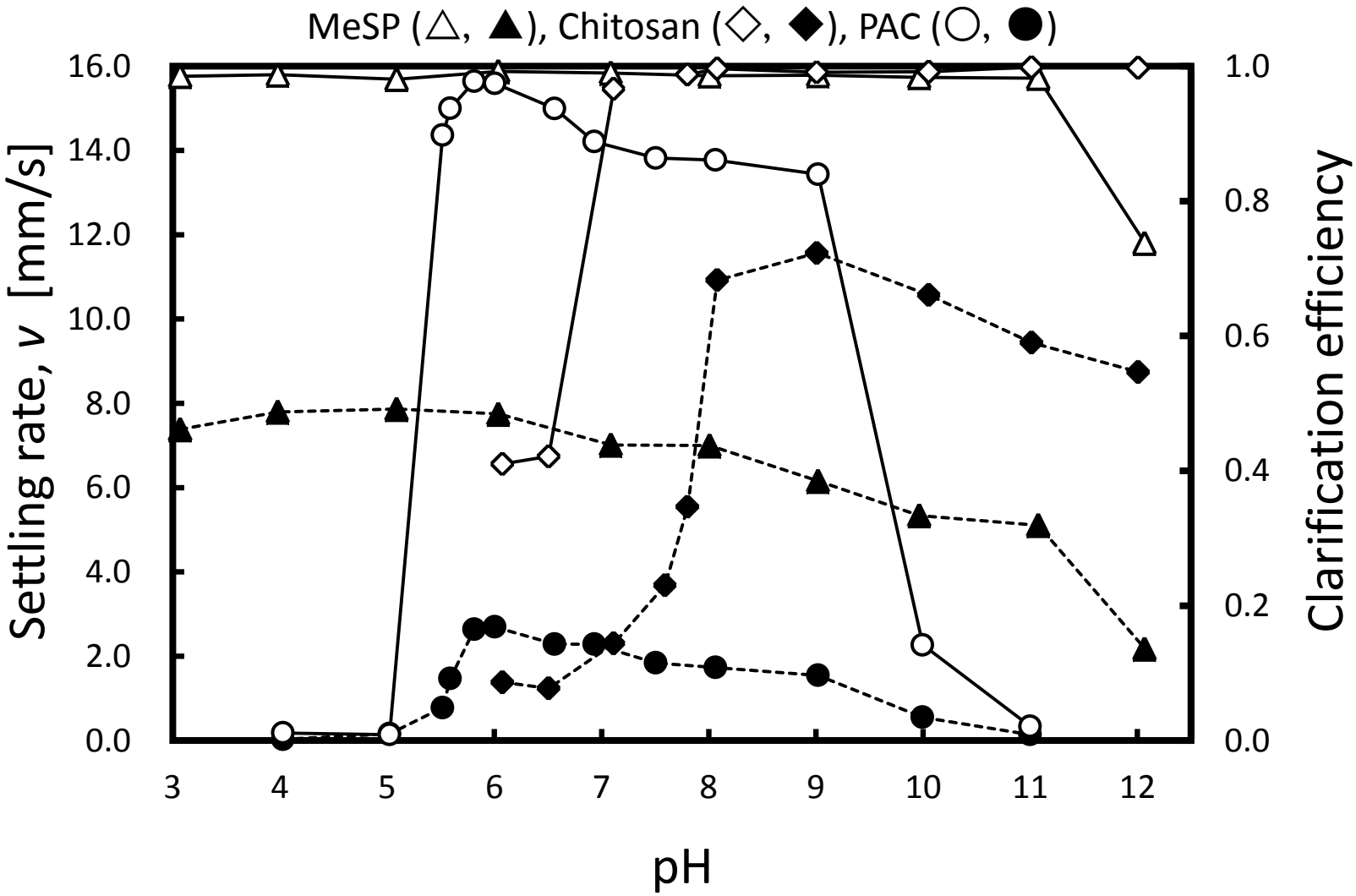


Fig. 8. Liu *et. al.*

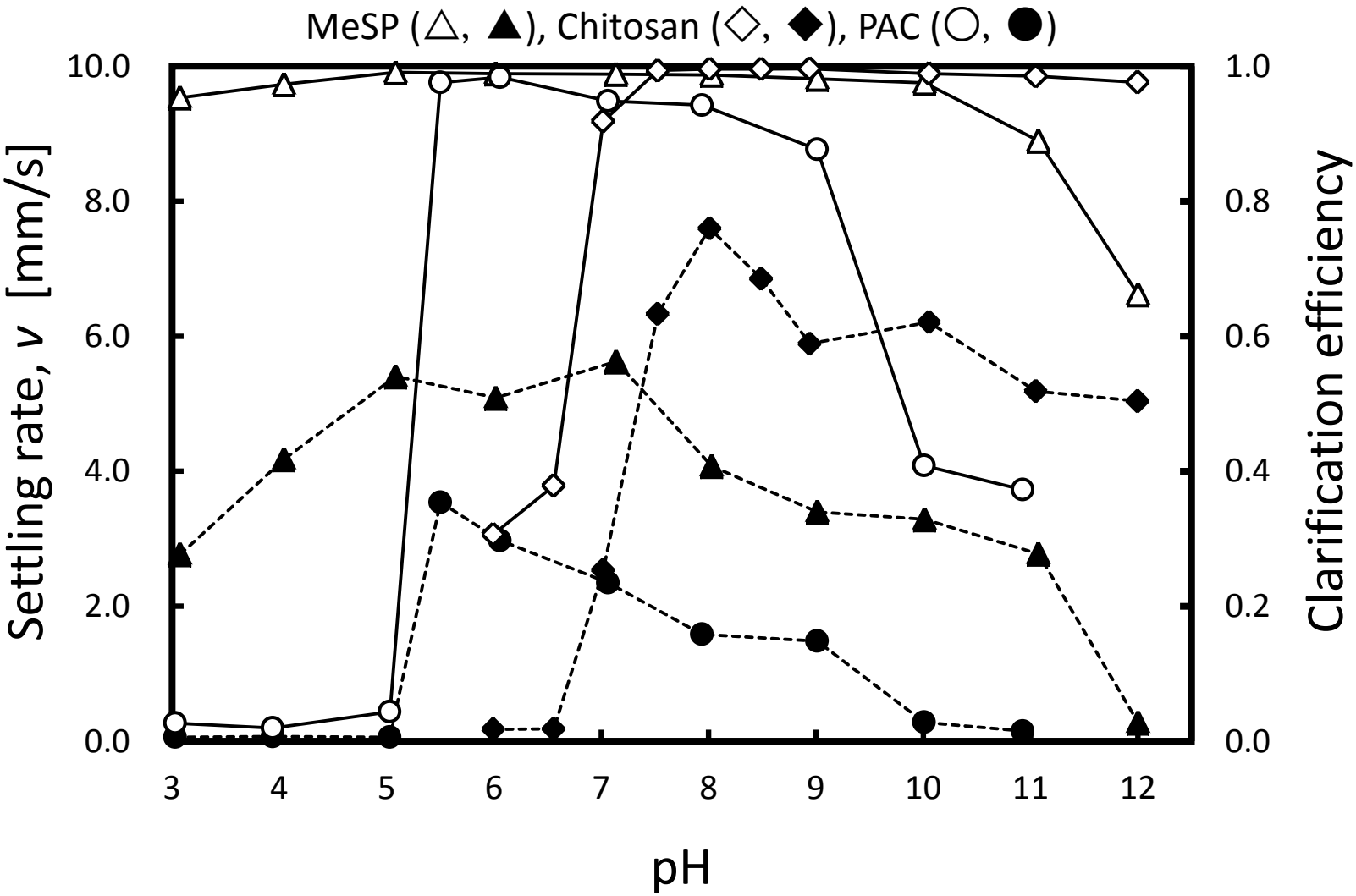


Fig. 9. Liu *et. al.*

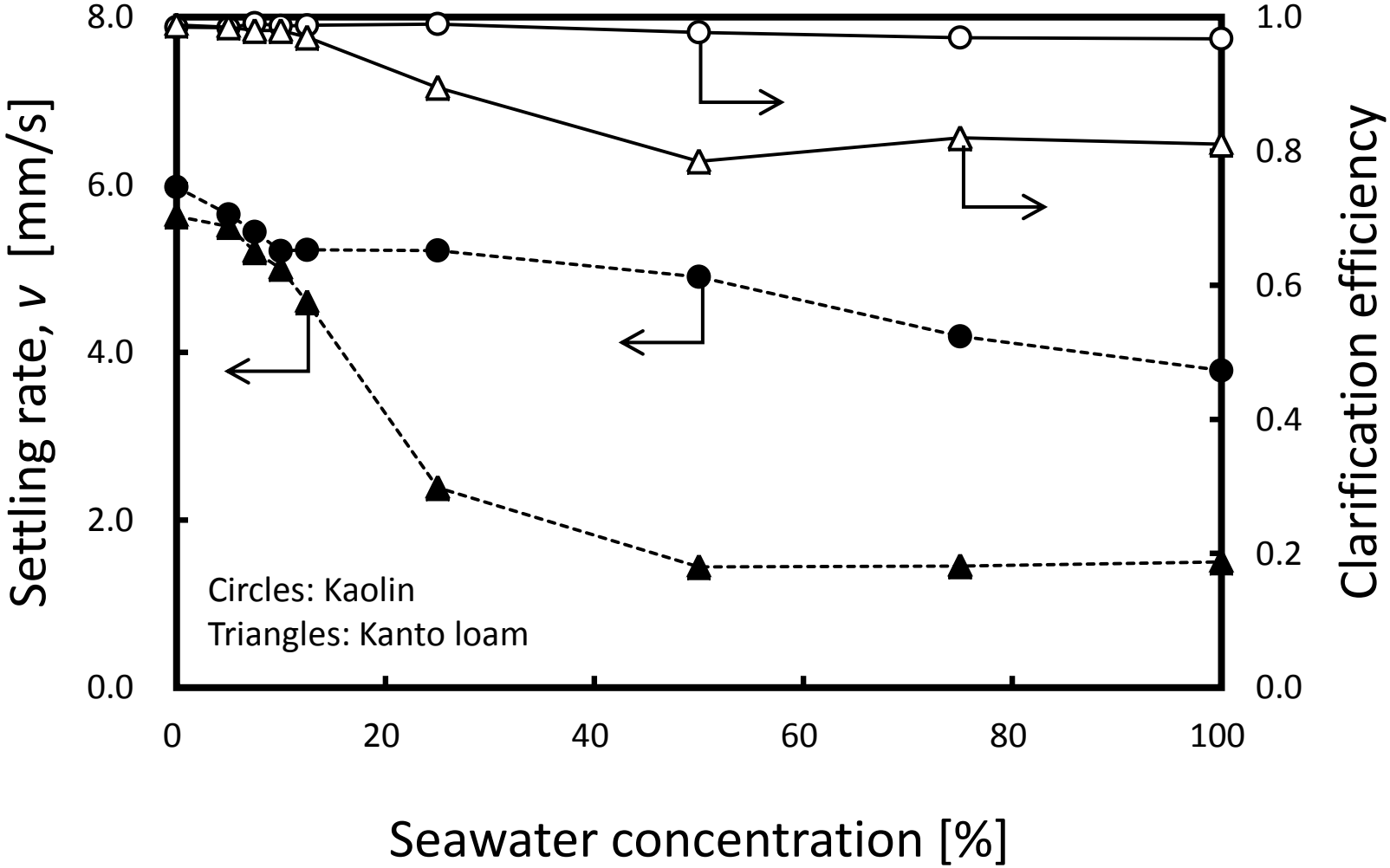
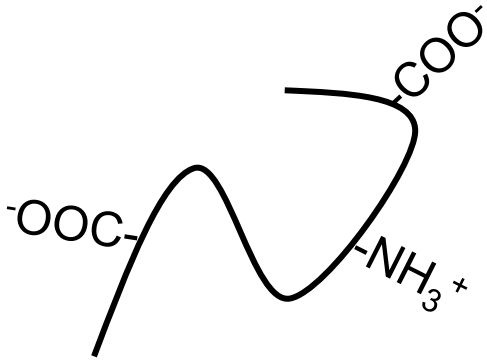


Fig. 10. Liu *et. al.*

Soy protein



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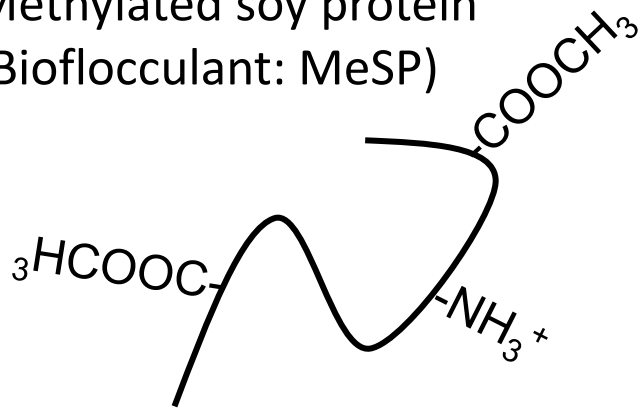
Methylation

0.1 M HCl-Methanol

Methylated soy protein
(Bioflocculant: MeSP)



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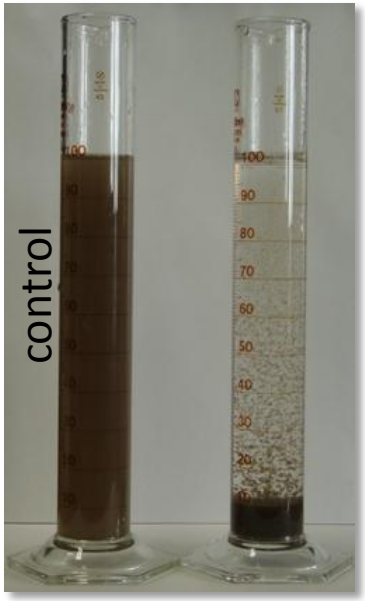
10 sec

20 sec



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