ε-polylysine biopolymer for coagulation of clay suspensions

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Abstract. The coagulation or flocculation of cohesive clay suspensions is one of the most widely used treatment technologies for contaminated water. Flocculated clay can transport pollutants and nutrients in ground water. Coagulants are used to accelerate these mechanisms. However, existing coagulants (e.g., polyacrylamide, polyaluminum chloride) are known to have harmful effects in the environment and on human health. As an alternative, eco-friendly coagulant, this study suggests ε-polylysine, a cationic biopolymer fermented by *Streptomyces*. A series of sedimentation experiments for various ε-polylysine concentrations were performed, and the efficiency of sedimentation with ε-polylysine was estimated by microscopic observation and light absorbance measurements. Two types of sedimentation were observed in the experiments: accumulation sedimentation (at 0.15%, 0.20%, 0.25% ε-polylysine) and flocculation sedimentation (at 0%, 0.1%, 0.5%, 1.0%, 2.0% ε-polylysine). These sedimentation types occur as a result of the concentration of counter ions. Additionally, the performance of ε-polylysine was compared with that of a previously used environmentally friendly coagulant, chitosan. The obtained results indicate that flocculation sedimentation is appropriate for contamination removal and that ε-polylysine functions more efficiently for clay removal than chitosan. From the experiments and analysis, this paper finds that polylysine is an alternative eco-friendly coagulant for removing chemical contaminants in groundwater.

Keywords: ε-polylysine; sedimentation; biopolymer; coagulation; flocculation

1. Introduction

According to the World Resources Institute, 54% of the Indian subcontinent is currently facing high to extremely high water stress (Fig. 1), 70% of its surface water is polluted, and more than 100 million people are living in areas of poor water quality (WBCSD 2015). The data clearly show that increasing water demand along with a serious decline in freshwater sources poses a major challenge to the management and reuse of groundwater. These conditions are also motivating researchers to improve and develop new water treatment technique, such as filtering (Kasim *et al.* 2016, Rajapakse *et al.* 2015).

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To reduce potential pollutants from groundwater, biological, chemical, and physical methods are widely used. Bioremediation is a method that uses natural biological activity to biologically degrade or destroy various organic contaminants (Vidali 2001). Chemical treatment involves the application of agents to promote the degradation of hazardous substances; for example, oxidation-reduction reactions such as ozonation, chlorination, and UV irradiation are applied to bread down organic substances (Marco *et al*. 1997). Meanwhile, physical treatments use physical means to remove contaminants and other hazards, using methods such as electro kinetics (Khoiruddin *et al*. 2014), soil washing and thermal desorption, air sparging/air stripping, and incineration (Shackelford and Jefferis 2000). In chemical and physical methods, coagulation, flocculation, and sedimentation are processes commonly used to improve the effectiveness of water treatment (Matilainen *et al*. 2010). Furthermore, coagulation and sedimentation are essential procedures in land reclamation, for example, to construct artificial islands through sedimentation processes (Chang and Cho 2010).

The mechanism for removing waste materials from groundwater involves the transformation of a stable clay colloidal suspension into coagulated or flocculated systems, by neutralization of the repulsive forces on the clay surface (Sengco *et al*. 2001). In order to accelerate the coagulation process of a clay suspension, specific coagulants are used in the suspension. Synthetic polymers, polyacrylamide, and mineral additives (aluminium sulfate, ferric chloride, ferric sulfate, and polyaluminum chloride) are the most commonly used coagulants (Aguilar *et al*. 2005, Amuda and Alade 2006, Csempesz 2000, Pierce *et al*. 2004, Tatsi *et al*. 2003).

However, these coagulants can pose serious health problems. For instance, while polyacrylamide is known to be an effective conditioner for stabilizing soil aggregates, reducing soil erosion and increasing water infiltration (Seybold 1994) or water and nutrient movements in soils (Kim *et al*. 2015) and itself is non-toxic to humans, animals, fish, and plants, its residual acrylamide monomer content is a neurotoxin to humans (Seybold 1994). Therefore, the residual content needs to be controlled at levels lower than 0.05% (Theodoro *et al*. 2013).

Recently, organic biopolymers are actively attempted in various environmental and geotechnical engineering purposes (Chang and Cho 2012, 2014, Chang *et al*. 2015a, b, c, d, 2016a, b) .Chitosan, an amino-biopolymer, has received a great deal of attention as a bio-flocculant in water treatment processes (Chatterjee *et al*. 2009, Pan *et al*. 2006, 1999, Roussy *et al*. 2005). However, because the amino groups of chitosan do not protonate at high pH, chitosan is insoluble in aqueous or alkali solvents. Therefore, the efficiency of chitosan decreases at high pH, and is restricted even at a neutral pH of 7.0 (Chatterjee *et al*. 2005). Furthermore, one kilogram of
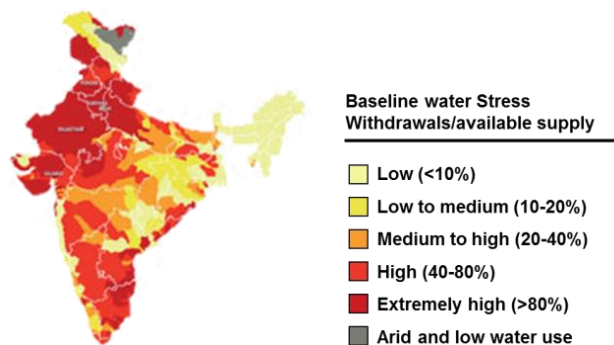

Fig. 1 Water stress in India (WBCSD 2015)

chitosan costs US $750-1,000[1]. Therefore, the practical use of chitosan as a water treatment agent is challenging from an economic point of view.

To overcome the aforementioned limitations of chitosan, this study proposes ε-polylysine, a food grade biopolymer, as a new alternative. In sedimentation aspect, the activity of ε-polylysine is not affected by pH or even the presence of high concentrations of salt (1.0M NaCl) (Chheda and Vernekar 2014). Furthermore, ε-polylysine is less expensive than chitosan, being just US $10 ~ 300[2] per kilogram. In addition, ε-polylysine is a completely non-harmful material, and was approved by the Japanese Ministry of Health, Labour and Welfare as a preservative in food in the late 1980s (Chheda and Vernekar 2014). Nonetheless, despite these advantages, there have been few studies on the application of ε-polylysine as a coagulant to precipitate soil suspensions.

In this study, a series of sedimentation experiments with varying concentrations of ε-polylysine was conducted. This study provides a detailed explanation of the interaction between ε-polylysine and clay. Furthermore, the effects of ε-polylysine in the sedimentation process are clarified.

2. Methods and materials

2.1 Materials

2.1.1 ε-Polylysine

ε-Polylysine is a type of polylysine, a lysine homopolymer that contains bonds between the carboxyl and ε-amino groups. It is produced by the fermentation of bacteria, *Streptomyces albulus*. At pH 7, ε-polylysine has positive charges due to positively charged hydrophilic amino groups at the L-lysine. The typical molecular formula for ε-polylysine is $C_{180}H_{362}N_{60}O_{31}$.

ε-Polylysine has both hydrophobic and hydrophilic characteristics. Methylene groups on the molecule are hydrophobic and carboxyl and amino groups are hydrophilic. This structure can prevent microbial activity and the decomposition of food. ε-Polylysine acts as a cationic surface active compound and can constrain the proliferation of microorganisms such as yeast, fungi, and bacteria species. Therefore, it can be used as a food preservative (Hiraki *et al*. 2003). Additionally, the cationic charges of ε-polylysine can interact with the negatively charged surfaces on a cell, a drug or fine clay. As a result of this electrical interaction, polylysine can be used as a coating agent for tissues or drugs in biotechnology (Hiraki *et al*. 2003, Mazia *et al*. 1975, Park *et al*. 2006).

In this study, ε-polylysine was used to accelerate the coagulation and flocculation of clay particles, due to the electrical interaction between the negatively charged facial surfaces of clay particles and the positively charged ε-polylysine. This interaction induces the clay suspension to form flocs, which sediment to the bottom. This phenomenon can be used to remove waste materials in groundwater.

Commercial ε-polylysine (BNF CO., LTD, CAS No. 28211-04-3) was chosen as the surfactant to coagulate clay particles in this study. Its molecular weight is 3,500 – 4,500 Da, and it is effective over a wide range of pH values, from about 4-10.

2.1.2 Chitosan

Chitosan is a linear polysaccharide produced from chitin, the second most abundant polysaccharide. Chitosan is extracted from the outer skeletons of crustaceans, shrimp, and lobsters

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[1] Material price quoted by Sigma-Aldrich Co., LLC. (www.sigmaaldrich.com) in 2016
[2] Material price quoted by Zhengzhou Bainafo Bioengineering Co., Ltd. (http://en.bnfsw.com) in 2016

(Dutta *et al.* 2004). It is randomly composed of *β*-1,4-D-glucosamine ($C_6H_{13}NO_5$) and N-acetlyglucosamine ($C_8H_{15}NO_6$). The amino group (N-) in chitosan has a positive charge and is more or less soluble in acidic to neutral solutions depending on pH (Goosen 1997).

Chitosan is a highly biodegradable material (Hirano *et al.* 1991) and is currently widely used in ecologically friendly fertilizers and biopesticides in agriculture and horticulture (Linden *et al.* 2000). Furthermore, because of its biocompatible, antibacterial, and polyelectrolytic characteristics (Kobayashi *et al.* 1996), chitosan is used in various other industrial applications including water treatment, chromatography, as additives for cosmetics, textile treatment for antimicrobial activity (Shin *et al.* 1999), novel fibers for textiles, photographic papers, biodegradable films, biomedical devices, and microcapsule implants for the controlled release in drug delivery (Bartkowiak and Hunkeler 1999, Sezer and Akbuga 1999, Suzuki *et al.* 1999)

In civil engineering, chitosan has been used to remove wastes in groundwater by flocculation (Chatterjee *et al.* 2009, Pan *et al.* 2006, 1999) or in coating processes (Gupta *et al.* 2013). Chitosan binds fine particles in suspension, and also removes phosphorus, heavy metals, and oils from water. Thus, it is important for water filtration (Juang and Shiau 2000). In particular, chitosan is suitable for the adsorption of metals, such as Cd^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+}, etc., due to its chelating property (Liu *et al.* 2002).

This study used commercial chitosan with low molecular weight, 50,000–190,000 Da, (Sigma-Aldrich, CAS No. 9012-76-4). Its viscosity is 20–300 cP at 1% weight to 1% acetic acid.

2.1.3 Kaolinite clay

In this study, kaolinite clay, which has the mineral composition Al_2O_3-$2SiO_2$, was chosen as a representative clay material. The kaolinite used in this study was mined in Indonesia and the kaolinite was used in powdered form with 3% moisture content. It was first mined and then crushed into a powder. Kaolinite has a mean particle size $D_{50} = 0.22$ *μ*m, specific gravity (Gs) of 2.65, and is classified as a clay of high plasticity, *CH* via USCS (Unified Soil Classification System).

2.2 Experimental set up

2.2.1 Sedimentation

To estimate and compare the coagulation efficiency of *ε*-polylysine and chitosan, sedimentation tests of clay suspensions were conducted. During the experiments, the temperature and relative humidity were controlled as 22±2°C and 25%, respectively, while the pH of the deionized water was near 6.5. For each experimental trial, 16 g of kaolinite clay and 80 g of deionized water were injected into a 100 ml graduated cylinder. The water content was determined as the point where the suspension could be mixed well by an electric stirrer machine (Kolaian and Low 2013). The effect of gravity on sedimentation is greater than that of the coulombic attraction when the water content of kaolinite slurry is below 500% (Imai 1980). Thus, the initial water content was fixed as 500% to render sufficient interaction between clay minerals and biopolymer additives. Previous researches also determined 500% water content for experiments (Imai 1981, Kondo and Torrance 2005). The inside diameter of the graduated cylinder was 26.5 mm, and was larger than the "yield diameter", which is the diameter at which settling cannot occur due to the wall effect, which was about 13 mm for this experiment (Michaels and Bolger 1962). As a result, the graduated cylinder is large enough to observe any accelerated sedimentation effect caused by the biopolymers. The volume, pH, and turbidity of the suspension were assumed to be the same since the same initial
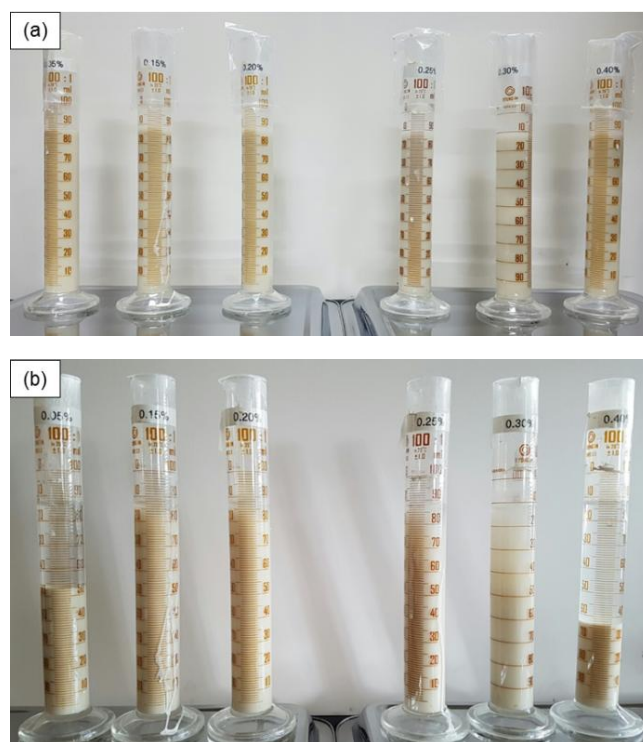
Fig. 2 Variation in sedimentation volume after (a) 0 minutes; and (b) 760 minutes from initial time

water content of 500% was set for each cylinder. Clay suspensions were then prepared by mixing kaolinite and deionized water in the graduated cylinder.

The cylinders were turned upside down about 20 times to uniformly spread clay particles throughout the suspension. Afterwards, chitosan and *ε*-polylysine were poured into each suspension with various biopolymer weight to water weight ratios, of 0%, 0.1%, 0.2%, 0.5%, 1%, and 2%, and then thoroughly mixed. For *ε*-polylysine, additional experiments were conducted at 0.05%, 0.15%, 0.25%, 0.3%, and 0.4% content to estimate the sedimentation effect at lower content more accurately.

After the mixing of the biopolymer and clay suspensions was completed (Fig. 2(a)), the volume of clay sediments was measured until the sediment volume change became constant (Fig. 2(b)). Then, the measured volumes were divided by the initial volume to generalize the variation in sedimentation volume, determined as the sediment ratio (instant height ratio to the initial height, %).

$$\text{Sediment ratio} = \frac{V_t}{V_i} \times 100 [\%] \tag{1}$$

where V_t is the sediment volume at time t and V_i is the initial sediment volume.

2.2.2 Spectrophotometer

Using spectrophotometry, the light reflection or transmission properties of a target material can be quantitatively measured. There have been many attempts to measure the clarification and

turbidity of a supernatant to determine the flocculation and sedimentation of clay suspensions using spectrophotometric experiments (Kurane *et al.* 1994, Li *et al.* 2008, Petzold *et al.* 2003). By comparing the absorbance values for each case, the clay particles remaining suspended in the supernatant can be evaluated. The amount of light absorbed during the propagation is expressed as a dimensionless value, absorbance. Absorbance (A) is the logarithmic ratio of the radiant flux received (ϕ_e^i) over the material to the radiant flux transmitted (ϕ_e^t).

$$A = \log_{10}\left(\frac{\phi_e^i}{\phi_e^t}\right) \qquad (2)$$

The absorbance property represents the amount of clay particles floating in the liquid. Therefore, higher absorbance means the sedimentation procedure has low efficiency.

After the sedimentation process was finished, 1000 μl volume of the uppermost layer was sampled with the use of a micropipette (Eppendorf Research® plus). The sample was then poured into a disposable cuvette (Eppendorf® UVette®) to fit into the spectrophotometer. The center height of the cuvette is 8.5 mm, the chamber volume is 2000 μL, and light in the wavelength range of 200-1600 nm can penetrate the cuvette. Afterwards, the cuvette was installed in the spectrophotometer (DR 5000TM UV-Vis Spectrophotometer) and visible light with a 640 nm wavelength, that is, red light, was used to measure the absorbance of each sample. Through the light penetration procedure, the effect of the biopolymer concentration on the absorbance variation was measured.

2.2.3 Microscopic observation

Microscopic observations were performed to identify the interaction behavior between kaolinite particles in the suspension and the ε-polylysine biopolymer. After the final constant sedimentation volume was measured, the clay aggregates at the bottom of the cylinders were sampled and smoothly spread out on carbon conductive tabs (Pelco Tabs[TM]) attached on an SEM mount (diameter 25 mm). The remaining moisture in the samples was eliminated using a manual air dryer for a minute. To improve the imaging of the specimen, osmium coating of the SEM mount was conducted using an osmium plasma coater (OPC 60-A). Using a scanning electron microscope (SEM; SU5000), high resolution images were taken, and they showed inter-particle bridges and structures between the clay and ε-polylysine particles.

3. Results and analysis

3.1 Sedimentation test

The sedimentation behavior of soils is governed by: (1) type of clay mineral; (2) type of dissolved electrolytes; (3) initial water content (water to solid ratio in mass); and (4) ionic concentration in water. Generally, gravitational force becomes dominant when the initial water content and ionic concentrations are relatively low, while inter-particle flocculation governs the sedimentation behavior when both water and ionic contents increase (Imai 1980).

Different sedimentation behaviors are exhibited based on the electrolyte concentration. The sedimentation behaviors of kaolinite clay suspensions can be divided into three categories, flocculation-sedimentation, accumulation-sedimentation and mixed-sedimentation (Ma and Pierre

1992). Mixed sedimentation can be observed when accumulation sedimentation occurs for heavy solid matters at the beginning, while flocculation occurs sequentially with remaining light suspensions. However, it is hard to distinguish pure accumulation and mixed (accumulation and flocculation) sedimentation behaviors clearly. Thus, we avoided mixed sedimentation behavior in this study. The left side of Figs. 3(a) and (b) shows flocculation-sedimentation.

In flocculation sedimentation, two layers, the flocculated sedimentation and the clear supernatant liquid, are clearly distinguishable. The flocculated sedimentation volume gradually drops down as sedimentation proceeds. This means that the density of the sediment continuously increases until reaching a final constant volume.

The right side of Figs. 3(a) and (b) shows accumulation-sedimentation. With accumulation sedimentation, there is a transition zone that remains in a suspended state. Accordingly, the division between the layers is less clear than in the case of flocculation sedimentation. Accumulation-sedimentation occurs due to the gravimetric settling of clay particles. Thus, the height of the interface between the accumulation-sedimentation zone and the transition zone continuously increases from the beginning to the end of sedimentation.

In general, flocculation sedimentation induces uniform sediment due to the identical settling behavior (e.g., similar size and gravimetric force) of each individual floc, while sediments formed via accumulation sedimentation shows gradual grain size distribution depending on the different size and density of distinguished solid matters. In the experiments, both flocculation and accumulation sedimentation behaviors were observed analyzed for ε-polylysine-treated kaolinite clay.

3.1.1 ε-polylysine

Sedimentation volume

The variation in sedimentation volume when ε-polylysine was used as a coagulant is shown in Fig. 4. The results show that for most ε-polylysine concentrations, the sedimentation follows flocculation sedimentation behavior. But from 0.15% to 0.25%, accumulation sedimentation became the dominant sedimentation mechanism. Each sedimentation type shows a different tendency in volume variation. For flocculation sedimentation, the total volumes of flocs decline together. Thus, the sedimentation volume is continuously decreased. In contrast, with accumulation sedimentation of 0.15% to 0.25%, clays were accumulated at the bottom of the cylinder from the heaviest particle. Therefore, the sediment volume increased until the volume became constant.

Flocculation sedimentation volumes are shown for ε-polylysine concentrations of 0.0%, 0.1%, 0.5%, 1%, and 2% in Fig. 4(a). For the flocculation sedimentation cases, even 0.1% injection of ε-polylysine produced a reduction in final sedimentation volume, by 74%, compared to the final volume at 0.0% injection. The lowest sedimentation volume, 27.5% of the initial sediment volume, occurred with an injection of 0.5% concentration ε-polylysine. The final sediment volume increases again as the ε-polylysine concentration exceeds 0.5%, but this value is smaller than that of the untreated clay suspension.

According to the DLVO theory (Pierre 1992), counter ions, cations for kaolinite clay, on the ε-polylysine surface neutralize the suspension charges and lower the repulsive forces between the colloidal particles. Therefore, the double layer around the clay particles is compressed and the clay particles collide more frequently. This rheology in the clay suspension flocculates the clay suspension and constitutes sedimentation.
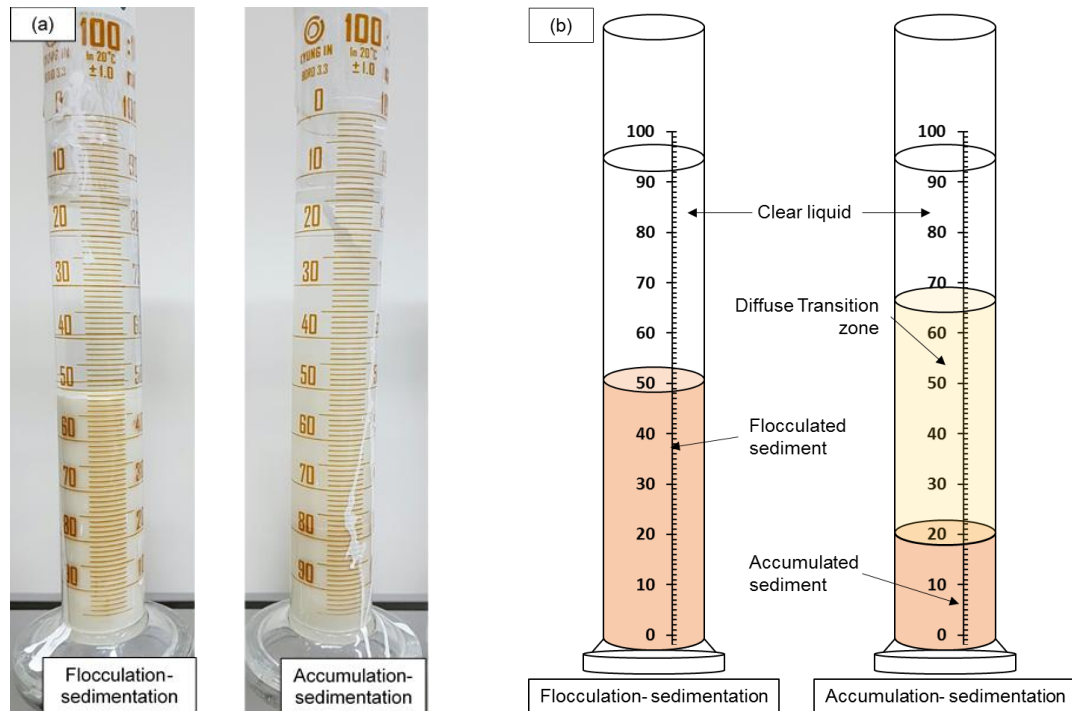
Fig. 3 Comparison of flocculation and sedimentation: (a) pictures after 8640 minutes settling; (b) schematic diagram
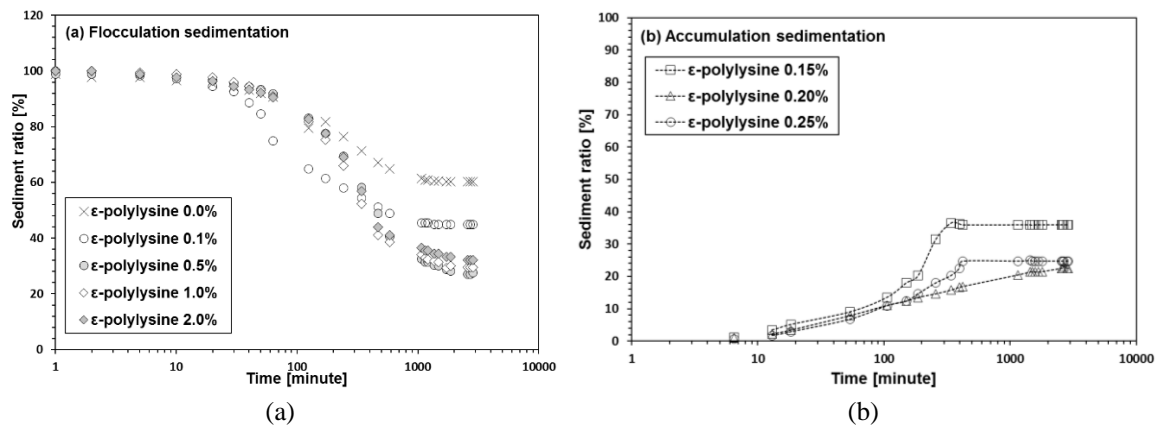

Fig. 4 Variation in sedimentation volume of ε-polylysine treated suspension for: (a) flocculation sedimentation; and (b) accumulation sedimentation

Acceleration of the sedimentation velocity is also observed in Fig. 4(a). The slope of the graph for the ε-polylysine treated suspension is steeper than that of the untreated case. This indicates that the injection of ε-polylysine in the clay suspensions accelerates the sedimentation process.

For the 0.15%, 0.20%, and 0.25% ε-polylysine concentrations, the dominant sedimentation mechanism was accumulation sedimentation (Fig. 4(b)). ε-Polylysine breaks the floc structure and

reconstitutes the floc structure by bonding clay particles face to face. However, for this case, the concentration of injected $ε$-polylysine was not sufficient to form bridges between clay particles. Hence, the dominant role of $ε$-polylysine was destroying clay structures and charge neutralization, and the heavy particles dropped by gravimetric force.

The volumes of accumulation sedimentation at 0.15%, 0.20%, and 0.25% are plotted in Fig. 4(b). Compared to the flocculation sedimentation, accumulation sedimentation became constant more rapidly. Flocculation sedimentation became constant after more than 1000 minutes, and accumulation sedimentation became constant at about 300 to 400 minutes. Additionally, the final sedimentation volume reached the lowest level of 0.20% and this indicates that at a $ε$-polylysine concentration of around 0.20%, most particles were separated and floating individually in the clay suspensions.

Sedimentation properties

During the sedimentation process, changes occur in the soil void ratio. Clay particles in the suspended state are not in contact and the repulsive forces between the clay particles are large enough to disperse the clay particles. Therefore, the void ratio is large, over 100%. After sedimentation proceeds, the clay particles interact with each other and create denser structures, and hence the void ratio decreases. The decrease in void ratio increases the dry unit weight.

The void ratio was derived using Eq. (3)

$$e = \frac{V_{sed}}{Gs \times \gamma_{water} \times W_{soil}} - 1 \qquad (3)$$

where e is the void ratio, V_{sed} is sediment volume, γ_{water} is the unit weight of water, W_{soil} is the weight of the soil.

For the flocculation sedimentation, changes in the sedimentation property by time were calculated, because it can be assumed that all the clay particles settled together. However, in the case of accumulation sedimentation, it could not be assumed that the accumulated volume contains all the clay particles in the suspension. Therefore, only the flocculation sedimentation cases were compared.
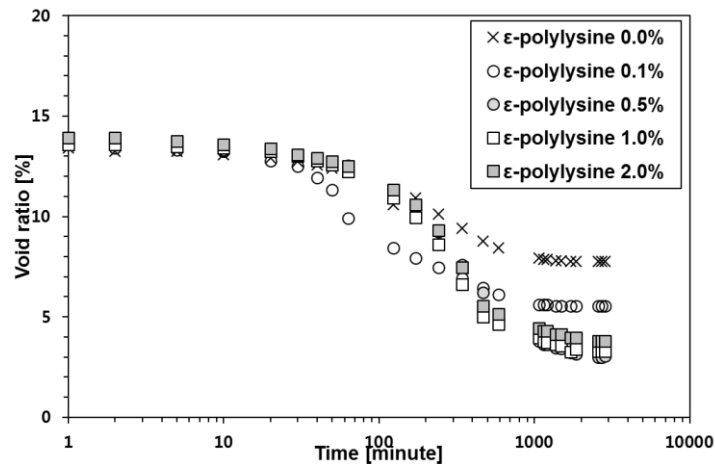

Fig. 5 Variation in void ratio of $ε$-polylysine treated suspensions

Flocculated sediment settles down together and so the volume of the sediment continues to shrink. Shrinkage in volume continuously decreased the void ratio (Fig. 5). The existence of ε-polylysine assisted the formation of flocs and accelerated the variation in sedimentation properties.

Additionally, structures in the flocculation sedimentation differed according to the ε-polylysine concentration. Because low ε-polylysine treated sediments had looser structures than high ε-polylysine treated sediments, the final void ratio decreases as ε-polylysine concentration decreases.

3.1.2 Chitosan

Sedimentation volume

Chitosan is one of the most commonly used, environmentally friendly coagulants for reducing pollutants in groundwater (Ghaee and Zerafat 2016, Roussy *et al.* 2005, Zou *et al.* 2006). To compare the sedimentation effects of chitosan and ε-polylysine, the same sedimentation experiment procedure was followed.

Unlike the ε-polylysine treated suspension, the use of chitosan increases the final sedimentation volume. This occurs due to the higher molecular weight of chitosan compared to ε-polylysine. Generally, bridge flocculation is formed by high molecular weight coagulants. Bridge flocculation renders larger flocs than those formed by ionic destabilization (e.g. via salts). However, although bridge flocculation forms large flocs, flocs have empty voids along bridge connections which results in overall strength reduction (Yukselen and Gregory 2004).

Compared to the final sediment volume of the untreated sample, the largest final volume, 118 %, occurred with the 2.0% chitosan treated suspension. This indicates that the chitosan formed more loose floc structures and the density of the sediment gradually decreased.

Additionally, chitosan used in this study has a higher molecular weight (50,000-190,000 g/mol) than that of ε-polylysine (3,200-4,500 g/mol). Thus, chitosan induces bridge flocculation between kaolinite particles regardless of chitosan concentration, while shows higher final void ratios (lower density) compared to ε-polylysine treatment. Therefore, flocculation sedimentation was observed for every experimented chitosan concentration, 0.0%, 0.1%, 0.2%, 0.5%, 1.0%, and 2.0%. Variation in sedimentation volume by time is plotted in Fig. 6.
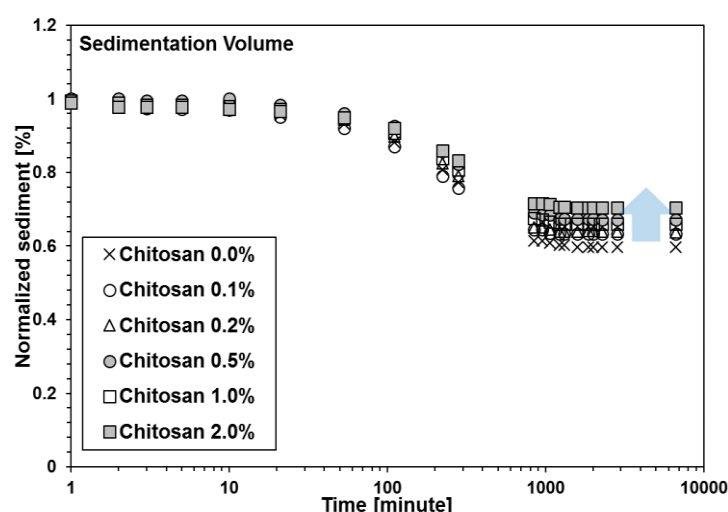

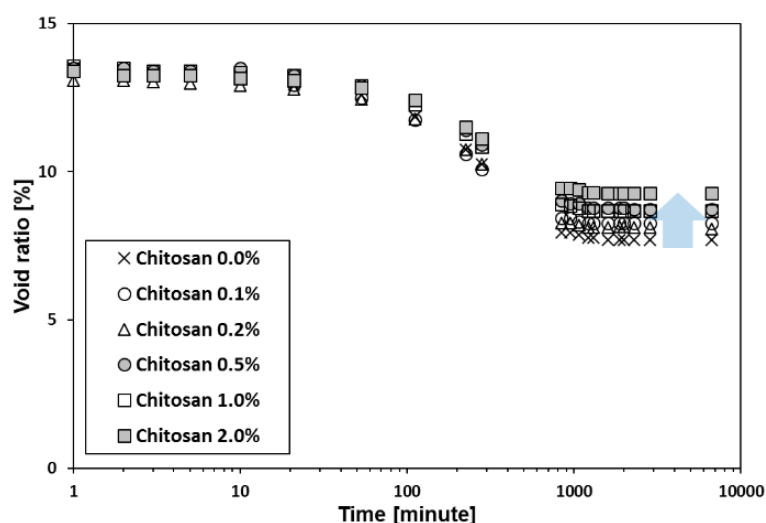Fig. 6 Variation in flocculation-sedimentation volume of chitosan treated suspensions

Fig. 7 Variation in sedimentation properties of chitosan treated suspensions; void ratio

Sedimentation properties

Sedimentation void ratio is plotted in Fig. 7. The void ratio was increased by increasing chitosan concentration.

The lowest final void ratio was also obtained for the untreated case, and the maximum, which was 120.4% larger than that of the untreated sample, was obtained for the 2.0% treated case. The results show that the structure of the chitosan treated sedimentation is looser than the $ε$-polylysine treated sediments, and even pure clay floc structures.

3.2 UV penetration

Laboratory sedimentation tests were proceeded for 8,640 minutes (6 days), since sediment heights converged after 1,000 minutes in most cases. Thus, 6 days were enough to collect uppermost supernatant fluid, which is totally separated from solid particles. The spectral absorbance values of the uppermost supernatant were measured with a spectrophotometer to determine the clarity of the supernatant. Absorbance refers to the amount of light that is absorbed while penetrating a media. It is expressed as the common logarithm of the ratio of incident to transmitted spectral power. For this study, the UV penetration method was used to measure the remaining clay particles in the upper liquid. After constant sedimentation volumes were observed, the uppermost part of the remaining liquid was collected using a micropipette and cuvette. Then, the spectrophotometer measured the amount of light penetrating the cuvette.

For the $ε$-polylysine treated clay suspension (Fig. 8(a)), the absorbance value increases at low concentration of $ε$-polylysine and it reaches the highest absorbance at 0.25% $ε$-polylysine concentration, which is 0.134. At this stage, the injected $ε$-polylysine detach kaolinite particles. Therefore, only heavy particles are submerged and light particles float in a suspended state.

Among the experimental conditions, the most clay particles remained at 0.25%. Additionally, the remaining liquid had a greater amount of impurities in the accumulation sedimentation state. Then, as addition concentrations of $ε$-polylysine were injected, the overall amount of $ε$-polylysine becomes sufficient to form $ε$-polylysine − kaolinite flocs which reduces suspensions in the
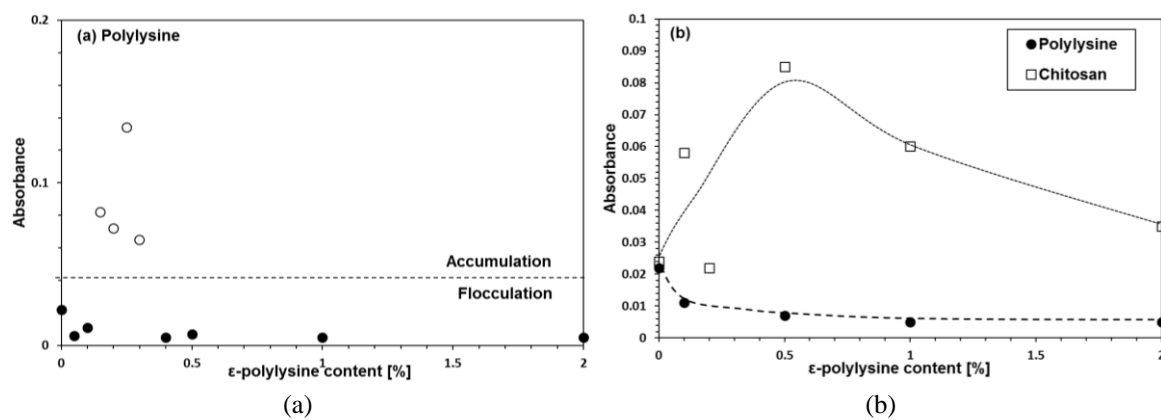
Fig. 8 Variation in absorbance with biopolymer concentration: (a) ε-polylysine; and (b) comparison between ε-polylysine (flocculation sedimentation) and chitosan

Fig. 9 SEM images of 0.05% ε-polylysine treated suspension; (a) and (b), flocculation sedimentation of 0.40% ε-polylysine treated suspension; (c) and (d) and chitosan treated specimen (e) and (f)

supernatant with gradual absorbance reduction with higher *ε*-polylysine concentration.

The lowest values were measured at 1% and 2% *ε*-polylysine concentration. Both absorbance values were 0.005. From the UV penetration results, it was verified that higher *ε*-polylysine injection improves the clay removal efficiency.

In the case of the chitosan treated clay suspension (Fig. 8(b)), the highest absorbance value, 0.085, was obtained at 0.5% concentration and the lowest value, 0.022, at 0.2% concentration. However, even the lowest value was not much higher than the absorbance value of the untreated case, 0.024. Additionally, the absorbance values of chitosan treated sediment is higher than that of *ε*-polylysine. This shows that the effect of chitosan on the clarity of the supernatant was smaller than that of *ε*-polylysine.

3.3 SEM images

A scanning electron microscopic analysis was conducted to compare the sediment structure of low concentration *ε*-polylysine treated specimens, high concentration *ε*-polylysine treated specimens, and chitosan treated sediments. Representative sedimentation samples of 0.05% concentration *ε*-polylysine and 0.40% *ε*-polylysine treated suspensions were selected. The SEM images for those samples are presented in Fig. 9, and they indicate different sedimentation structures, altered by different concentrations and coagulants.

First, the effect of the *ε*-polylysine concentration on the sediment structure is compared in Figs. 9(a) and (b) and Figs. 9(c) and (d). At low concentration, 0.05%, the amount of *ε*-polylysine was not enough to transform the edge to face structures into face to face structures. Thus, the main role of *ε*-polylysine was breakage of the edge to edge contacts. In Figs. 9(a) and (b), face to face aggregated particles are not observed in notable quantities. At high concentration, 0.40%, the injected *ε*-polylysine amount was enough to bond clay particles and form face to face contacts (Figs. 9(c) and (d)).

Face to face structures were more abundantly observed than in the case of lower concentration. Additionally, *ε*-polylysine formed clay conglomerates by bonding clay particles, and the surface of the higher concentration *ε*-polylysine suspension was coarser than that of the lower concentration treated specimen. The microstructure of the chitosan treated sediment was also different from that of the *ε*-polylysine treated samples. The surface of the chitosan treated sediment in Fig. 9(e) and (f) indicates that the face to face structures are indirectly bonded.

4. Discussions

Floc structure affects the final sedimentation thickness and densities (Sidik *et al.* 2014). At small concentrations of *ε*-polylysine, the dominant structure of flocs was an edge to face structure. Naturally, clay has negative charges on its face and positive charges on its edges. Therefore, the edges of the clay particles bond with the faces of the clay particles and these edge to face (EF) bonding structures form flocs (Rand and Melton 1977). Normally, the EF structure has higher porosity than other types of structures. Thus, the final sedimentation volume is the highest in the untreated case.

Fig. 10 presents possible inter-particle interaction mechanisms between clay particles and *ε*-polylysine with *ε*-polylysine content variation, as obtained from this study. When *ε*-polylysine is initially injected into a clay suspension, its positive charges break the EF bonds between clays and reconstitute clay particles by bonding the particles. If the injected amount of *ε*-polylysine is not

enough to bridge or bond clay particles, the dominant effect of ε-polylysine in the floc structure is the breakage of EF bonding. During this stage, clay particles are separated and float in the suspension individually and accumulate from the heaviest particles. Hence, the accumulated particles of this stage became coarse. Light particles remain in suspension and these particles increase the absorption value. Therefore, the clarity of the supernatant was the lowest in the 0.25% ε-polylysine treated suspension. Additionally, the injected ε-polylysine decreases the liquid double layer and the distance between clay particles. Due to the larger amount of remaining clay particles and the compressed liquid double layer, the final sedimentation volume reached the smallest value at 0.20%.

At ε-polylysine content above 0.25%, the amount of ε-polylysine is sufficient to form bonds between clay particles. ε-Polylysine interacts with negative charges on the clay face and connects clay particles by face to face (FF) structures. Thus, FF flocs are the dominant floc structure at this stage. As the ε-polylysine content increases, injected ε-polylysine bonds with clay particles forming face to face structures, and this bonding effect increases the clay floc size. Floc density decreases when the floc size increases (Tambo and Watanabe 1979). Therefore, the final sedimentation volume is increased by the ε-polylysine concentration at this stage.

In the case of sedimentation tests performed with ε-polylysine, both accumulation and flocculation sedimentation were observed. For chitosan treated suspensions, only flocculation sedimentation was discovered. Additionally, ε-polylysine decreased the final sedimentation thickness, whereas chitosan increased the final sedimentation thickness. These phenomena occurred due to the molecular size difference between ε-polylysine and chitosan. Chitosan has a 10-50 times higher molecular weight than ε-polylysine. Therefore, flocs formed by chitosan have higher molecular weight than flocs formed in the ε-polylysine treated suspension. Floc size and floc density have an inverse proportional relation. Small floc density resulted in a large final sedimentation volume.

ε-polylysine and chitosan can be applied for different purposes, to form different sediments. With low concentration ε-polylysine, accumulation sedimentation occurs, and sediments have low final volume while the supernatant liquid is turbid. For this reason, it is inappropriate for waste removal in contaminated groundwater, but could be applied in the reclamation industry, such as for artificial island construction.

The supernatant liquids that remained after flocculation sedimentation had lower absorbance values than those in the case of accumulation sedimentation. This means that the clay and the
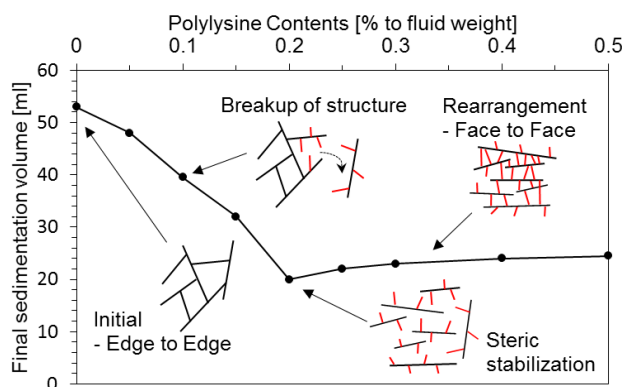

Fig. 10 Interaction mechanisms of clay suspension by the varying the ε-polylysine content

liquid were more effectively separated in the suspension with flocculation sedimentation. This characteristic is suitable for the contaminant removal field.

Additionally, the injection of *ε*-polylysine decreased the final sedimentation thickness. For chitosan, the final sedimentation volume increased. This volume difference indicates that *ε*-polylysine and chitosan produce final sediments with different densities. Lower sediment volume indicates that settled materials can be more effectively removed.

In conclusion, higher concentrations of *ε*-polylysine were more effective than chitosan for the removal of floating matter in suspension, kaolinite in this study. However, it should be noted that the results in this study are based on the use of deionized water. In practical implementations, ground water in field contains numerous ions such as calcium, magnesium, sodium, potassium, bicarbonate, carbonate, sulphate, chloride, and so on. Higher ionic concentration should promote inter-particle flocculation due to enhanced ionic bonds. In this study, deionized water was used to evaluate the pure effect of biopolymer additives on the sedimentation behavior of kaolinite clay regardless of additional ions. However, further considerations with ground water and seawater are required before empirical applications.

5. Conclusions

In this study, *ε*-polylysine was proposed as a new environmentally friendly coagulant to remove pollutants in contaminated groundwater. The rheology and the properties for each situation were analyzed using sedimentation experiments and SEM image analysis. The amount of clay particles remaining in the fluid were measured by the spectrophotometer. Additionally, the performance of *ε*-polylysine as a coagulant was compared with the performance of chitosan.

ε-Polylysine detaches clay flocs at low concentrations and reconstitutes clay flocs at high concentrations. Based on the structural changes, the sedimentation mechanisms of the kaolinite clay suspensions were classified into two types, accumulation sedimentation and flocculation sedimentation. The final sedimentation volume for *ε*-polylysine was smaller than that of chitosan. This indicates that more highly compacted sediments were obtained for *ε*-polylysine treated sedimentation. Therefore, *ε*-polylysine sedimentation has greater applicability to clay removal.

For accumulation sedimentation, separation between the clay and liquid was not clear because light clay particles remained in the suspension. Thus, accumulation sedimentation is not appropriate for clay removal.

This study can be a guideline for designing the injection concentration of *ε*-polylysine coagulant. From the results of this study, the concentration of coagulant that is appropriate for the given purpose can be determined.

Acknowledgments

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