Removal efficiency of Gram-positive and Gram-negative bacteria using a natural coagulant during coagulation, flocculation, and sedimentation processes

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ABSTRACT

Staphylococcus sp. as Gram-positive and *Escherichia coli* as Gram-negative are bacterial pathogens and can cause primary bloodstream infections and food poisoning. Coagulation, flocculation, and sedimentation processes could be a reliable treatment for bacterial removal because suspended, colloidal, and soluble particles can be removed. Chemical coagulants, such as alum, are commonly used. However, these chemical coagulants are not environmentally friendly. This present study evaluated the effectiveness of coagulation, flocculation, and sedimentation processes for removing *Staphylococcus* sp. and *E. coli* using diatomite with standard jar test equipment at different pH values. *Staphylococcus* sp. demonstrated 85.61% and 77.23% significant removal in diatomite and alum, respectively, at pH 5. At pH 7, the removal efficiency decreased to 79.41% and 64.13% for *Staphylococcus* sp. and *E. coli*, respectively. At pH 9, there was a decrease in *Staphylococcus* sp. after adding diatomite or alum compared with that of *E. coli*. The different removal efficiencies of the Gram-positive and Gram-negative bacteria could be owing to the membrane composition and different structures in the bacteria. This study indicates that diatomite has higher efficiency in removing bacteria at pH 5 and can be considered as a potential coagulant to replace alum for removing bacteria by the coagulation process.

Key words | coagulation, diatomite, *Escherichia coli*, natural coagulant, *Staphylococcus* sp., turbidity removal

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INTRODUCTION

Water is composed of hydrogen coupled with oxygen and is an important resource, currently with an inadequate supply. The need for clean water is increasing due to the discharge of industrial wastewater and urbanization. Water treatment is used to remove turbidity and dissolved substances in water. Polyaluminium chloride (PAC) is widely used to treat water. However, it is not eco-friendly or cost-effective when used in large amounts (Pan *et al.* 2006; Wu *et al.* 2011). Furthermore, concerns have been raised regarding the effect of the aluminium (Al) concentration in treated water (Ohno *et al.* 2009). An elevated Al concentration could lead to potential risks to public health (World Health Organization 2004; Gupta *et al.* 2005), such as Alzheimer's disease and other related diseases associated with residual Al (Flaten 2001). Therefore, it is important to obtain affordable and effective natural coagulant.

Recently, natural polymers have been used to treat water (Antov *et al.* 2018; Boulaadjoul *et al.* 2018; Braga *et al.* 2018; Mohd-Asharuddin *et al.* 2018; Zhang *et al.* 2018). Seventeen years ago, slow sand filters were applied for water purification (Wotton 2002). Diatomite or diatomaceous earth (a natural coagulant) is a silica mineral with a highly porous structure composed of 80–90% voids and a density of 1.9–2.3 g/cm^3 (Khraisheh *et al.* 2004). Diatomite has a high permeability (0.1–10 mD), porosity of approximately 35–65% (Murer *et al.* 2000), low thermal conductivity, small particle size (Hassan *et al.* 1999), and high surface area (Gao *et al.* 2005). In addition, diatomite can be modified through physical and

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chemical modification. These unique properties make diatomite a suitable candidate for water treatment.

Staphylococcus sp., which is a Gram-positive bacterium, is one of the most common causes of food poisoning. The Staphylococcus strain can form biofilms, providing protection to the bacteria (Yong *et al.* 2019). These bacteria multiply rapidly at room temperature to produce a toxin that can cause illness. *Staphylococcus* sp. is found in the environment and in normal human flora, on the skin and mucous membranes of individuals in good health. Transmission can occur from direct contact; however, some infections involve other transmission methods (Rasigade & Vandenesch 2014). *Staphylococcus aureus* is the most harmful of the numerous staphylococcal bacteria and is the leading cause of hospital-acquired infections in developed countries (DeLeo & Chambers 2009). Approximately 30% of the human population is colonized with *Staphylococcus aureus* (Wertheim *et al.* 2005). Regardless, the prevention of *Staphylococcus* sp. infections remains complicated.

Escherichia coli is the most common Gram-negative bacterium and is frequently found in drinking water. *E. coli* is a member of the family *Enterobacteriaceae* and is a rod-shaped, facultative anaerobe, lactose-fermenting, and non-endospore-forming microorganism. It is generally found in the gut of humans and warm-blooded animals. *E. coli* can be differentiated by its growth and color reaction on certain media and can be differentiated from most other coliforms by its ability to ferment lactose at 44 °C in the fecal coliform test. Serious illnesses are attributed to it (Rebello & Regua-Mangia 2014), such as severe diarrhea and kidney damage owing to contaminated water supplies. Therefore, the waterborne pathogen should be understood to ensure safe water supply.

Low removal efficiency of the pathogen in water treatment is common, thus impacting society. Pathogenic contamination of water causes disease outbreaks and contributes to background disease rates around the world, severely impacting the developing world. Strategies to combat antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARGs) in wastewater have been reviewed by Barancheshme & Munir (2018). There are several treatment processes that have been used to remove ARB and ARGs. One of the treatment processes is coagulation. Li *et al.* (2017) used an inorganic coagulant ($FeCl_3$) and inorganic polymer coagulant, polyferric chloride (PFC). They examined the removal of *sul* genes, *tet* genes, and integrase genes. A significant removal correlation was shown between the dissolved NH_3-N and dissolved organic carbon and ARGs.

In Australia, inadequate on-site wastewater treatment systems (OWTS) release pathogens into the environment with a failure rate of up to 40% (Jelliffe 1995). Because of the lack of simple and dependable methods, a majority of the waterborne pathogens are difficult to detect or quantify (Saxena *et al.* 2015). To ensure there is no presence of pathogens, including bacteria, viruses, and protozoa, which could potentially cause disease if digested, water should be monitored before human consumption. Although most of the pathogens can be removed during wastewater treatment, numerous pathogens are discharged into wastewater and entering and receiving waters. Establishing suitable methods of treating wastewater and preventing pathogens from entering the drinking water system is a key component of optimizing water usage in the future. Owing to its simplicity, coagulation–flocculation is used worldwide in the water treatment industry, and it is a common process applied before water can be distributed to consumers (Ndabigengesere & Narasiah 1998). Eman *et al.* (2014) reported that variations in turbidity are a serious complication of surface water treatment.

Therefore, this study evaluated the efficiency of diatomite in removing bacteria, which are *Staphylococcus* sp. as Gram-positive bacteria, and *E. coli* as Gram-negative bacteria. The pH affects the surface charge of the coagulant. A pH study is required to determine the optimum pH condition of the treatment system. This present study has demonstrated, for the first time, the response of *Staphylococcus* sp. and *E. coli* to coagulation, flocculation, and sedimentation processes using diatomite, focusing on the optimization of pH.

MATERIALS AND METHODS

Micro-organisms

Staphylococcus sp. and *E. coli* were isolated using the procedure described in the previous study (Zulkeflle *et al.* 2016; Hara *et al.* 2018). *Staphylococcus* sp. was collected from hospital wastewater samples from the University of Malaya Medical Centre hospital sewage treatment plant, while *E. coli* was collected near the Gombak River near Kuala Lumpur, Malaysia. For the measurement of colony forming units (CFU), *Staphylococcus* sp. were recovered using membrane filtration while *E. coli* were vacuum-filtered through cellulose nitrate membrane filters. Then, *Staphylococcus* sp. were grown and assayed on mannitol salt agar (MSA), while *E. coli* was assayed on eosin methylene blue

(EMB) agar. Prior to seeding into synthetic turbid water, a culture from the agar plate was inoculated into 25 mL of sterile Luria Bertani broth until the optical density OD_{600} reached 1.0.

Field-Emission scanning electron microscope (FESEM)

The sample diatomite powder specimen obtained from YunNan Qing Zhong Science Tech Co. Ltd (Beijing, China) was observed microscopically. The sample was sputter-coated with platinum to avoid charging using an auto-fine coater by JEOL (Tokyo, Japan) for viewing under a field-emission scanning electron microscope (FESEM). SEM imaging was performed using the back-scattered electron emission mode with JS-7800F PRIME from JEOL (Tokyo, Japan). The sample was further analyzed for the compositional element by using energy dispersive X-ray spectroscopy (EDS).

Synthetic turbid water

To create synthetic turbid water, 10 g of kaolin (R&M Chemicals, Malaysia) was prepared by adding 1 L of distilled water. The suspension was mixed rapidly at 200 rpm for 60 min to uniformly disperse the particles. The solution was settled for 24 h for complete hydration before extracting the supernatant. This solution is stock turbidity water, and it was diluted again with distilled water to prepare synthetic turbid water at 50 ± 10 NTU before the coagulation test.

Jar test

Powder alum ($Al_2(SO_4)_3.18H_20$) (Sigma-Aldrich) was used as a control because this salt is commonly used in practice. One percent of alum stock solution was prepared by dissolving 1 g of alum in 100 mL of distilled water. The sample specimen of diatomite powder purchased from YunNan QingZhong Science Tech Co. Ltd (Beijing, China) was stored in a plastic resealable storage bag and placed in the desiccator until further use. A diatomite stock solution for coagulant dosage was prepared by diluting 1 g of diatomite in 1 L of distilled water.

Coagulation tests were performed using a standard jar test apparatus with six stainless steel $1'' \times 3''$ paddles (PB 900, Phipps & Bird, USA) to optimize turbidity reduction, such as the coagulant dose, pH, flocculation time, and flocculation speed for both diatomite and alum. The standard procedure involved 4 min of rapid mixing at 125 rpm, followed by 25 min of slow mixing at 40 rpm for the flocculation process. Then, the solution was allowed to settle for 60 min (Muyibi *et al.* 2003). The supernatant sample was collected for analysis using a syringe from an approximately 2 cm depth. The turbidity was measured in a 2100P turbidimeter (HACH, USA) and calculated using Equation (1) as follows:

$$\text{Removal (\%)} = \frac{\text{Initial concentration of the sample} - \text{Final concentration of the sample}}{\text{Initial concentration of the sample}} \times 100 \quad (1)$$

To evaluate the removal of the bacteria, a 1 L beaker containing synthetic turbid water (50 ± 10 NTU, in 1 L) was spiked with bacteria (*Staphylococcus* sp. or *E. coli*, ~10^6 CFU/mL) without coagulant. The addition of the bacteria did not affect the turbidity. The other beaker was injected with an optimum dose of alum or diatomite stock solution (1.0–2.5 mg/L). The same coagulation standard procedure was performed. A 300 μL sample was obtained from below the water (approx. 2 cm depth) for physical and microbiological analyses. The jar test was conducted at room temperature (30 ± 2.0 °C). All the experiments were conducted at three different pH values (pH 5, pH 7, and pH 9) and in triplicate to ensure the reproducibility of the results. The pH of the sample was measured using a pH meter (Eutech Instrument Pte. Ltd).

Physical and microbiological measurements

After the coagulation test, a microbiological analysis was performed by first serially diluting tenfold in sterile saline (0.7% NaCl). *Staphylococcus* sp. was assayed on MSA, and *E. coli* was assayed on EMB agar (0.1 mL). This experiment was performed using the spread plate method. The colonies of test bacteria could be differentiated from those of the background bacteria by their specific colony morphology based on color on their specific media. The colony formation was counted after overnight incubation.

Statistical analysis

Statistical analyses were performed using EXCEL (Microsoft Corporation). EXCEL was applied to create descriptive statistics and to conduct independent *t*-tests to compare the removal rates of *Staphylococcus* sp. and *E. coli*. The removal efficiency was calculated using Equation (2),

and the statistical significance level was set at $P = 0.05$:

$$\text{Percentage of removal } (\%) = \frac{A - B}{A} \times 100 \quad (2)$$

Here, A is the number of viable CFUs before treatment, and B is the number of viable CFUs after treatment.

RESULTS AND DISCUSSION

FESEM analysis

FESEM observed the surface of the diatomite. Figure 1 shows the porosity of diatomite with open pores, indicating a high porosity and large surface area. This structure provided high strength for diatomite to remove contaminants. Furthermore, Qian *et al.* (2016) reported several modification methods including acid treatment, calcination, alkali leaching and nano-silica decoration on the fine structure of diatomite in order to enhance its pore size and surface area. Moreover, there were fewer impurities. Thus, the pores on the surface of diatomite became more obvious, and the average size of the silica particles in the sample was in the range of 1–10 μm. The chemical characterization (data not shown) showed that the refined diatomite consisted of oxygen, followed by silica and carbon. The composition mixture of this compound provided stable refined diatomite. Generally, 86% to 94% of the diatomite is comprised of silica (Kennedy 1990). The honeycomb silica structure provides the chemical stability, high absorptive capacity and surface area, and low bulk density of the diatomite. A small number of other elements could be secreted in the diatom skeleton. The reactivity of diatomite is connected to the reactive sites on its surface (e.g., hydroxyl groups), which are the main reactive sites similar to that of synthetic amorphous silica. In addition to the hydroxyl groups, the acid sites, iron or aluminum oxides, are also considered reactive sites on the surface of diatomite (Yuan *et al.* 2004).

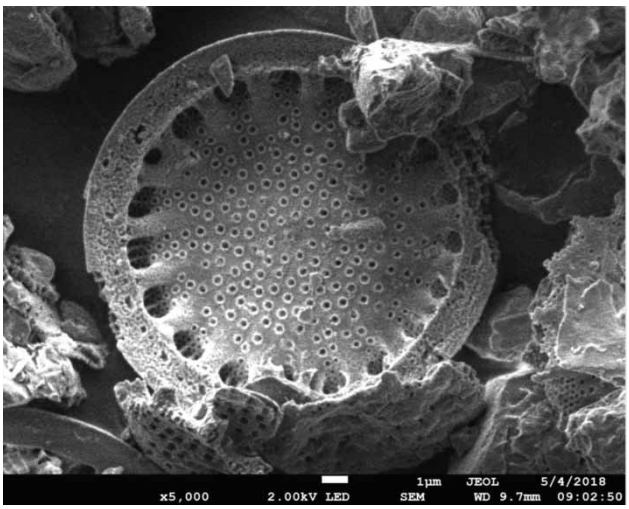

Figure 1 | The morphology of diatomite obtained via FESEM.

Optimum turbidity of diatomite at 50 NTU

In this study, a kaolin suspension was used to represent synthetic turbid water. Synthetic model water does not completely represent real surface water; however, it is a stable suspension suitable for studying turbid water, and the turbidity and pH can be controlled easily compared with that of surface water. Generally, according to the Interim National Water Quality Standards (INWS) in Malaysia, 50 NTU is used as a standard starting turbidity (Supplemental Table 1(a) and (b)) and considered as needing conventional treatment. The results in Supplemental Figure 1 show that 2.5 mg/L diatomite successfully removed 84.0% of synthetic turbid water, providing 8.0 NTU. This result demonstrated that diatomite could operate efficiently to treat low turbidity water. Low turbidity waters are difficult to coagulate because of the low concentrations of stable particles, and frequently, turbidity is synthetically added to water to form heavier flocs that can settle. This is supported by previous findings using a jar test in low turbidity water (45 NTU) showing a 76% removal with *Moringa oleifera* (Pritchard *et al.* 2010). *Moringa oleifera* is well studied and the best natural coagulant discovered so far that can replace usage of alum, which is used widely for water treatment around the world. However, it was found that the residual turbidity of samples is inversely proportional to the initial turbidity at the optimum dosage of *Moringa oleifera* (Gaikwad & Munavalli 2019). This indicates that *Moringa oleifera* may not be an efficient coagulant to treat low turbidity water and other potential natural coagulants are preferable. For the *Moringa oleifera* coagulant, high turbidity water has high coagulation activity. However, the activity is low for low turbidity water (Muyibi & Evison 1995). Optimization of the coagulant dosage is required as the coagulation process is a surface phenomenon. Thus, coagulation performance can be affected by the surface change owing to the mass of the coagulant. When the diatomite concentration exceeded the optimum dosage, the turbidity increased because all colloids were neutralized and precipitated with the optimum dosage. Thus, the excess coagulants cause turbidity in

Table 1 | Log_{10} of CFU removal of *Staphylococcus* sp. and *Escherichia coli* at 50 NTU in pH 5, pH 7 and pH 9 after coagulation process by diatomite or alum

	***Staphylococcus* sp.**			***Escherichia coli***		
Coagulants	**pH 5**	**pH 7**	**pH 9**	**pH 5**	**pH 7**	**pH 9**
Diatomite	0.99 ± 0.42^a	0.80 ± 0.41	0.17 ± 0.05	0.87 ± 0.30	0.51 ± 0.31	0.67 ± 0.25
Alum	0.70 ± 0.27^a	0.29 ± 0.23	0.27 ± 0.04	0.43 ± 0.18	0.21 ± 0.08	0.62 ± 0.17

[a]$P < 0.05$ compared with diatomite/alum.

water, as they do not interact with oppositely charged colloidal particles.

Effect of different pH values on the removal of bacteria

Based on plate counting, there was a greater than 30% removal efficiency of the bacteria for each plate. When cultured, *Staphylococcus* sp. on MSA turned phenol red into yellow, while a positive result for *E. coli* showed metallic green colonies on a dark purple medium and an EMB plate. These tests were performed at three pH values, 5, 7, and 9, which were adjusted using HCl or NaOH. Table 1 shows log_{10} of CFU removal of bacteria before and after adding coagulant in pH 5, pH 7 and pH 9 at 50 NTU. At a lower pH (acidic condition), there was a significant difference in the removal of the bacteria (*Staphylococcus* sp.) using diatomite and alum of 85.61% ±14.62% (0.99 ± 0.42 log_{10} of bacterial removal) and 77.23% ±13.56% (0.70 ± 0.27 log_{10} of bacterial removal), respectively with initial concentration 4.26 log_{10}. The acidic condition of the water was preferable for diatomite to work effectively. For certain organic and inorganic compounds, 'natural mineral clay' (diatomite) has cation exchange and adsorptive affinity (Jiang *et al.* 2004). In addition, *E. coli* demonstrated a higher removal of 84.65% ±8.05% (0.87 ± 0.30 log_{10} of bacterial removal) at pH 5 using diatomite with initial concentration 4.36 log_{10}. At pH 7, there was a reduction in the removal efficiency of *Staphylococcus* sp. and *E. coli* in diatomite of 79.41% ±15.20% (0.80 ± 0.41 log_{10} of bacterial removal) with initial concentration 4.62 log_{10} and 64.13% ± 22.96% (0.51 ± 0.31 log_{10} of bacterial removal) with initial concentration 2.77 log_{10} respectively. At a higher pH (pH 9), *Staphylococcus* sp. was killed more rapidly than *E. coli.* at 31.67% ±7.64% (0.17 ± 0.05 log_{10} of bacterial removal) with initial concentration 3.09 log_{10} and 75.83% ±14.22% (0.67 ± 0.25 log_{10} of bacterial removal) with initial concentration 3.10 log_{10}, respectively. This is because residual turbidity increases at higher pH due to diatomite not aggregating. Diatomite is a silica-containing material, and thus it does not coagulate when in contact with an alkaline suspension (Khraisheh *et al.* 2004).

Effect of different pH values on the zeta potential

Changes in the pH of the solution influence bacteria removal by affecting the surface charge of the coagulation and stabilization of the suspension (Saritha *et al.* 2017) and by affecting the zeta potential. Diatomite has a strong negative surface charge in water; therefore, it will remain negative when the pH decreases to as low as 3. Hence, diatomite could significantly remove bacteria in acidic conditions. The surfaces of diatomite are negatively charged in a broad pH range 2–12 (Sheng *et al.* 2009). The zeta potential of diatomite becomes progressively negative with increasing pH (Gao *et al.* 2005, 2018; Sheng *et al.* 2009). Therefore, the quantity of negatively charged functional groups on the diatomite surface increases when pH increases. Thus, in this study at pH 5, the removal efficiency in both bacteria increases because of the higher progression in the coagulation process owing to the higher zeta potential. At pH 9, the zeta potential of flocs constituted after coagulation could decrease and move from positive to negative. Pearson *et al.* (1987) showed that a pH range of 9.0–9.5 is considered a lethal level for pathogen survival. Moreover, there have been several attempts to investigate the effect of pH on the microorganism. The pH of water is one of the key factors that affect the removal of viruses in coagulation and pH values around 5–6.5 are widely recommended for coagulants with metallic coagulants (USEPA 1980; Harrington *et al.* 2001; Bell *et al.* 2002). This shows that pH is a critical factor as it affects the result intrinsically.

Different removal rates between *Staphylococcus* sp. and *E. coli*

Conventional physiological and chemical water treatment processes could be effective against *Staphylococcus* sp. because *Staphylococcus* sp. attaches to particles based on

the membrane composition and the different structures of Gram-positive and Gram-negative bacteria (e.g., Gram-negative bacteria contain a thinner cell wall than that of Gram-positive bacteria) in a neutral condition. In addition, *Staphylococcus* sp. is highly hydrophobic. *E. coli* has a less hydrophobic cell wall, and there is less tendency to attach to the particle. This was supported by LeChevallier (2004) and Vaerewijck *et al.* (2005) who studied *Mycobacterium avium* subspecies *hominissuis* (MAH). They hypothesized that conventional physiological and chemical water treatment could be effective against MAH because MAH attaches to particles owing to the highly hydrophobic cell wall. The pH during coagulation impacts effectiveness during the destabilization process. In addition to controlling the speciation of the coagulant and the solubility, the pH also affects the speciation of the contaminants. To lower the pH to the optimal pH ranges (alum pH 6–7 and iron pH 5.5–6.5) for high alkalinity water, an excessive amount of coagulant could be required (USEPA 2017). Ismail *et al.* (2014) used a coagulation process as a pre-treatment to remove a high content of suspended solids. The results showed that the pretreatment process could remove the suspended solids in palm oil mill effluent (POME) at an optimum pH of 6.5.

Regardless of the exposure to temperature and humidity, *Staphylococcus* sp. can survive and persist in the environment for months to years (Wagenvoort *et al.* 2005). As the water activity and pH work synergistically, microorganisms also have different pH limits, below which they are not able to grow. Bulson *et al.* (1984) found that during the alum treatment of a lake, 90% of the fecal coliform (FC) population and ca. 70% of the fecal streptococci populations were removed from the water column within 72 h. Based on Van Oss (1994), the Van der Waals free energy interaction and the Lewis acid–base free energy interaction is due to bacterial adhesion to surfaces. Bacteria either donate or accept electrons on the surface of the substrate (gas bubbles). The pH value also affects the removal of bacteria. The high porosity, large surface area, and high chemical stability of diatomite make it applicable for the adsorption of organic pollutants. Furthermore, the crystal structure of diatomite comprises ion-exchangeable cations, which can be exchanged with organic and inorganic cations (Erdem *et al.* 2005). A study by Bhatia *et al.* (2007) using *Moringa oleifera* on POME by a coagulation–flocculation process showed that, at a low pH, the solution appeared clear with smaller colloidal particles. However, as the pH increased toward an alkaline value, the POME turned a darker color. This was because of the higher suspension creating poor removal. Therefore, the study of pH is essential for determining the optimum pH condition for the removal of microbes. Saritha *et al.* (2017) showed that the highest performance of alum for removing turbidity from the water was achieved at pH 7, followed by pH 6.

Marois-Fiset *et al.* (2013) evaluated the significant effect of pH on water treatment performance. Minimal studies on the removal of bacteria using other coagulants are available. The first systematic study of the removal of *Mycobacterium avium* subspecies *hominissuis* (MAH) was reported by Wong & Shin (2014) using alum. The most recent study by Garcia-Alonso *et al.* (2018) reported a good affinity between ciprofloxacin and diatomite. Charge neutralization, precipitation, bridge-aggregation, adsorption, and sweep-flocculation are several mechanisms that explain the coagulation of particles and organic substances. Diatomite enhances the physical and chemical properties of the flocs, in particular antiparticle bridging and settling velocity. Thus, the cells are incorporated into flocs more efficiently, producing settleable flocs of greater density, size, and strength (Wu *et al.* 2011). The pH of the water influences the surface charge of the coagulants, apart from the degree of stabilization of the suspension (Altaher 2012). The effect of raw diatomite on coagulation performance and residual aluminum has been studied by Hu *et al.* (2015). A broader perspective has been adopted by Sruthi *et al.* (2018), who reported that bacterial removal by electrocoagulation was significantly greater than that by chemical coagulation using alum at optimum doses. In addition, the surface charge of the microorganism is an important factor in the removal of microorganisms in the coagulation process, in addition to charge neutralization (Amirtharajah & O'Melia 1990). *Streptococcus pneumoniae* (pneumococcus) has a negative charge (Li *et al.* 2013). The outer cell surface of pneumococcus displays various degrees of negative charge, owing to the presence of acidic sugars, pyruvate, or phosphate in the capsular polysaccharides of different serotypes (Kamerling 2000), with additional contributions from cell surface structures (Swiatlo *et al.* 2002). Shin & Sobsey (2015) studied the removal of the norovirus from water by coagulation, flocculation, and sedimentation. The study showed that the coagulation, flocculation, and sedimentation processes reduced the number of noroviruses. Therefore, additional studies on the mechanism of *Staphylococcus* sp. removal by coagulation, flocculation, and sedimentation processes are recommended.

At pH 9, there was a lower removal of *Staphylococcus* sp. than that of *E. coli*. Gram-negative bacteria have an outer membrane and a periplasmic space that exists

between the cytoplasmic and outer membranes. This periplasmic space is involved in a variety of functions, such as nutrient binding, transport, electron transport, and alteration of substances toxic to the cell (Seltmann & Holst 2002; Matsuno & Yumoto 2015). In contrast, Gram-positive bacteria do not have this structure. Thus, periplasmic space could play a key role in bioenergetic adaptation to an alkaline environment. The removal of *Staphylococcus* sp. was greater in alum than in diatomite at pH 9. Aluminum salts offer multiple products, including cationic species that can adsorb on negatively charged particles, neutralizing the charge (Malik 2018). However, the removal of *E. coli* at pH 9 differs from *Staphylococcus* sp. bacteria, where diatomite has higher removal than that of alum, requiring further investigation of the mechanism behind this occurrence.

The *Staphylococcus* sp. in this study was grown in a nutrient-rich laboratory medium (MSA) and on the exponential growth phase. However, *Staphylococcus* sp. in natural water is generally grown under oligotrophic conditions and on a stationary growth phase. Therefore, the response of *Staphylococcus* sp. in natural water to the coagulation process could be different than the results of this study. Moreover, the pH in the coagulation process in a real water treatment plant could be different from the pH used in this study. Hence, it is necessary to understand the physico-chemical treatment processes by using natural coagulants in order to remove *Staphylococcus* sp. and *E. coli* that are not only applicable in laboratory-based experiments but also in field-based.

CONCLUSION

In this study, the removal of bacteria using coagulation, flocculation, and sedimentation processes with diatomite at different pH values (5, 7, and 9) was evaluated. Based on the laboratory experiment, the result of this study indicated that the coagulation, flocculation, and sedimentation processes using diatomite at pH 5 could be reliable and cost-effective treatment processes for removing microbes at low turbidity, particularly *Staphylococcus* sp. The result showed that diatomite has higher efficiency in removal of bacteria at pH 5. In the case of high water-turbidity, based on our knowledge, the effectiveness might be comparable with alum, and diatomite can be considered as a potential coagulant to replace alum for removing bacteria by the coagulation process. However, further studies are required to understand the mechanism behind this process.

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SUPPLEMENTARY MATERIAL

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