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Effects of Small-Size Suspended Solids on the Emission of *Escherichia coli* from the Aeration Process of Wastewater Treatment

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ABSTRACT

The concentrations of bioaerosols near pretreatment facilities are often higher than those near activated-sludge aeration tanks in wastewater treatment plants (WWTP). The reason for this difference is not yet clear and the differences between the characteristics of suspended solids (SS) in these two processes might play a critical role. In this study, a lab-scale wastewater treatment system was tested with *Escherichia coli* (*E. coli*) in order to investigate the effects of the type and concentration of SS on the concentration and size distribution of emitted bioaerosols. Two types of SS, activated sludge from a hospital WWTP and kaolin clay, were selected to represent floc-type mixed-liquor SS (MLSS) and non-floc-type SS, respectively. An Andersen six-stage sampler was used to analyze the size distribution of the airborne *E. coli* aerosols. When the tested aeration rate was as low as 0.5 L min^{-1}, it was found that the presence of bioaerosols slightly decreased in air/water ratio (AWR, CFU m^{-3} air/CFU 100 mL^{-1} water) when the SS increased from 500 to 2000 mg L^{-1} for both floc and non-floc SS. However when the aeration rates went up to 5–15 L min^{-1}, the pattern of AWR vs. SS curve was different with an increase trend for non-floc SS and a decrease trend for floc SS, with an exception of increase trend for floc SS as the MLSS increased from 0 to 500 mg L^{-1}. The major sizes of emitted bioaerosols ranged from 0.65 to 1.1 μm. A peak was observed at an aerodynamic diameter greater than 7 μm when the aeration rate was 15 L min^{-1}. In conclusion, floc-type activated sludge can inhibit the emission of *E. coli* from an aeration tank. In contrast, the non-floc-type small-size kaolin clay can enhance the emission of *E. coli* from the pretreatment process.

Keywords: Bioaerosols; Suspended solids; Wastewater treatment plant; *E. coli*.

INTRODUCTION

Wastewater, especially hospital wastewater, contains a variety of potentially pathogenic microorganisms (Laitinen *et al*., 1994; Pillai *et al*., 1996; Tsai *et al*., 1998). Particles containing bacteria can be aerosolized from the bubbling surface during the aeration process in wastewater treatment plants (WWTP) (Ulevicius *et al*., 1997), which increases the risk of communicable infections and other health effects associated with wastewater treatment to sewage workers and the public. (Katzenelson *et al*., 1976; Pahren and Jakubowski, 1980; Teltsch *et al*., 1980).

The characteristics of airborne bacteria generated from WWTPs have been studied to some extent (Muller, 1980; Fannin *et al*., 1985; Laitinen *et al*., 1992; Sawyer *et al*., 1993; Ranalli *et al*., 2000; Goyer and Lavoie, 2001; Gangamma *et al*., 2011; Li *et al*., 2013; Guo *et al*, 2014; Li *et al*., 2016). It was found that the concentration of bacterial aerosols can be higher in pretreatment than in an activated sludge tank. (Pascual *et al*., 2003; Fracchia *et al*., 2006; Korzeniewska, 2011; Teixeira *et al*., 2013). The effect of aeration parameters on the characteristics of emitted bioaerosols, and the emission of bioaerosols were found to be dependent on the operating conditions, related to the amount of dispersion of water and particles into the air (Goyer and Lavoie, 2001).

The major function of aeration is to maintain appropriate aerobic conditions and prevent anaerobic odor from occurring in pretreatment tanks, such as aerated grit chambers and equalization basins, while offering sufficient oxygen and mixing in activated sludge tanks. However, aeration will inevitably generate emission of bioaerosols. The emission of health-related bioaerosols from WWTPs in hospitals even

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become a major concern after epidemic outbreaks such as severe acute respiratory syndrome (SARS) in 2004 in Taiwan. However, the reason for the difference between bioaerosol concentrations in pretreatment and in an activated sludge tank remains under-documented (Li *et al*., 2016), especially for the characteristics of suspended solids (SS). This study evaluates the concentrations and particle size distributions of *Escherichia coli* aerosols emitted from an aeration treatment tester under controlled concentrations of floc-type and non-floc-type SSs and aeration rates. To our knowledge, this is the first study to investigate the characteristics of suspended solids for the emission of bioaerosols in laboratory scale studies.

MATERIALS AND METHODS

Test System

The test system used for these experiments is shown schematically in Fig. 1. A lab-scale activated sludge treatment tester, measuring 140 (L) × 85 (W) × 520 (H) mm and made of polyvinyl chloride was used to generate the bioaerosols. A perforated tube (13 mm in diameter) with five bubble holes (1.5 mm in diameter) was located near the bottom inside the aeration tank. Four aeration rates of 0.5 (the minimum value under well-controlled conditions), 5, 10, and 15 L min^{-1} were used for this study. The air sampling system was directly connected to the top of the tank, and a makeup air inlet with a HEPA filter was installed on the other side to balance the mass of the air flow and evaporate the water moisture from the emitted bioaerosols, in order to simulate the process in the field.

Test Bacteria and Suspended Solids

We obtained the activated sludge and a strain of acclimated *E. coli* from a local hospital WWTP, and the strain was identified by the medical laboratory. *E. coli* is often one of the major microbial aerosols generated from WWTPs (Fannin *et al*., 1985; Brandi *et al*., 2000; Fracchia *et al*., 2006), and has been used as a challenge bioaerosol in other laboratory studies (Li *et al*., 1999; Li and Lin, 1999; Hung *et al*., 2010). The preparation of solution containing *E coli* followed the procedure described by Hung *et al*. (2010). The bacterial cells were harvested after 18-hr incubation since the number and cultivability of harvested cells would be suitable to be the challenge bioaerosols after 18- to 24-hr incubation. The cells were then suspended in the solution at a concentration of approx. 8.50 ± 0.73 × 10^6 CFU mL^{-1}, which was about 25 times that of the total coliforms in an activated sludge tank in the field (3.6 × 10^5 CFU mL^{-1}) (Tsai *et al*., 1998), where the colonies recovered from the bacterial species already present in the activated sludge were rare compared to the added *E. coli*, and the colonies from the other species were easy to be differentiated from the *E. coli* using selective agar. Two types of SS were used, namely activated sludge collected from the activated sludge tank of a hospital WWTP (denoted here as mixed-liquor suspended solids, MLSS) (Peavy *et al*., 1985) and kaolin clay. In the first stage of experiments, the kaolin clay served as the SS. The particle size of the kaolin clay ranged from 0.1 to 4.0 μm, which was used to simulate small-size non-floc-type particles in pretreatment process. In the second stage, the floc-type activated sludge was separated from wastewater and added back into the remaining wastewater to obtain concentration of MLSS of 500, 1000, 1500, and 2000 mg L^{-1}. The aeration provides mixing and oxygenation for the activated sludge and kaolin clay with *E. coli* suspension, similar to the operation in the full-scale WWTP.

Bioaerosol Sampling

An Andersen six-stage viable sampler (Andersen Samplers, Inc., Atlanta, GA) with a flow rate of 28.3 L min^{-1} was used to collect the emitted bioaerosols (Lundholm and Rylander, 1983; Andersen, 1958; Fannin *et al*., 1985; Lin and Li, 1996; Brandi *et al*., 2000; Heinonen-Tanski *et al*., 2009; Hung *et al*., 2010; Chien *et al*., 2011; Li *et al*., 2013; Guo *et al*., 2014). The airborne *E. coli* were impacted onto plates filled with an *E. coli* selective agar, m-Endo Agar LES (Difco, Detroit, MI) (Tsai *et al*., 1997) for 1 to 15 minutes depending on

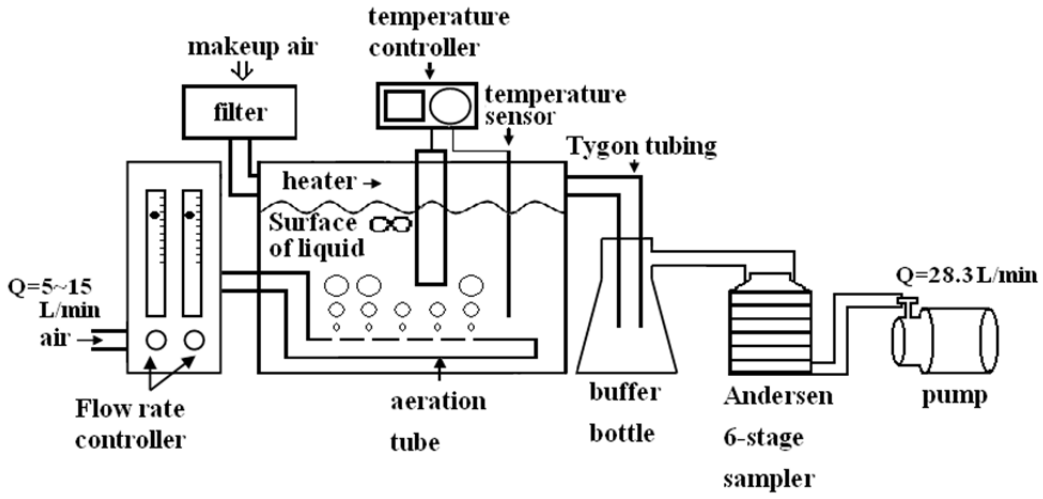

Fig. 1. Schematic drawing of the experimental and measurement systems. Cells of *E. coli* and test sludge were introduced into the aeration tester before each test.

the concentration of *E. coli* in the emitted bioaerosols. Shorter sampling time, such as 1−2 min could reduce the bioaerosol culturability during the field sampling (Mainelis and Tabayoyong, 2010). However, sampling time less than 15 min would not significantly influenced the recovery of airborne *E. coli* cells in the laboratory experiments (Li and Lin, 1999). After sampling, the samples were incubated for 24 to 48 hours at 37°C before colony counting. For each evaluated condition, the experiments were performed at least three times with sequential sampling.

Definition of Emission Potential of Bioaerosols

The emission potential of bioaerosols from the aeration tester was defined as air/water ratio (AWR) and calculated using the following equation (Sorber and Sagik, 1980):

$$AWR = \frac{C_{air}(CFU / \mathrm{m}^3)}{C_{water}(CFU / 100\ \mathrm{mL})} \quad (1)$$

where, C_{air} is the bioaerosol concentration in air (CFU m^{-3}), and C_{water} is the bacterial concentration in water (CFU 100 mL^{-1}), with CFU denoting for colony forming unit.

RESULTS

The AWRs of bioaerosols resulting from the treatment of wastewater containing kaolin clay are shown in Fig. 2(a). When the aeration rate was 0.5 L min^{-1}, the AWRs were less than 3×10^{-6} and slightly decreased as the amount of SS increased from 0 to 2000 mg L^{-1}. When the aeration rate rose to 15 L min^{-1}, the AWRs also increased from less than 1×10^{-5} to as high as 2.5×10^{-5}. The relationships between AWR and MLSS under various air flow rates from wastewater containing activated sludge are shown in Fig. 2(b). When the aeration rate was 0.5 L min^{-1}, the AWR was less than 1×10^{-6} and decreased as the amount of MLSS increased from 0 to 2000 mg L^{-1}. Under the same MLSS conditions, the AWRs were between 1×10^{-6} and 2×10^{-6} and the peak appeared at 500 mg L^{-1} for aeration rates of 5 L min^{-1}. Similar patterns occurred for aeration rates of 10 and 15 L min^{-1}. In addition, Fig. 2 shows a general pattern that the AWR increased as the aeration rate increased both for kaolin clay and activated sludge.

The size distribution of bioaerosols emitted from kaolin clay and activated sludge obtained from the Andersen six-stage sampler are plotted in Fig. 3 and Fig. 4, respectively. The mode of the particle size distributions from the sampler is located in the range of 0.65 to 1.1 μm for the four test aeration rates.

DISCUSSIONS

The Role of Suspended Solids and MLSS

The relationship between bacterial aerosol concentrations and SS concentrations was further examined. As shown in Figs. 2 (a) and 2(b), at the aeration rate from 5 to 15 L min^{-1}, the concentration of bioaerosols increased as the concentration of kaolin clay increased from 0 to 2000 mg L^{-1}. However, the concentration of bioaerosols decreased as the MLSS increased from 500 to 2000 mg L^{-1}, while the concentration of bioaerosols increased as the MLSS ranged from 0 to 500 mg L^{-1}. The enhancement effects of kaolin clay and the masking effects of activated sludge to the bioaerosol emissions might were also observed by many previous studies in which higher bioaerosol concentrations were found in pre-treatment processes (Pascual *et al*., 2003; Fracchia *et al*., 2006; Korzeniewska, 2011; Teixeira *et al*., 2013).

The ambient environment of real WWTPs can be more complex, such as the varying air velocity would change the bioaerosol concentrations measured near the WWTPs. It may be difficult to compare these air/water ratios with previously published AWRs of bacteria released from real waste water treatment plants. However, this is an important issue and deserves further investigation.

In our current study, *E coli* was used as the challenge bioaerosol since it was one of the most important indicator for WWTP operation and health concern. However, the results might not be entirely applicable to viruses, hydrophobic bacterial spores and fungal spores because their survival characteristics during emission and sampling may be different from *E coli*. Therefore, the effects of bioaerosol emissions for different species of microorganisms deserve further investigation.

The Enhancement Effect of Bioaerosols by Kaolin Clay

The bioaerosol concentrations increase with the increasing kaolin concentration from 0 to 2000 mg L^{-1} for aeration rates of 5 to 15 L min^{-1} (Fig. 2(a)). This suggests that the kaolin particles may effectively adsorb the bacterial cells and carry them out of the surface of the water along with air bubbles. Based on our microscopic examination, the particle size of kaolin is smaller than that of activated sludge. Therefore, the kaolin particles are more easily emitted into air, compared to activated sludge, during aeration. In addition, kaolin clay is free of inter-particle bridging, which leads to a greater free surface for the cells to attach onto the surface. Therefore, the emission of *E. coli* cells became easier when the kaolin clay was added into the test tank. When the kaolin particles were emitted with the bubbles formed by the aeration tubes, the bacterial cells became airborne with the emitted kaolin particles. Therefore, the emission of more *E. coli* cells could be expected as the concentration of kaolin clay increased.

Furthermore, the AWR values increased with the increasing concentrations of kaolin clay at the aeration rate from 5 to 15 L min^{-1} and the positive gradient increased with the increasing aeration rate (Fig. 2(a)), indicating the enhancement effects of kaolin clay. In general, bioaerosols generated for laboratory investigations are prepared as a pure solution containing only the test microorganisms (Simon and Duquenne, 2013). Therefore, it might be possible to enhance the generation of test bioaerosols by adding small-size non-floc-type particles, such as kaolin clay, if the added particles will not affect the quality of the generated bioaerosols.

The Masking Effect of Bioaerosols by Activated Sludge

The relationship between AWR and the concentration of

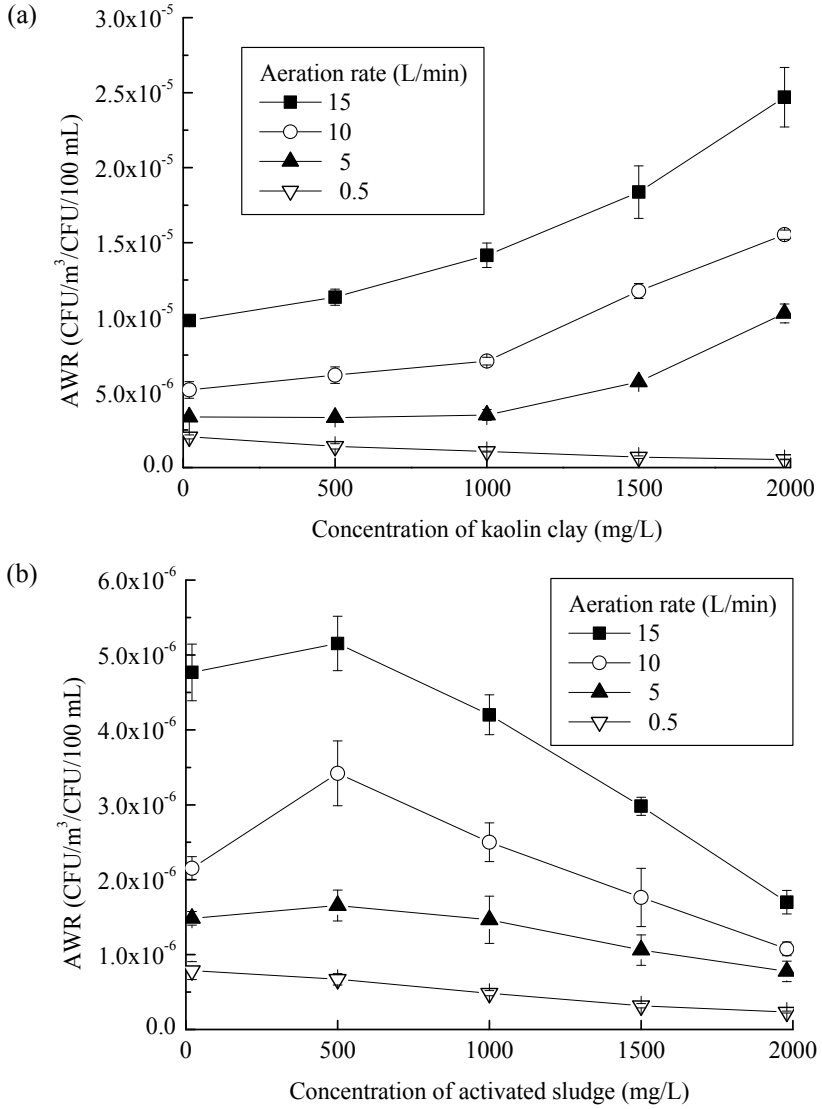

Fig. 2. Air/water ratios (AWR) when (a) kaolin clay and (b) activated sludge serve as the suspended solid. Each error bar represents one standard deviation on mean of at least three tests.

activated sludge (Fig. 2(b)) was different from that of a kaolin clay system (Fig. 2(a)). From Fig. 2(b), it was found that the emission of bioaerosols increased when a low concentration of activated sludge was added to the aerator, i.e., the MLSS increased from 0 to 500 mg L^{-1}. This enhancement effect is similar to kaolin clay tests. However, the AWRs decreased with the increasing concentration of MLSS from 500 mg L^{-1} to 2000 mg L^{-1} as aeration rates ranged from 5 to 15 L min^{-1}. Most of the high values of AWR appeared at an MLSS of 500 mg L^{-1} for tests at these three different flow rates (Fig. 2(b)).

Over a range of MLSS concentrations, from 500 to 2000 mg L^{-1}, a decreasing trend of emission rates versus MLSS concentration was observed. This decreasing trend for the AWR was even more pronounced as the air flow rate increased from 5 to 15 L min^{-1}. It was suggested that the additional activated sludge might form flocs to trap the cells when the sludge concentration increased. While the kaolin clay is a dense material with a smoother surface, the activated sludge is a filamentous and porous material with a higher surface area with which *E. coli* can adhere. Using fluorescence in-situ hybridization (FISH) method, Morgan-Sagastume *et al*. (2008) found that 60 to 70% of bacterial cells.in the activated sludge tank were associated with flocs. The additional activated sludge in wastewater might result in flocculation to trap the bacterial cells. Those bacterial cells were then trapped by the matrices. The effects of masking and/or coagulation of sludge decreased the emission potential of bioaerosols.

When the aeration rate was as low as 0.5 L min^{-1} for both the kaolin clay and activated sludge tests, there was insufficient mechanical capability to enhance the generation of bioaerosols. Instead, masking effects could occur, since the AWR decreased with the increase in the SS/MLSS concentration.

Based on the result of these experiments, the bioaerosol concentrations above the grit chamber or flow equalization tank without flocs could be even higher than those above the aeration tanks. Several studies have indicated that the

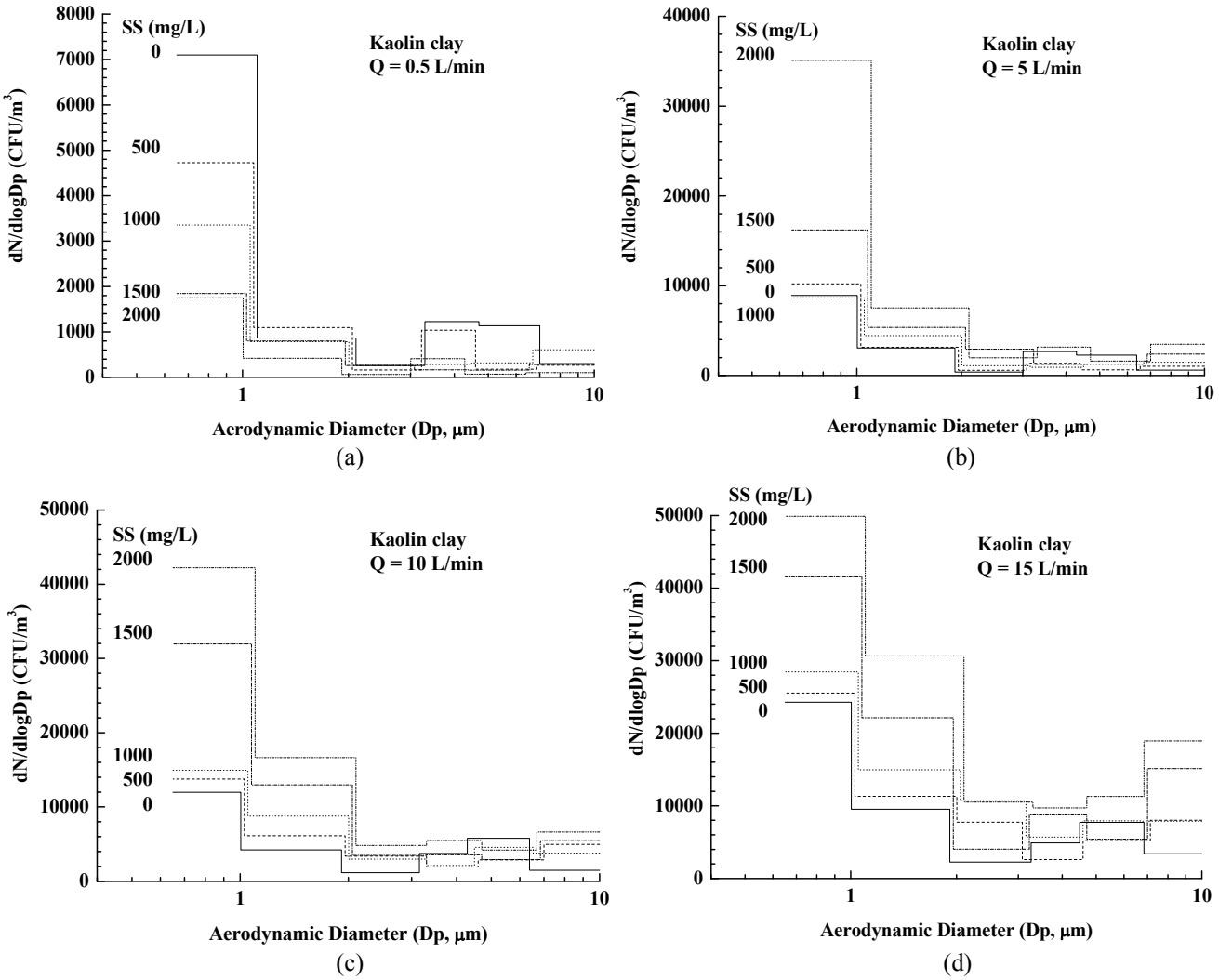

Fig. 3. Size distributions of emitted *E. coli* when kaolin clay serves as the SS for aeration rates of (a) 0.5, (b) 5, (c) 10, and (d) 15 L min^{-1}.

aeration tanks were not the only place for bioaerosol emissions in WWTPs. Karra and Katsivela (2007) observed a trend of gradual decrease in bioaerosol concentrations from pretreatment to the primary and secondary treatment areas. The mean concentration of airborne total coliforms in the aerated grit chambers was 172 CFU m^{-3}, and it was as low as 1 CFU m^{-3} in the aeration tanks. Fernando and Fedorak (2005) also found that the concentration of airborne microorganisms could be as high as 620 CFU m^{-3} in the uncovered grit tank, and at the concentrations in the aeration sections could be controlled at below 100 CFU m^{-3} when fine bubbling was applied.

According to the masking effect, when the bacterial cells were carried by the aeration bubbles, they could be intercepted or trapped by the floc before they were carried to the surface of the tank, and thus the flocs in the activated sludge tanks might serve as a control measure to eliminate the emission of bacteria suspended in the tanks. Recently, layers of balls several centimeters in diameter covering the bubbling liquid surface were reported to effectively control microorganism emissions in a lab-scale aeration system (Hung *et al*., 2010). The *E. coli* cells might have been trapped by the floc formed by the activated sludge and could not be aerosolized. The mechanism and results were similar to those of layers of small balls added to the surface.

The success of the activated-sludge process depends on the establishment of a mixed community of microorganisms that will aggregate and adhere to the sludge in a process known as bio-flocculation, and then settle to produce a concentrated sludge (called return activated sludge) for recycling. If this process fails, then bio-flocculation might collapse, resulting in decreased concentrations of MLSS. Under these conditions, according to our results, not only did the secondary treatment lose its function, but also the potential for bioaerosol emission could increase. However, the mechanism by which the addition of sludge and clay affect the amount of emitted bioaerosols from wastewater should be further investigated.

Particle Size Distributions

The Andersen six-stage bioaerosol sampler can collect bioaerosols larger than 0.65 μm. In this study, the mode of

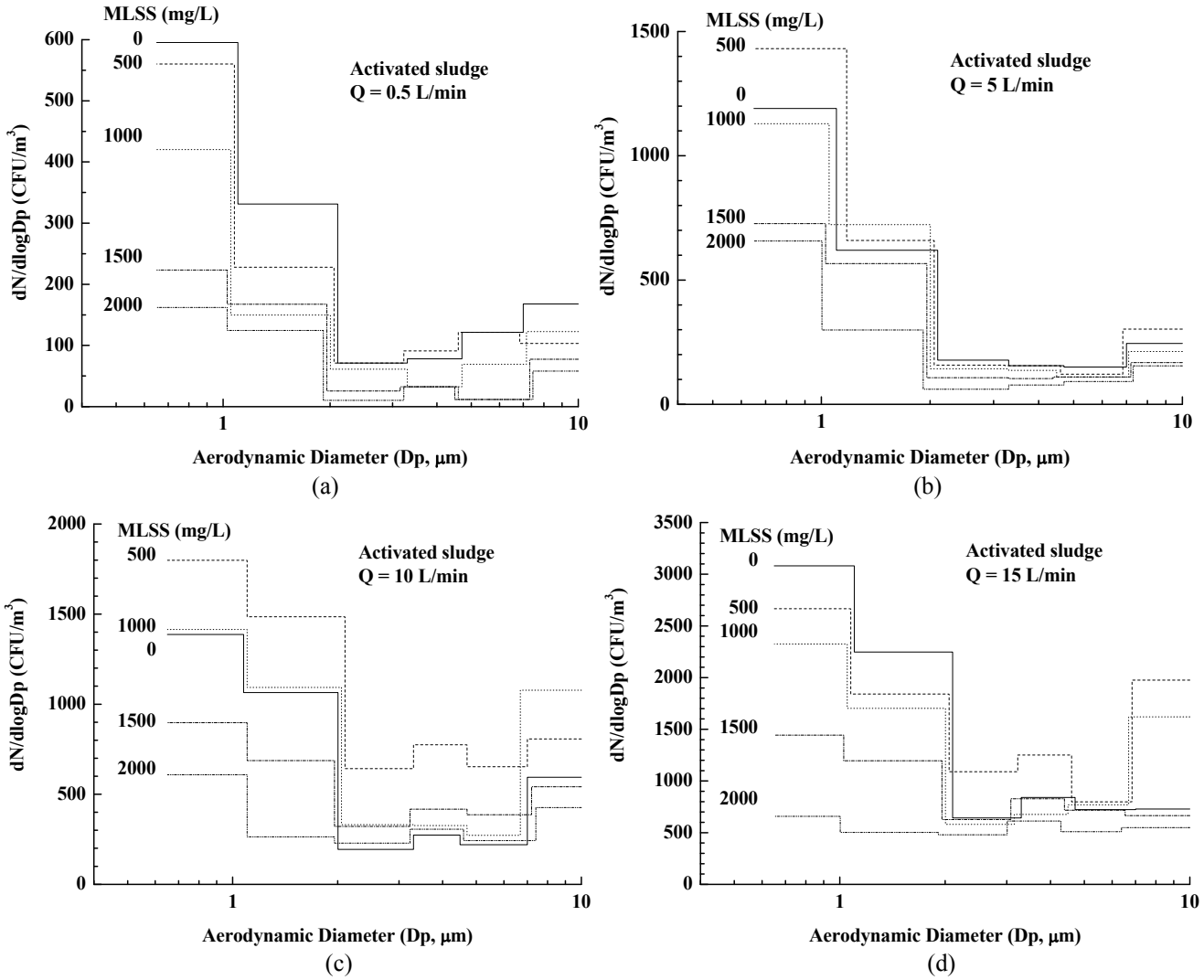

Fig. 4. Size distributions of emitted *E. coli* when activated sludge serves as the MLSS for aeration rates of (a) 0.5, (b) 5, (c) 10, and (d) 15 L min^{-1}.

the particle size distribution was located in the sixth stage of the Andersen sampler, meaning the predominant aerodynamic particle size of the bioaerosols ranged from 0.65 to 1.1 μm. When measured by the aerodynamic particle sizer instruments, the aerodynamic particle size of the single cells of *E. coli* was estimated to be in the range of 0.7 to 0.9 μm (Li *et al*., 1999; Lee *et al*., 2002) and 97% of the recovered colonies of *E. coli* cells were smaller than 2.1 μm (Li and Lin, 1999). According to the mode of the distribution in the sixth stage of the Andersen sampler, most of the emitted bioaerosol particles from the surface of the tester might contain only one *E. coli* cell, which was similar to the generation of *E. coli* in laboratories (Li *et al*., 1999; Li and Lin, 1999) and the likelihood of aerosol coagulation was low (Chien *et al*., 2011). A study on the size distribution of particles in the supernatant from activated sludge also found that the majority of particles (99%) were small particles with a diameter smaller than 2.5 μm (Morgan-Sagastume *et al*., 2008). However, some of the results from the field samples showed that the percentage of bacteria recovered from the 5th and 6th stages of the Andersen apparatus ranged from about 20 to 40% of the total bacteria samples (Brandi *et al*., 2000). In addition, the concentration of *E. coli* in the bioaerosols with an aerodynamic diameter smaller than 0.65 μm could be neglected, since the size of a single *E. coli* cell is seldom smaller than 0.65 μm from microscopy, which was one of the major reasons for designing the Andersen sampler (Andersen, 1958).

From Figs. 3 and 4, it can be seen that the modes of the particle sizes decreased as the amount of MLSS increased. The amount of emission of the single-cell-type bioaerosols decreased as a result of the higher trap ability of the flocs of the activated sludge. The calculated particle size distributions demonstrated the pronounced effects of coagulation of the sludge in the presence of the smaller particles, especially at higher flow rates. When the small-size single-cell particles were attached to activated sludge, the particle size distribution in the aerosols shifted to a larger particle size range.

In Fig. 4, it was found that the mode of the particle size decreased as the amount of MLSS increased. The decrease

of the AWRs shown in Fig. 2(b) led to a decrease in the amount of emission of the single-cell-type bioaerosols, which might be trapped in the activated sludge flocs. In contrast, the peak in the kaolin tests became higher as the kaolin concentrations increased (Figs. 3(b), 3(c), and 3(d)). This indicated that the increased AWRs of *E. coli* might be due to the increased emission of small particles.

CONCLUSIONS

During aeration, the bacterial aerosol concentrations could increase as the concentration of kaolin clay increased. In contrast, the bacterial aerosol concentrations could decrease as the concentration of activated sludge increased from 500 to 2000 mg L^{-1}. The mode value of the particle size distributions (0.65–1.1 μm) of the emitted bacterial aerosols was estimated with the sixth stage of the Andersen sampler, which indicated that most of the emitted particles contained only one single *E. coli* cell. It can be concluded that the types and concentrations of suspended solids may affect the emission characteristics of bacterial aerosols. In addition, it is critical to provide low aeration rates in the grit chambers and equalization basins to reduce bioaerosol emissions, especially if the major objectives are only to avoid anaerobic odor and sedimentation of SS. In an aeration tank, the MLSS concentration should be controlled as closely to the maximum value of the operating range as possible in order to reduce bioaerosol emission. If the MLSS concentration is decreased, then the aeration rate should be controlled at the lower value of the range.

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