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Collection and inactivation of airborne microorganisms using combination of water trap and electrical technologies

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Abstract

The elimination and inactivation of airborne microorganisms using the combination of a water trap and electrical technologies were investigated as a potential air purifier using readily available resources, such as water, salt, and electricity. A water trap with a single nozzle for trapping airborne microorganisms was used. The collection efficiencies in the experiment using water traps with 4 and 0.4 mm outlet diameters of the nozzles were 28 and 35%, respectively. Corona charging to airborne microorganisms just before blowing into the water trap decreased the particle concentration in the outlet gas to the same extent as in the experiment with just bubbling in trapping solution. The airborne microorganisms collected in the water trap were inactivated by the free chlorine generated by the electrolysis of saline as the trapping solution. The electrolysis for 30 min resulted in a sufficient concentration of free chlorine to inactivate the collected microorganisms completely, and the concentration could be maintained by only 1 min of electrolysis every hour. Electrostatic precipitation (ESP) collected 90% of airborne microorganisms, and the combination of a water trap and ESP collected 99.7% of airborne microorganisms. Our results demonstrate that an air purifier using readily available resources can be realizable using the combination of a water trap and electrical technologies.

Keywords: Corona charging, electrostatic precipitation, water trap, microorganisms, electrolysis.

1. Introduction

Biological clean rooms with airborne microorganisms eliminated from the room air are essential in various industries to maintain safety and ensure certainty of the work conducted in them. In medicine, the air of the operating room must be kept clean to prevent airborne microorganisms from entering the body through the incision site. Clean rooms are also used for the therapy of patients with a weakened immune system. In food industries, not only the microorganisms found in ingredients but also airborne microorganisms cause the decay of food products and food poisoning in the worst case [1, 2]. Therefore, microorganisms should be eliminated from food processing facilities, including those in room air. In addition to these industries, elimination of airborne microorganisms through the use of air purifiers is also in great demand in household. Various types of microorganisms, including bacteria and fungi, exist in indoor environments as airborne microorganisms and some cause undesirable reactions in the human body [3–5]. For instance, mold is one of the most common microorganisms that cause allergic reactions such as asthma and rhinitis [6–8]. Household air purifiers help eliminate these harmful microorganisms to maintain human health.

The elimination of airborne microorganisms using conventional air cleaning equipment mainly depends on filtration with a high-efficiency particulate air (HEPA) filter. Filtration with a HEPA filter has a proven track record and is the most reliable method, and regular replacement of the filter is necessary to maintain the performance of the air-cleaning equipment. However, in industrial facilities, the cost of the HEPA filter itself, the replacement process, and the shutdown of the facility during replacement are large burdens. Moreover, the

HEPA filter in household equipment is seldom, if ever, replaced, and there are concerns of growth and secondary diffusion of the microorganisms trapped in the filter during long-term usage.

The alternative or supplementary technologies for filtration in air cleaning are electrostatic precipitation (ESP) and wet scrubbing. ESP has been widely used in industrial plants, such as thermal power plants, to control particulate emission and also in indoor air purifiers. The application of ESP to the collection of airborne microorganisms and the elementary charges carried by airborne microorganisms were already investigated by the end of the last century [9, 10]. Wet scrubbing has been used in industrial plants emitting high-temperature, high-humidity, and corrosive gases to control particulate emission and neutralize gases. Airborne microorganism removal by the wet scrubbing was also investigated [11], and recently, indoor wet-scrubber-type air purifiers have also become commercially available. Both technologies are promising for the elimination of airborne microorganisms using industrial indoor and household air purifiers, and need only water and electricity for continuous operation. In this study, we investigated the efficiency of airborne microorganism collection by the combination of a water trap as a wet scrubber and electrostatic technologies including ESP and corona charging. In addition to the collection, the inactivation of collected airborne microorganisms is important to preventing their growth and secondary diffusion. In the purifier with the combination of a water trap and electrostatic technologies, the collected airborne microorganisms can be inactivated without adding a bactericidal agent by the free chlorine generated by the electrolysis of the trapping solution of the water trap. The electrolyzed water produced by the membrane-less electrolysis of the NaCl or HCl solution contains free chlorine (ClO^-, HClO, and Cl_2), which is a major bactericidal component, and it is easier and cheaper to produce than the conventional membrane electrolyzed water [12]. Therefore, the collected airborne microorganisms were also inactivated by the free chlorine produced by the membrane-less electrolysis. In this study, the overall potential of the air purifier using inexpensive and readily available resources, such as water, salt, and electricity, was investigated.

2. Materials and methods

2.1 Preparation of microorganisms

The *Staphylococcus epidermidis* NBRC 100911 strain was cultivated in liquid medium [1% (w/v) Bacto Peptone, 0.2% (w/v) yeast extract, 0.1% $MgSO_4 \cdot 7H_2O$] at 37 °C for 1 day with shaking. After cultivation, *S. epidermidis* cells were collected by centrifugation (10,000 × g, 10 min, 4 °C) and washed twice with sterile distilled water. The washed cells were frozen at -80 °C for 1 day, lyophilized with Freeze-Dryers DC401 (Yamato Scientific Co., Ltd., Tokyo, Japan) for 1 day, and ground with mortar and pestle to homogenize the dried cells. The colony forming unit (CFU) was used as an estimate of the number of viable cells. CFU was determined by counting the number of colonies formed on agar medium [1% (w/v) Bacto Peptone, 0.2% (w/v) yeast extract, 0.1% $MgSO_4 \cdot 7H_2O$, 1.5% (w/v) agar]. The CFU of the dried cells was approximately 2.1×10^5 CFU mg^{-1}.

2.2 Experimental apparatuses

Fig.1 shows the schematic of the experimental setup. As the model of airborne microorganisms, air containing dried cells of *S. epidermidis* was intermittently blown into the water trap through a single nozzle with outlet diameters of 4 (glass tube) or 0.4 mm (polypropylene pipet tip). The pressure reduction valve was adjusted to 50 kPa and the air solenoid valve was controlled to open 50 ms every 15 s by an Arduino microcontroller. The volume of the chamber was 13 L, and 1 L of saline was used as the trapping solution of the water trap. To exhaust the air in the chamber and to circulate the trapping solution, air was supplied into the trapping solution in the chamber at a rate of 5 L min^{-1}. For the electrodes used in the electrolysis to generate free chlorine, platinum wires (ϕ 0.02 mm, 100 mm) connected to each conductor of an AC adaptor (12 V, 1 A) were used. The free chlorine was measured with total chlorine colorimeter-checker HI711-25 (HANNA Instruments Japan Inc., Chiba, Japan) by using an aliquot of electrolyzed solution. A corona discharge unit equipped with a wire to plate electrode (Fig. 2a) consisted of glass tubes, a stainless-steel plate, and tungsten wire (300 × 80 μm). DC negative voltage (–6 kV, 15 mA) was applied to the tungsten wire by a high-voltage power supply NUNA-10N15 (MATSUSADA Precision Inc. Shiga, Japan) to generate a corona discharge. An ESP unit consisted of silicone sheets, tungsten wires, and stainless-steel plates, and has an inner volume of 0.1 L and 10 high-voltage

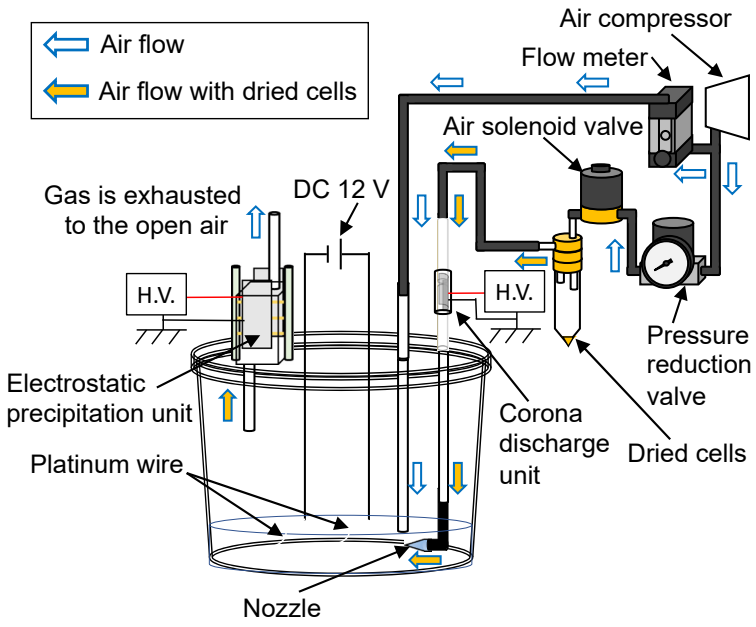

Fig. 1. Schematic of experimental setup.

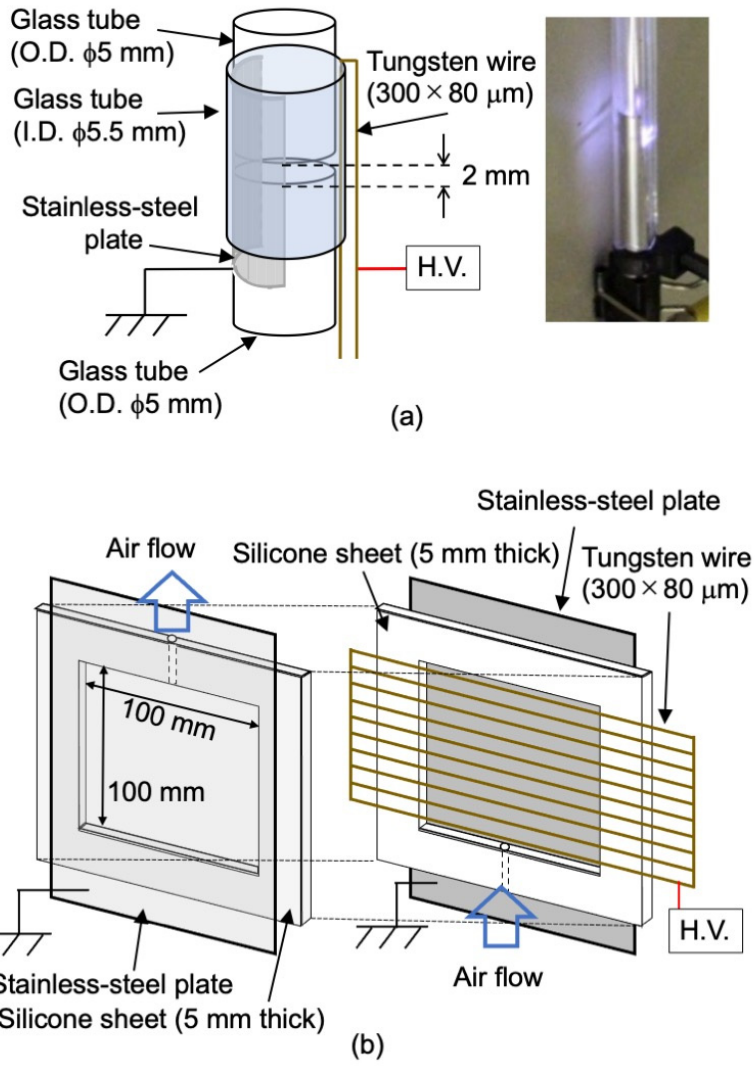

Fig. 2. Schematic (left) and picture (right) of corona discharge unit (a), and schematic of electrostatic precipitation unit (b).

wire electrodes in its center (Fig. 2b). DC negative voltage (-6 kV, 15 mA) was also applied to the tungsten wire by a NUNA-10N15 high-voltage power supply to generate a corona discharge. The concentration of particles of 1.0 to 5.0 μm size in the outlet gas from the chamber was measured with an airborne particle counter KC-52K (RION Co., Ltd., Tokyo, Japan), and particle counting in 1 L of gas took 21 s. For a measurement of CFU in trapping solution, aliquot of the trapping solution was appropriately diluted and plated on agar medium, then obtained CFU was converted to per 1L of trapping solution. In this study, all particle counting experiments were performed in at least triplicate, and the particle concentration (particles L^{-1}) is shown as the average ± standard deviation.

3. Results and discussion

3.1 Collection of airborne microorganisms using combination of water trap and corona charging

The air containing dried cells of *S. epidermidis* as the model of airborne microorganisms was blown into the water trap through a nozzle with an outlet diameter of 4 or 0.4 mm. The number of viable cells (CFUs) in the whole trapping solution (1 L) increased with time in the experiments with both nozzle diameters (Fig. 3). The change in CFU was larger in the first 5 min than after 5 min, and this would be because the smaller and easily transportable particles of the dried cells were firstly ejected by the blowing air. The CFU in the water trap increased almost linearly after 5 min, indicating that constant number of airborne microorganisms were continuously being blown into the chamber. The number of airborne microorganisms collected in the experiment with the nozzle of 0.4 mm outlet diameter was larger than with the nozzle of 4 mm outlet diameter. It is considered that the smaller the outlet diameter of the nozzle, the smaller the bubbles that are generated in the water trap and the larger the surface area becomes, and thus the collection efficiency is improved. However, we could not employ a single nozzle with an outlet diameter smaller than 0.4 mm in the experiment because of technical limitations. The efficiency of airborne microorganism collection by the water trap was evaluated from the CFU and reduced weight of stock of dried cells at each data point, and collection efficiencies in the experiment with nozzles of 4 and 0.4 mm outlet diameter of nozzle were 28 and 35%, respectively.

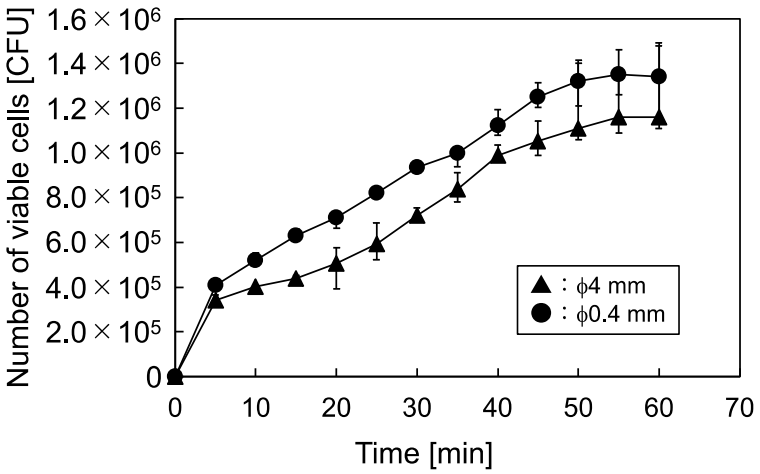

Fig. 3. Time course of number of viable cells (CFUs) in 1 L of trapping solution. The diameters of the nozzles for blowing the airborne microorganisms were 4 (triangles) and 0.4 (circles) mm.

The particle concentration in the gas at the chamber outlet was measured with a particle counter in the combination of following 4 conditions (Fig. 4); with blowing only air (A), with blowing airborne microorganisms (M), with bubbling of trapping solution (B), and with corona charging (C). The particle concentration was 10 ± 4 particles L^{-1} when only air (A) was circulated in the chamber. When the dried cells of *S. epidermidis* as the airborne microorganism were blown into the chamber without a water trap (M), the particle concentration in gas at the chamber outlet was 55,834 ± 4,034 particles L^{-1}. The relationship between

the aerosol resulting from the bubbling of water at the water trap and the airborne microorganisms not trapped at the water trap in the chamber was investigated. The particle concentration at the chamber outlet in the experiment with only bubbling of water by the air supplied at 5 L min^{-1} (B) was 30,450 ± 4,463 particles L^{-1}, and large amounts of aerosols were generated by bubbling. On the other hand, the particle concentration at the chamber outlet in the experiment with airborne microorganisms blown through the nozzle of 0.4 mm outlet diameter into the water and bubbling of the trapping solution (MB) was 35,601 ± 2,011 particles L^{-1}. From the comparison of particle concentration between these two (B and BM) experiments, the increase in the particle concentration by blowing airborne microorganisms was about 5,200 particles L^{-1}. As shown above, the collection efficiency of airborne microorganisms in the water trap was 35%. Therefore, the remaining 65% of airborne microorganisms would be 36,300 particles L^{-1}, and this almost agrees with the particle concentration at the chamber outlet in the experiment with blowing airborne microorganisms and with bubbling of the trapping solution (MB). From these results, in the gas at the chamber outlet, at least 9.1% (5,200 particles L^{-1}) of the airborne microorganisms were emitted from the chamber without an interaction to the aerosol, and a large part of airborne microorganisms not trapped in the water trap might stick to the aerosol. To improve the collection efficiency in the water trap, corona charging of airborne microorganisms was carried out immediately before blowing the microorganisms into the water trap. The particle concentration at the chamber outlet in the experiment with blowing airborne microorganisms, bubbling of water, and corona charging (MBC) was 31,238 ± 1,541 particles L^{-1}. Statistical analysis using the t-test confirmed that there is no significant difference ($p > 0.05$) between the results of the B and MBC experiments. Therefore, airborne microorganisms were rarely emitted without an interaction to aerosol and almost all airborne microorganisms would be trapped in the water trap or stuck to the aerosol. The corona charging before water trapping is significantly effective in interacting airborne microorganisms to the water and the aerosol, and this might be due to the increase of a Coulomb force between charged cell and water/aerosol.

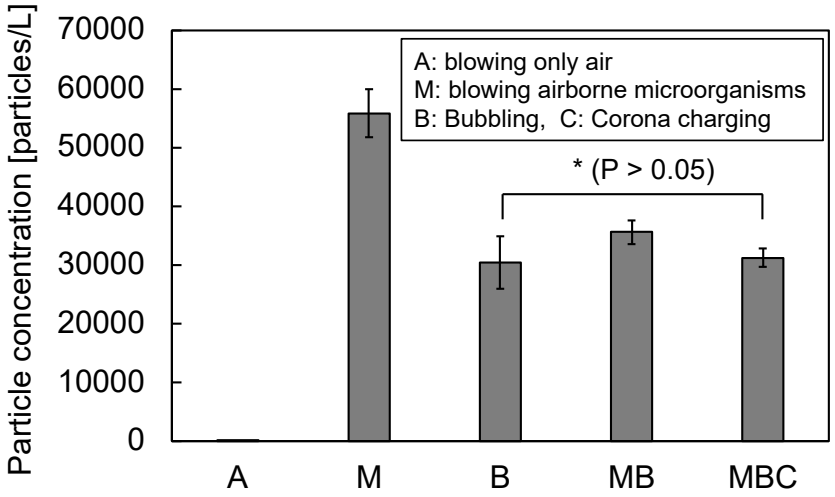

Fig. 4. Particle concentration in gas at chamber outlet. Each experiment was carried out with the following combinations; with blowing only air (A), with blowing airborne microorganisms (M), with bubbling of trapping solution (B), and with corona charging (C).

3.2 Inactivation of trapped airborne microorganisms by free chlorine generated by electrolysis

The time course of the free chlorine generation by the membrane-less electrolysis of saline in this experimental setup was determined (Fig. 5). The free chlorine concentration linearly increased with time within 60 min and the generation rate was 1.1 ppm min^{-1}. The inactivation of *S. epidermidis* under the same electrolysis condition was also investigated. After 10 min of electrolysis when the free chlorine concentration reached 11 ppm, the survival ratio of *S. epidermidis* decreased to 0.21 (79% inactivation). Zhao *et al*. (2014) reported 98% and complete inactivation of airborne bacteria collected from an aviary hen house after 0.5 and 5 min of treatment at 10.2 ppm free chlorine concentration [12]. In this study, the process from sampling, dilution, and plating on agar medium took over 5 min. From the comparison of our results with those of Zhao *et al*., it was found that *S. epidermidis* and probably *Staphylococcus* spp. have higher tolerance toward free chlorine than airborne

microorganisms from poultry production facilities. The survival ratio of *S. epidermidis* decreased to 4.4×10^{-5} (99.996% inactivation) after 20 min of electrolysis, and no viable cells were detected after 30 min of electrolysis. The free chlorine concentration after 30 min is sufficient for the inactivation of collected airborne microorganisms. The decrease in free chlorine concentration with time was also measured, and it was almost 1 ppm h^{-1} (data not shown). Therefore, we investigated the survival ratio of continuously collected airborne microorganisms with 30 min of electrolysis before the collection of airborne microorganisms and with 1 min of electrolysis every hour. In the experiment for a total of 7 h, no viable cells were detected in the trapping water with NaCl (data not shown). From these results, the electrolysis of trapping water with NaCl is effective in inactivating airborne microorganisms collected in the water trap.

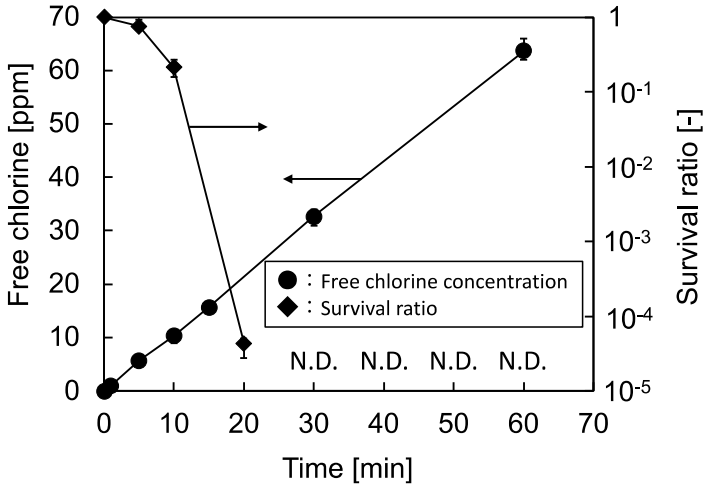

Fig. 5. Time courses of free chlorine generation and inactivation of *S. epidermidis*: free chlorine concentration in the electrolyzed 1 L of saline (circles) and survival ratio of *S. epidermidis* in it (diamonds).

3.3 Collection of airborne microorganisms using the combination of a water trap and ESP

It was suggested that the aerosol generated by bubbling contain untrapped airborne microorganisms. The particle concentration in the gas at the chamber outlet was measured with a particle counter in the combination of following 4 conditions: with blowing airborne microorganisms (M), with electrostatic precipitation (P), with bubbling of trapping solution (B), and with electrolysis of trapping water (L). The ESP unit was used at the outlet of the chamber to collect them. The particle concentration in the gas after the ESP unit in the experiment without a water trap (MP) was 5,479 ± 877 particles L^{-1}, and 90% of the airborne microorganisms were collected in the ESP (Fig. 6). The ESP is highly efficient for collection of airborne microorganisms; however, the inactivation efficiency of the collected airborne microorganisms on ESP would be poor. Kikuchi *et al.* (2020) reported that even airborne fungal spores adhered to the dielectric barrier discharge reactor by electrostatic precipitation were not inactivated [13]. The combination of the water trap and electrolysis could be one of the powerful tools for indoor air purifiers to collect and inactivate airborne microorganisms. In the experiment using the combination of bubbling and electrolysis without (BL) and with (MBL) blowing airborne microorganisms, the particle concentrations in the outlet gas were 35,904 ± 2,999 and 42,915 ± 3,832 particles L^{-1}, respectively. There were also untrapped airborne microorganisms in the gas, and the percentage of untrapped airborne microorganisms (12.5%) was slightly higher than that without electrolysis (9.1%). This might result from changes in the trapping solution characteristics, such as ion species and ion concentration, caused by electrolysis, and emphasizes the importance of corona charging before blowing airborne microorganisms into the water trap to efficiently collect the microorganisms. The particle concentration in the gas in the experiment with blowing airborne microorganisms, bubbling of trapping solution, electrolysis, and ESP (MBLP) was 174 ± 162 particles L^{-1}, and the ESP efficiently collected the aerosol and airborne microorganisms. The total elimination efficiency of airborne microorganisms from the air was 99.7% even in this experimental setup. The airborne microorganisms trapped on the collection electrode might cause growth and secondary diffusion, and accumulation of them might also cause re-entrainment and of collected particles [14]. One of the applicable ideas for inactivating and removing the airborne microorganisms trapped on ESP

is periodic washing of collection electrode using trapping solution, because the airborne microorganisms trapped in water trap were efficiently inactivated by free chlorine in the rapping solution. Therefore, the combination of a water trap as the web scrubber, electrolysis of NaCl solution, and electrostatic technologies is promising for air cleaning by elimination and inactivation of airborne microorganisms. In addition, each component has been widely applied to air-cleaning, for instance, corona discharge was applied to collection of oil mist emitted during cooking [15]. Wet scrubber was applied to removal of ammonia from lagoon biogas [16], and cleaning of cooking fume [17] and grilling gas [18]. In these applications in food industry and agriculture, simultaneous collection and inactivation of airborne microorganisms are also preferable for keeping hygiene. From also these view point, the system proposed in this study is a promising for air-cleaning technology.

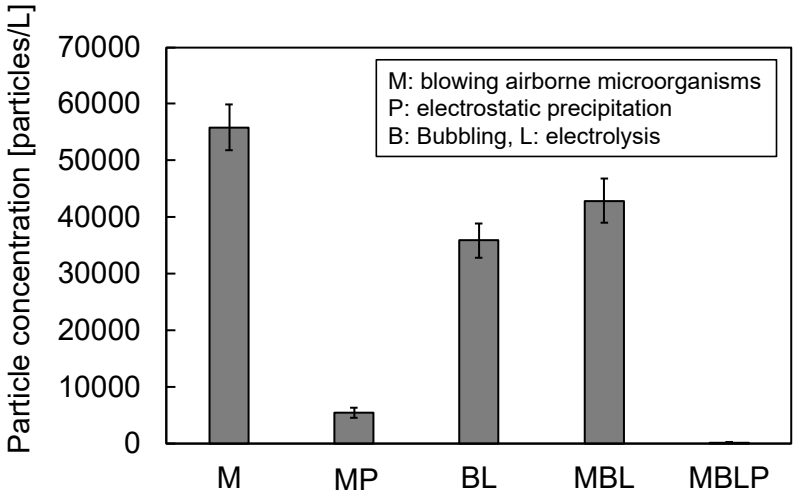

Fig. 6. Particle concentration in gas at chamber outlet. Each experiment was carried out with the following combinations: with blowing airborne microorganisms (M), with electrostatic precipitation (P), with bubbling of trapping solution (B), with electrolysis of trapping water (L).

4. Conclusion

In this study, eliminating airborne microorganisms with the combination of a water trap as the wet scrubber and electrical technology was investigated. The water trap with a single ϕ 0.4 mm nozzle could collect 35% of airborne microorganisms, and corona charging before the water trap improved the collection efficiency of airborne microorganism in the water trap. Electrolysis of saline as the trapping solution of the water trap generated sufficient free chlorine to completely inactivate *S. epidermidis* after 30 min of treatment. The water trap with 30 min of pre-electrolysis of the trapping solution and 1 min of electrolysis of the trapping solution every hour completely inactivated and continuously collected airborne microorganisms for 7 h. The high collection efficiency of ESP for airborne microorganisms (90%) was confirmed, and the combination of ESP and a water trap eliminated 99.7% of airborne microorganisms from the air. The combination of a water trap and electrical technologies is promising for the development of air-cleaning devices and using inexpensive and readily available resources, such as water, salt, and electricity, would be suitable for indoor and household air purifiers. In the future study, we'd like to investigate the utilization of active species such as ozone generated by corona discharge on the inactivation of microorganisms in the trapping solution, and configuration and form of ESP unit and wet scrubber for improvement of collection efficiency.

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