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Emission Characteristics of Microbial Aerosols in a Municipal Sewage Treatment Plant in Xi'an, China

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ABSTRACT

To characterize the emission of microbial aerosols from the widely used municipal sewage treatment plants (MSTP) in China, an Andersen six-stage impactor and the culture method were employed to determine the concentrations and size distributions of airborne viable bacteria, fungi and actinomycetes in a sewage treatment plant with an oxidation ditch process in Xi'an in summer. The results showed that the concentrations and size distributions of each of the airborne microorganisms varied greatly at different phases of sewage treatment process. The highest bacteria (7866 ± 970 CFU/m^3) and actinomycetes concentrations (2139 ± 229 CFU/m^3) were found in the sludge-dewatering house while the highest fungi concentration (2156 ± 119 CFU/m^3) at the oxidation ditch. The particle size distributions showed that similar single-peak pattern for airborne actinomycetes, bacteria and fungi. Another important finding was that about 52% of airborne bacteria, 62% of airborne fungi and 65% of airborne actinomycetes were in respirable size range (less than 3.3 µm), indicating that most microbial aerosols from MSTP could easily penetrate into the human alveolus. Finally, the order of the count median diameters of different microbial aerosols was found to be similar at each phase, that is, airborne bacteria > airborne fungi > airborne actinomycetes. This implied that airborne actinomycetes emitted from MSTP might have a more significant effect on public health and urban air quality than bacteria and fungi.

Keywords: Municipal sewage treatment plant; Microbial aerosols; Emission characteristics; Size distribution; Actinomycetes.

INTRODUCTION

With the acceleration of urbanization recently, more and more municipal sewage treatment plants (MSTP) have been built and put into operation in China. As a result, many MSTPs and related sewage works become surrounded by new residential and shopping districts. In such situation the question of hygienic sustainability of MSTP site location arises not only in terms of frequent noxious odours, but also of intermittent enteric illness and related syndrome of unknown origin among nearby residents (Stellacci *et al.*, 2010).

During handling sewage to protect water environment, sewage treatment plants have also been proved to generate some hazardous air pollutants like microbial aerosols and thus have become growing concern in the public (Pascual *et al.*, 2003; Fracchia *et al.*, 2006; Heinonen-tanski *et al.*, 2009). Sewage is well known to contain high numbers of pathogens like viruses, bacteria and fungi. It is inevitable to lead to aerosolization of these microorganisms in many phases of the treatment process, particularly in those phases containing aeration and mechanical agitation operations (Fernando and Fedorak, 2005; Sánchez-Monedero *et al.*, 2008). Consequently, microbial aerosols generated from MSTP may produce serious impact on human health and air quality (Brandi *et al.*, 2000; Thorn and Kerekes, 2001; Grisoli *et al.*, 2009). Therefore, it is significantly important to require knowledge of emission characteristics of microbial aerosols from MSTP in order to evaluate a potential risk to plant workers and surrounding residents.

So far, many studies have been conducted on the topic of microbial aerosols generated from MSTP and most of existing research work has focused on two aspects. One is to evaluate the biological risks by determining the emission and transport characteristics of microbial aerosols generated from MSTP, among which compositions and concentrations of viable microorganisms have been intensively investigated using different sampling and detection methods (Carducci *et al.*, 2000; Dowd *et al.*, 2000; Oppliger *et al.*, 2005; Karra and Katsivela, 2007; Patentalakis *et al.*, 2008). Concentrations have been found to vary widely between different studies, depending upon such influence factors as kind and capacity of sewage treated, operation activities, site location and

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weather conditions. Another is to develop control measures to reduce or remove airborne microorganisms generated from MSTP. Some available measures proposed in literatures involve: submerged aeration for sludge digestion (O'Hara, 2005), air diffuser as an aeration system for the biological treatment (Sánchez-Monedero *et al.*, 2008), using floating balls in the aeration system (Hung *et al.*, 2010), adsorption on activated carbon (Li *et al.*, 2011a) and ultraviolet irradiation technique in indoor environments (Lee, 2011).

Effects of microbial aerosols on human health depend not only on their concentration and species, but also on their size distribution. Bioaerosol particles with different aerodynamic diameters are recognized to be deposited in different positions of the respiratory system and result in various respiratory illnesses (Thomas *et al.*, 2008; Lee *et al.*, 2011). Hence, it is necessary to study the size distribution of microbial aerosols from MSTP. In addition, the existing literatures focused mainly on the characteristics of airborne bacteria and fungi whereas few studies investigated that of airborne actinomycetes in sewage treatment plants. In fact, several studies concerning actinomycetes quantification in agricultural areas and urban outdoor environments (Fang *et al.*, 2008; Martin *et al.*, 2010) indicated that actinomycetes had a worldwide distribution in soil, decaying organic material and sewage, and contributed to air pollution and serious respiratory diseases (Byeon *et al.*, 2008). Therefore, there is a need to study emission characteristics of airborne actinomycetes generated from MSTP.

The objective of the present study is to gain comprehensive information about the emission characteristics of microbial aerosols, especially for actinomycetes aerosols, generated in typical wastewater treatment plants in China. For this purpose, a Xi'an sewage treatment plant with the oxidation ditch process was selected because this process is most widely used in sewage treatment plants in China due to the good treatment efficiency and inexpensive running costs. The concentration and size distribution of airborne bacteria, fungi and actinomycetes were determined at the different treatment phases by using an Andersen six-stage impactor and the culture method.

MATERIALS AND METHOD

Plant Description

Bioaerosol samples were collected at a MSTP with oxidation ditch process located in the eastern part of Xi'an city, China. This plant has been operated continuously throughout the year since 2006, with a treatment capacity of 2×10^5 m^3/day, corresponding to about 290,000 inhabitants served. In this plant, influent wastewater is pre-treated by screens and aerated grit chamber (AGC) and the biological treatment is then conducted in an Orbal oxidation ditch (OD) with horizontal rotor aeration, followed by primary and secondary settling tank (SST). Purified wastewater is finally discharged through an effluent outlet (EO) to a drainage ditch flowing to the Ba River. The sludge is thickened by centrifugation in a sludge dewatering house (SDH).

Sampling

Sampling sites in this study were arranged at different treatment phases as shown in Fig. 1: AGC, OD, SST, SDH and EO. In addition, a background site (BGS) located about 100 m upwind of the plant was chosen as a background air sample according to the prevailing wind direction (also shown in Fig. 1).

Sampling period selected between 10:00 am–12:00 pm from June to August 2011. Each sampling was performed on the sunny days with similar climatic conditions to reduce the measurement uncertainty resulted from weather variations. At the same time as aerosol sampling, the wind speed, ambient temperature and relative humidity were monitored. During sampling, the outdoor temperature and relative humidity ranged from 26.9 to 30.2°C, and 47–62%, respectively. The prevailing wind direction was southwest with average wind speed of 0.6 m/s.

An Andersen six-stage impactor (Westech, UK) with six glass petri dishes of 93 mm in diameter was employed to collect bioaerosol samples with different size ranges in the present study. The range of aerodynamic diameter at each stage is: ≥ 7.0 µm (stage 1), 7.0–4.7 µm (stage 2), 4.7–3.3 µm (stage 3), 3.3–2.1 µm (stage 4), 2.1–1.1 µm (stage 5) and 1.1–0.65 µm (stage 6). According to definition above, respirable fraction of particles corresponds to the size range between stages 3–6. At each of the sampling sites, the sampler was mounted at 1.5 m above the floor surface and 0.5–1.5 m far from the treatment units. All outdoor samples were taken downwind. The samples were collected for about 10 min by sucking the air at the rate of 28.3 L/min with three repetitions each time.

Microbial Cultivation

The airborne viable microorganisms studied here were bacteria, fungi and actinomycetes, which could be captured on petri dishes with appropriate cultivation agars. After sampling, the agar plates were immediately transported to the laboratory for incubation. Bacteria were incubated in

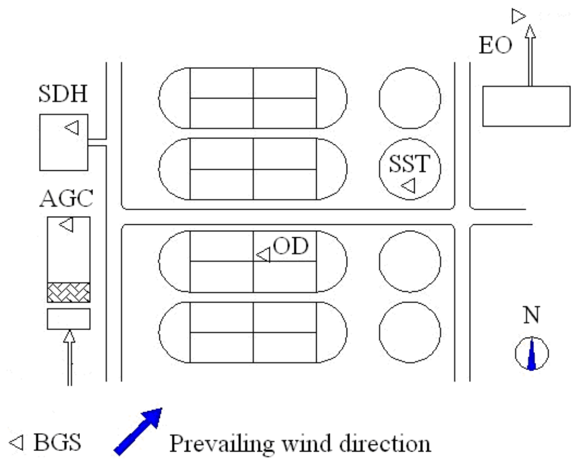


Fig. 1. Sketch of sampling sites at a municipal sewage treatment plant in Xi'an, China. AGC: aerated grit chamber; OD: oxidation ditch; SST: secondary settling tank; SDH: sludge dewatering house; EO: effluent outlet; BGS: background site; Δ: sampling sites.

nutrient agar at 37°C for 48 h. Fungi were incubated in Sabouraud dextrose agar at 28°C for 72 h. Actinomycetes were incubated in Gause's synthetic agar at 28°C for 120 h. Note that it is necessary that the sampler should be disinfected with 75% ethanol to prevent contamination before and after each sampling. After incubation, the colonies were counted followed by the positive-hole correction method (Andersen, 1958) to revise colony overlapping by Eq. (1).

$$Pr = N \cdot [\frac{1}{N} + \frac{1}{N-1} + \frac{1}{N-2} + \cdots + \frac{1}{N-r-1}] \tag{1}$$

where Pr, r and N denote the revised colonies, sampling colonies and number of sieve pore at each stage of sampler, respectively.

The concentration of airborne microorganisms which is generally expressed as total colony-forming units (CFU/m^3) was then calculated as follow:

$$C = \frac{Pr \times 1000}{t \times F} \tag{2}$$

where C presents the concentration of airborne microorganisms (CFU/m^3), t is the sampling time, and F is the sampling flow rate.

Statistical Analysis

The concentration results reported below were the mean and standard deviation of the plate counts obtained at each sampling site. The standard deviation was presented in the respective figure in the form of error bars. The difference of bioaerosol concentration at different sites was also compared by t-test, in which p values of < 0.05 were considered to be statistically significant. In addition, the count median diameter (CMD) was defined in this study as the diameter dividing the total number into two halves, which could be obtained directly from the cumulative fraction curve of particle size distribution, that is, the diameter corresponding to the fraction value of 0.5.

RESULTS AND DISCUSSION

Concentrations of Airborne Viable Bacteria, Fungi and Actinomycetes

Fig. 2 shows the concentrations of airborne viable bacteria, fungi and actinomycetes in aerosol samples at all sampling sites. It could be clearly seen that the higher numbers of viable bacteria, fungi and actinomycetes were detected at each phase of sewage treatment process compared to their background levels (bacteria: 1065 ± 39 CFU/m^3, fungi: 599 ± 87 CFU/m^3, actinomycetes: 473 ± 46 CFU/m^3) at the background site (p < 0.05). The result confirmed the conclusions obtained by several authors (Fracchia *et al.*, 2006; Karra *et al.*, 2007; Korzeniewska *et al.*, 2009), that is, sewage treatment process was an important source of microbial aerosols. Furthermore, the concentration of each of airborne microorganisms studied here varied greatly at different phases of sewage treatment process. The mean concentration of airborne fungi was in a range from 2156 ± 119 CFU/m^3 at the oxidation ditch to 822 ± 70 CFU/m^3 at the effluent outlet. Bacterial concentration ranged from 7866 ± 970 CFU/m^3 in the sludge-dewatering house to 1696 ± 96 CFU/m^3 at the effluent outlet, while actinomycetes concentration ranged from 2139 ± 229 CFU/m^3 to 902 ± 54 CFU/m^3 at the same positions.

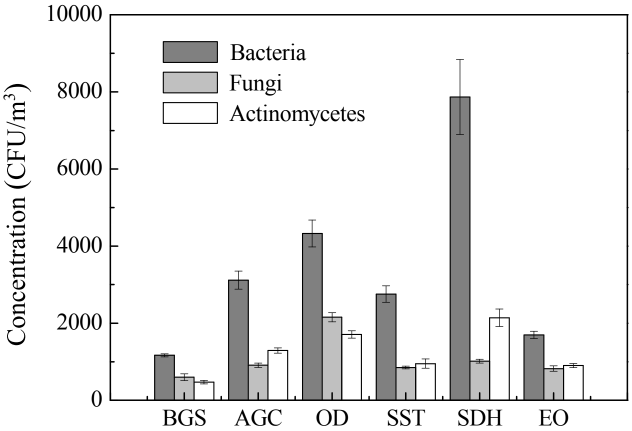


Fig. 2. Concentrations of airborne microorganisms in aerosol samples at all sampling sites.

The lowest concentrations of all airborne viable microorganisms found at the effluent outlet indicated that the effluent after biological treatment contained much lower amounts of microorganisms. The numbers of airborne microorganisms near the secondary settling tank could be found to be usually low (bacteria: 2755 ± 212 CFU/m^3, fungi: 850 ± 41 CFU/m^3, actinomycetes: 949 ± 120 CFU/m^3), which implied that settling process in sewage treatment plant seemed not to be a major bioaerosol source. In contrast, higher numbers of airborne microorganisms were detected in the oxidation ditch. Obviously consistent with other studies (Pascual *et al.*, 2003; Fracchia *et al.*, 2006; Li *et al.*, 2011), the concentrations of airborne microorganisms generally decreased as the wastewater became further treated. Our higher result of microbial aerosols in the OD supports the finding of Brandi *et al.* (2000) and Sánchez-Monedero *et al.* (2008) who reported that mechanical agitation of wastewater could increase the numbers of airborne microorganisms.

It is interesting to note that the airborne microbes detected in this investigation shows higher concentrations, of 1–2 orders of magnitude, in comparison to previous studies in MSTP with similar biological process (Brandi *et al.*, 2000; Sánchez-Monedero *et al.*, 2008; Grisoli *et al.*, 2009; Heinonen-tanski *et al.*, 2009; Li *et al.*, 2011b). This may be attributed mainly to the larger volume of treated water (2 × 10^5 m^3/day), of 2–3 orders of magnitude, applied in the present study. Brandi *et al.* (2000) proved that the numbers of airborne microorganisms correlated well with the amount of sewage treated in MSTP. Another important reason may be due to different aeration systems used in those plants. As Sánchez-Monedero *et al.* (2008) reported, the impact of the aeration methods on bioaerosol release was: air diffusers < surface turbine < horizontal rotors. Compared to aeration systems with air diffusers (Brandi *et al.*, 2000; Sánchez-Monedero *et al.*, 2008; Heinonen-tanski *et al.*, 2009) and turbines (Sánchez-Monedero *et al.*, 2008; Grisoli *et al.*, 2009), rotating aeration brushes were installed in the oxidation ditch in the present MSTP. While rotating a number of paddles mounted on a large-diameter horizontal shaft to provide liquid motion and wastewater aeration, the rotating brushes also produced a greater dispersion of microorganisms into the environment. Moreover, the difference of bacterial concentration may be due to heavier air pollution in Xi'an than in other cities examined in the world (Chen *et al.*, 2012; Hu *et al.*, 2012). This has been confirmed by Li *et al.* (2012), who thought that the high microbial concentrations was a reflection of recent rapid economic growth in Xi'an in the past decades.

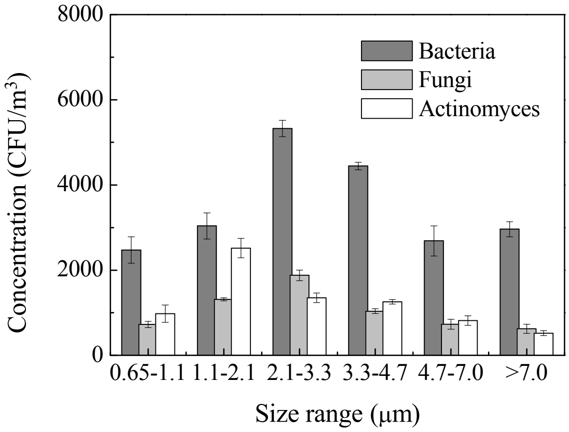
In addition, the highest concentrations of both airborne bacteria and actinomycetes were found in the indoor environment (SDH), implying that the largest emission source of bacteria and actinomycete aerosols was the area of mechanical agitation of sludge. Similar results were also reported by other authors (Fracchia *et al.*, 2006; Breza-Boruta and Paluszak, 2007; Sánchez-Monedero *et al.*, 2008; Heinonen-tanski *et al.*, 2009; Haas *et al.*, 2010). As indicated by Breza-Boruta and Paluszak (2007), the strongest emission of bacteria and actinomycetes at the composting piles resulted from favorable conditions for their proliferation in composted biomass. It is worthy of noting that only the sludge-dewatering house among selected sampling sites is an enclosed room. As indicated by Li *et al.* (2011b), insufficient ventilation and reduced die-off rates from limited solar radiation led to the accumulation of microorganisms in the room, thereby increased the concentrations of microbial aerosols. Adequate ventilation is therefore needed in enclosed spaces to protect the health of workers. Moreover, according to Haas *et al.* (2010), the survival time of bacteria in the emitted aerosol increased in closed spaces due to the high relative humidity. In the present study, the relative humidity in the SDH was higher than that in other outdoor environments, which may be another reason for the higher levels of airborne bacteria.

Since there are no official standards to regulate the permissible content of actinomycetes, fungi and bacteria in outdoor and indoor air in China, it is not easy to evaluate the level of air contamination with microorganisms in the present MSTP. Nevertheless, compared to the threshold value (100 CFU/m^3) of airborne actinomycetes and that of airborne bacteria (1000 CFU/m^3) recommended by American Conference of Governmental Industrial Hygienist (ACGIH), the mean concentrations of airborne actinomycetes and bacteria detected at the SDH are about 21 times and 8 times as much as the above permissible limits, respectively. Such comparison reveals to some extent that substantially strong air pollution occurs in the present MSTP in Xi'an. It can be also seen that more attention should be paid to the potential health risk related to actinomycetes, an often neglected issue in previous studies on wastewater treatments.

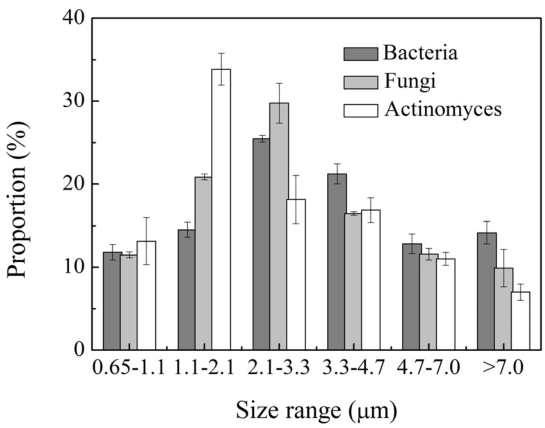
Size Distributions of Airborne Viable Bacteria, Fungi and Actinomycetes

Fig. 3 presented the size distributions of total airborne bacteria, total fungi and total actinomycetes in this sewage treatment plant. It could be seen that there were similar distribution characteristics among three types of airborne microorganisms: single-peak distribution pattern. The highest concentrations of both total bacteria and total fungi appeared between 2.1 and 3.3 μm (stage 4) and they were considerable fractions of 26% and 30% respectively. The highest concentration of total actinomycetes aerosol was in a size range of 1.1–2.1 μm (stage 5) with considerable fractions of 34% of total actinomycetes. In contrast, the lowest concentration of airborne bacteria, fungi and actinomycetes was detected in stage 7 (0.65–1.1 μm), stage 1 (> 7 μm) and stage 1 (> 7 μm), respectively, and each fractions was less than 10%.

In addition, results shown in Fig. 3(b) revealed that about 52% of airborne bacteria, 62% of airborne fungi and 65% of airborne actinomycetes were in respirable size range (less than 3.3 μm), which implied that most of the airborne fungi and actinomycetes were in single cells or pore patterns. Although the total concentration of actinomycetes was smaller than that of bacteria in the MSTP, the respirable fraction for actinomycetes was higher than that for bacteria. Therefore, it is necessary to pay more attention to such high respirable fraction for actinomycetes emitted from MSTP with regard to the higher prevalence rates of asthma, pneumonia, and influenza.



(a) Size distribution by number



(b) Size distribution by number frequency

Fig. 3. Size distributions of airborne microorganisms in the municipal sewage treatment plant of Xi'an, China.

Fig. 4 presents the size distribution of airborne bacteria at different phases of sewage treatment process. It could be found that the size distributions of airborne bacteria differed greatly among four phases selected. At each phase of the AGC, OD and SST, the highest proportions of airborne bacteria were detected on stage 4 (2.1–3.3 μm) with about 30%, 31% and 30% of airborne bacteria, respectively, while the lowest values were found on stage 6 (0.65–1.1 μm) with 2%, 7% and 8%, respectively. At the SDG, however, the highest (26%) and lowest proportion (8%) appeared on stage 3 (3.3–4.7 μm) and stage 1 (> 7.0 μm), respectively. The distribution of airborne bacteria in respirable size generated from AGC, OD, SST and SDG was 38%, 56%, 49% and 57%, respectively. This meant that OD and SDG could generate more respirable microbial aerosols than AGC. The proportion differences of respirable microbial aerosols among OD, SST and SDG were not statistically significant ($p > 0.05$).

Fig. 5 shows the size distribution of airborne fungi at different phases of sewage treatment process. The size distribution pattern of airborne fungi was approximately similar at four sites. The maximum proportions of airborne fungi were found on stage 4 (2.1–3.3 μm) at all of four phases, whereas the minimum proportions were observed on stage 1 (> 7.0 μm) and 6 (0.65–1.1 μm) at all four phases. The distribution of airborne fungi in respirable size generated from AGC, OD, SST and SDG was 66%, 67%, 58% and 65%, respectively, indicting that, the distribution proportion of respirable airborne fungi did not show obvious variations for four phases.

Fig. 6 is the diagram of the size distribution of airborne actinomycetes at different phases of sewage treatment process. At the OD, the highest distribution appeared on stage 3 (3.3–4.7 μm) with 30.9% of airborne actinomycetes. In contrast, at other three phases, the highest distributions appeared on stage 4 (2.1–3.3 μm) with 31.1%, 37.5%, 32.0%, respectively. Moreover, the lowest distributions were detected on stage 1 (> 7.0 μm) at all of four phases. It was

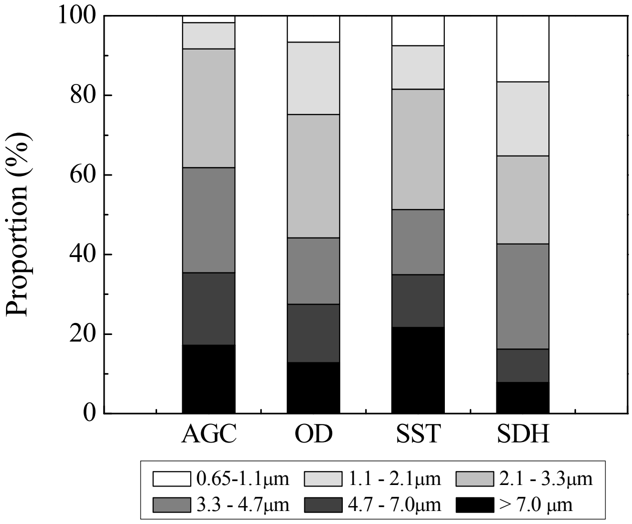


Fig. 4. Size distribution of airborne bacteria at different sewage treatment phases.

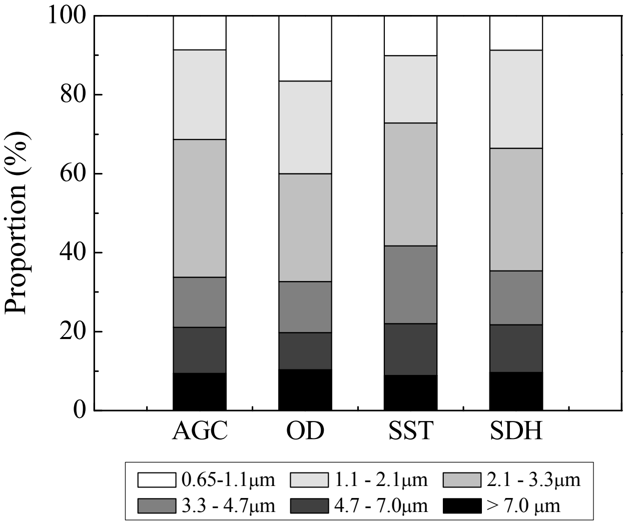


Fig. 5. Size distribution of airborne fungi at different sewage treatment phases.

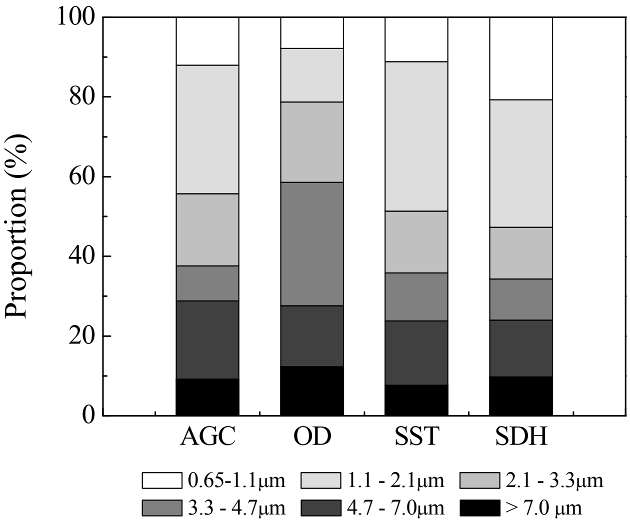


Fig. 6. Size distribution of airborne actinomycetes at different sewage treatment phases.

9.1%, 11.8%, 8.0% and 9.8% at the AGC, OD, SST and SDG, respectively. The proportions of airborne actinomycetes in respirable size from AGC, OD, SST and SDG accounted for about 62%, 41%, 64% and 66%, respectively. The distribution differences of respirable microbial aerosols were not statistically significant ($p > 0.05$) among AGC, SST and SDG.

Table 1 presented comparisons of the count median diameters (CMD) of airborne microorganisms at different phases of sewage treatment process. The CMD order of different microbial aerosols was found to be similar at each phase, that is, airborne bacteria > airborne fungi > airborne actinomycetes. This revealed once again that airborne actinomycetes emitted from MSTP might have more significant effect on public health and urban air quality than bacteria and fungi. The reason of different CMDs among airborne microorganisms may be that airborne bacteria

Table 1. Comparisons of CMD of airborne microorganisms at different phases (unit: μm).

	Bacteria aerosols	Fungi aerosols	Actinomycetes aerosols
Aerated grit chamber (AGC)	4.0	2.7	2.4
Oxidation ditch (OD)	3.1	3.0	3.7
Secondary settling tank (SST)	3.4	2.9	2.2
Sludge dewatering house (SDH)	2.9	2.4	2.0

exist primarily in clusters or attached to particles whereas airborne fungi and actinomycetes may exist mainly in single cells or pore patterns (Rajasekar and Balasubramanian, 2011; Li *et al.*, 2012).

In addition, the CMD of each microbial aerosol at different sewage treatment phases was different. The largest CMD of bacteria, fungi and actinomycetes aerosols appeared at the AGC, OD and OD, respectively, while the smallest values were all in the SDH. This meant that the CMD of microbial aerosols generated from the AGC and OD were larger than that in the SDH. The size of microbial aerosols generated from the AGC and OD is generally larger than that from the SDH. This fact may be relative to different activities among these phases of sewage treatment plant. The vigorous bubbling aeration mode used at the AGC and horizontal rotor aeration mode using in the OD seemed to produce relatively larger aerosols than centrifugation operation in the SDH. This should be further studied in the future.

CONCLUSIONS

Compared to background levels, the significantly higher numbers of airborne viable bacteria, fungi and actinomycetes were all detected at each phases of the sewage treatment process, indicating that the municipal sewage treatment plant was really an important source of microbial aerosols. Both aeration and sludge thickening operations were found to be able to generate much high levels of microbial aerosols. For different airborne microorganisms detected in this study, similar single-peak distribution pattern was also observed in the sewage treatment plant. About 52% of airborne bacteria, 62% of airborne fungi and 65% of airborne actinomycetes were in respirable size range (less than 3.3 μm), which meant that they were more likely to reach bronchi and alveolus easily and cause adverse health effects. Moreover, sampling results revealed that size distribution of each of airborne microorganisms varied greatly at different phases. The count median diameters of microbial aerosols at the aerated grit chamber and oxidation ditch were generally found to be larger than that in the sludge-dewatering house due to the different operation mode. Another important finding was that the order of the count median diameters of different microbial aerosols was similar at each phase, that is, airborne bacteria > airborne fungi > airborne actinomycetes. This implied that airborne actinomycetes emitted from MSTP might have more significant effect on public health and urban air quality than bacteria and fungi. Emission characteristics of airborne viable bacteria, fungi and actinomycetes determined in this study can be useful for understanding their potential health impacts and establishing the proper control measure in sewage treatment plants.

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