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Original Research

Concentration and Size Distribution of Airborne Actinomycetes in a Municipal Wastewater Treatment Plant

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Abstract

To quantify the emission of airborne actinomycetes from the wastewater treatment plant (WWTP), the concentration and size distribution of airborne culturable actinomycetes were examined with a six-stage cascade impactor in a municipal WWTP with oxidation ditch process in Xi'an, China from June to July 2011. Simultaneously, characteristics of airborne bacteria were also measured to compare with data of actinomycetes. Similar to airborne bacteria, the concentration and size distribution of airborne actinomycetes were found to vary greatly at different phases of the wastewater treatment process. The mean concentration of actinomycetes in the WWTP ranged from 2139±229 at the sludge dewatering house (SDH) to 902±54 CFU·m^{-3} at the effluent outlet (EO). The largest emission source of actinomycete aerosols was detected at the SDH, with 21-fold exceeding the permissible standards recommended in the literature. The particle size distributions showed that similar single-peak distribution patterns appeared for both airborne actinomycetes and bacteria. The count median diameter (CMD) of total actinomycetes and bacteria concentration were 2.3 and 3.2 μm in the WWTP, respectively. Although the total concentration of actinomycetes was smaller than that of bacteria in the present WWTP, the respirable fraction (particles smaller than 4.7 μm in aerodynamic diameter) for actinomycetes (81.9%) was higher than that for bacteria (64.6%). The results obtained in the present research suggest that more attention should be paid to the potential health risk related to actinomycetes in studies on wastewater treatments.

Keywords: municipal wastewater treatment plant, microbial aerosols, concentration, size distribution, airborne actinomycetes

Introduction

With the acceleration of urbanization in recent years, more and more wastewater treatment plants (WWTP) have been built and put into operation in China. As a site of wastewater treatment, however, wastewater treatment plants have been regarded recently as a major source of microbial aerosols and thus have become of growing concern to the public [1-3]. It is well known that wastewater contains high numbers of pathogens like bacteria, fungi, and viruses. Aerosolization of these microorganisms is then the present inevitably in many phases of the wastewater treatment process, particularly in those phases containing aeration and mechanical agitation operations [4]. As a consequence, microbial aerosols generated from WWTP may transport pathogens over long distances and may cause serious impact on human health and air quality [5-7].

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So far, numerous studies have been conducted to assess exposure levels to airborne culturable bacteria and fungi generated from WWTP. The exposure of sewage workers to airborne bacteria and fungi has been reported to vary depending on site location, type and amount of wastewater, treatment technology, and weather conditions [4, 8-11]. Such exposure has also been found to cause a variety of infectious diseases as well as allergic and toxic effects [5, 12, 13]. However, few studies have investigated the concentration of airborne actinomycetes from WWTP while most of studies examine that of airborne bacteria and fungi from WWTP. Furthermore, a few studies concerning actinomycetes quantification reported in agricultural areas and urban outdoor environments [14-17] have indicated that actinomycete have a worldwide distribution and are usually found in soil, decaying organic material, and sewage. Many actinomycete species are human pathogens that can enter the body through wounds or by inhalation to cause illnesses, such as allergic alveolitis, lung abscesses, appendicitis, and mucous membrane irritation [18]. Therefore, it is important to study emissions of airborne actinomycetes from WWTP for further assessment of potential risk in relation to the wastewater treatment process.

Note that effects of microbial aerosols on human health depend not only on their concentration and species, but also on 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. According to Pastuszka et al. [19], particles with diameter less than 4.7 μm (the so-called respirable particles) can penetrate the human alveolus easily and can lead to allergic alveolitis and asthma. In contrast to many studies on size distribution of microbial aerosols in other environments such as composting plants and indoor environments [19-23], only limited studies on size distribution of airborne bacteria and fungi in WWTP have been reported in the literature [8, 24]. Until now, no reference literatures relate to the size distribution of airborne actinomycetes in the WWTP environment. Hence, it is critical to study the size distribution of airborne actinomycetes emitted from WWTP in order to correlate human disease.

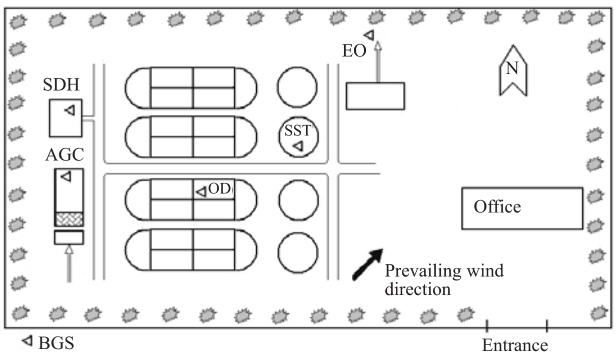

Fig. 1. Sketch of sampling sites at a municipal wastewater treatment plant in Xi'an, China. AGC – aerated grit chamber, OD – oxidation ditch, SST – secondary settling tank, SDH – sludge dewatering house, EO – effluent outlet, BGS – background site, Δ – sampling sites.

The objective of the present study is to gain comprehensive information about the emission characteristics of actinomycete aerosols generated in typical WWTP in China. For this purpose, a WWTP with the oxidation ditch process in Xi'an was selected because this process is most widely used in chinese WWTPs due to good treatment efficiency and inexpensive running costs. The concentration and size distribution of airborne actinomycetes were examined emphatically at different wastewater treatment phases by using an Andersen six-stage cascade impactor and the culture method. Additionally, the concentration and the size distribution of airborne bacteria were also measured to compare with data of actinomycetes detected here and with data of previous literatures.

Materials and Methods

Plant Description

Bioaerosol samples were collected at a municipal WWTP with an oxidation ditch process located in the eastern part of Xi'an, China. This plant has been operated continuously since 2006, with a treatment capacity of 2×10^5 $m^3\cdot d^{-1}$ 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 tanks (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).

Sampler

An Andersen six-stage cascade impactor (Westech, UK) with six 93 mm diameter glass petri dishes was employed to collect bioaerosol samples with different size ranges. 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 the definition above, respirable fraction of particles corresponds to the size range between stages 3-6.

Sampling Method

Sampling was conducted from June 2011 to July 2011 at the six sampling sites, among which five sites 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 (Fig. 1). At each of the sampling sites, the sampler was mounted 1.5 m above the floor surface and 0.5-1.5 m far from the treatment units. All outdoor samples were taken downwind.

Table 1. Concentrations of airborne actinomycetes and bacteria at all sampling sites in the WWTP (Unit: CFU·m^{-3}).

	Actinomycetes		Bacteria		p-value*
	Mean ± SD	Range	Mean ± SD	Range	
Background site (BGS)	473±46 [a]	445-526	1165±39 [a]	1120-1194	0.005
Aerated grit chamber (AGC)	1291±69 [b]	1233-1367	3117±233 [b]	2867-3329	0.004
Oxidation ditch (OD)	1706±96 [c]	1639-1816	4328±347 [bc]	3936-4598	0.0049
Secondary settling tank (SST)	949±121 [bdf]	872-1088	2755±212 [bd]	2628-3000	0.0089
Sludge dewatering house (SDH)	2139±229 [ce]	1879-2233	7866±969 [e]	7060-8942	0.0073
Effluent outlet (EO)	902±54 [fd]	869-964	1696±97 [f]	1597-1780	0.0018

a, b, c, d, e, f means that mean values within the column by the same letter are not significantly different ($p<0.05$).
*The differences between airborne actinomycetes and bacteria are compared by t-test.

At each site, sampling was performed twice: once in June and once in July. The samples were collected for about 10 min. by sucking air at a rate of 28.3 L/min with three repetitions each time. Therefore, a total of 12 samples (72 plates) were collected including 6 samples (36 plates) for actinomycetes and 6 samples (36 plates) for bacteria at each site. A total 72 of samples (432 plates) for six sampling sites were analyzed in this study.

To reduce the measurement uncertainty resulting from weather variations, the sunny days with similar climatic conditions and the same period between 10-12 a.m. were selected for sampling. In order to ensure similar climatic conditions wind speed, ambient temperature, and relative humidity were monitored simultaneously. During sampling, the outdoor temperature and relative humidity ranged from 26.9 to 30.2ºC and 47-62%, respectively. The prevailing wind direction was southwest with average wind speed of 0.6 m/s.

Microbial Cultivation

The airborne culturable bacteria and actinomycetes were captured on petri dishes with appropriate cultivation agars in the present study. After sampling, the agar plates were immediately transported to the laboratory for incubation. Bacteria were incubated in nutrient agar (3 g beef extract, 10 g peptone, 5 g sodium chloride, 15 g agar, 1000 ml distilled water, PH=7.2) at 37ºC for 48 h [16]. Actinomycetes were incubated in Gause's synthetic agar (20 g soluble starch, 1 g KNO_3, 0.5 g NaCl, 0.5 g $K_2HPO_4·3H_2O$, 0.5 g $MgSO_4·7H_2O$, 0.01 g $FeSO_4·7H_2O$, 20 g agar, 1000 ml distilled water, PH=7.2, 0.01% potassium dichromate added to inhibit bacterial and fungal growth) at 28ºC for 120 h [16]. The sampler was 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 [25] to revise colony overlapping. Each concentration of airborne microorganisms, which is generally expressed as total colony-forming units (CFU·m^{-3}) for respective particle size, was then calculated by dividing the number of colonies cultured separately for each stage by the sampling air volume.

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 Culturable Actinomycetes

The concentrations of airborne culturable actinomycetes and bacteria in aerosol samples at all sampling sites of the WWTP are shown in Table 1. It can be clearly seen that the concentrations of airborne actinomycetes varied at different wastewater treatment phases, ranging from 2,233 to 445 CFU·m^{-3}. The highest mean concentration of actinomycetes was observed at SDH, and the lowest was found at EO. Significantly, actinomycete concentrations at SDH and OD were higher than those at other sites ($p<0.05$), but no significant differences were found between SDH and OD, or SST and AGC, or SST and EO ($p>0.05$). In addition, the concentrations of airborne actinomycetes at each site were observed to be significantly lower than those of airborne bacteria ($p<0.01$) as shown within the rightmost column in Table 1. Bacterial concentrations tested in the present study ranged from 1,120 to 7,068 CFU·m^{-3}. The highest mean concentration of bacteria was detected at SDH while the lowest was found at EO in WWTP ($p<0.05$).

As also observed in Table 1, the concentrations of airborne actinomycetes and bacteria were significantly higher at each phase of the wastewater treatment process than those at the background site ($p<0.05$), indicating that WWTP was not only an emission source of airborne bacteria, but actinomyces. The present finding regarding the latter confirmed the conclusions drawn by Fracchia et al. [2], Korzeniewska et al. [10], and Breza-Boruta and Paluszak [17].

In the present study, the lowest concentration of airborne actinomycetes found at EO indicated that the sewage after biological treatment contained much lower amounts of microorganisms. In contrast, the highest concentrations of airborne actinomycetes were found at SDH, implying that the largest emission source of actinomycete aerosols was the area of mechanical agitation of sludge. Similar results were also reported by Fracchia et al. [2] and Breza-Boruta and Paluszak [17]. As stated by Breza-Boruta and Paluszak, the strongest emission of actinomycetes at the composting piles resulted from favorable conditions for their proliferation in composted biomass. It is worth noting that only the sludge-dewatering house among selected sampling sites is an enclosed room. Therefore, as indicated by Li et al. [24], 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.

The concentrations of actinomycetes and bacteria obtained in this study were also found to be higher than those of other studies on WWST [3, 8-11, 17]. This might be attributed mainly to the larger volume of treated water (2×10^5 $m^3\cdot d^{-1}$) applied in the present study. Brandi et al. [8] proved that the numbers of airborne microorganisms correlated well with the amount of sewage treated in WWTP. Another important reason might be due to heavier air pollution in China than in other cities in the world. This has been confirmed by Fang et al. [16], who thought that the high microbial concentrations was a reflection of recent rapid economic growth in China in the past decades.

Since there are no official standards to regulate the permissible content of actinomycetes and bacteria in outdoor and indoor air in China, it is hard to evaluate the level of air contamination with actinomycetes in this WWTP. Nevertheless, compared to the threshold value (100 $CFU\cdot m^{-3}$) of airborne actinomycetes recommended by Polish Standards [17], both of the highest and lowest mean concentrations detected at the SDH and EO far exceed this permissible limit with 21-fold and 9-fold excess, respectively. Such comparison reveals to some extent that substantially strong air pollution occurs in the present WWTP in Xi'an. Consequently, 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 Culturable Actinomycetes

Fig. 2 illustrated the size distributions of total airborne actinomycetes and total bacteria emitted from the present WWTP. It can be seen in Fig. 2a that there was a similar distribution pattern between two kinds of airborne microorganisms: a single-peak distribution pattern. The highest concentration of airborne actinomycetes was found in a size range of 1.1~2.1μm (stage 5), with considerable fractions of 34% of total actinomycetes. The highest bacteria concentrations appeared between 2.1 and 3.3 μm (stage 4), accounting for 26%. In contrast, the lowest concentration of airborne actinomycetes and bacteria was detected in stage 1 (>7μm) and stage 6 (0.65~1.1μm), respectively, and each fraction was less than 10%.

Since there were no precedent studies on size distributions of airborne actinomycetes in the environment of WWTP, a comparison with those from the outdoor atmospheric environments in Beijing reported by Fang et al. [16] was carried out in this study. In their study, most of the actinomycete particles were collected at stages 1, 5, and 6 and the relatively uniform percentages were detected in stages 2-4. This difference of distribution pattern was most likely due to different emission sources. In the present study, the source of actinomycetes is attributed to sewage and sewage sludge, while the source of actinomycetes is considered to be traffic flow and vegetation coverage in Fang et al.'s study [16]. Another comparison with size distribution of airborne bacteria emitted from WWTP was also conducted in this study. The experiments carried out by Li et al. [25] in a Beijing municipal WWTP with similarly Orbal oxidation ditch process found that the maximum and minimum proportions of airborne bacteria were detected in

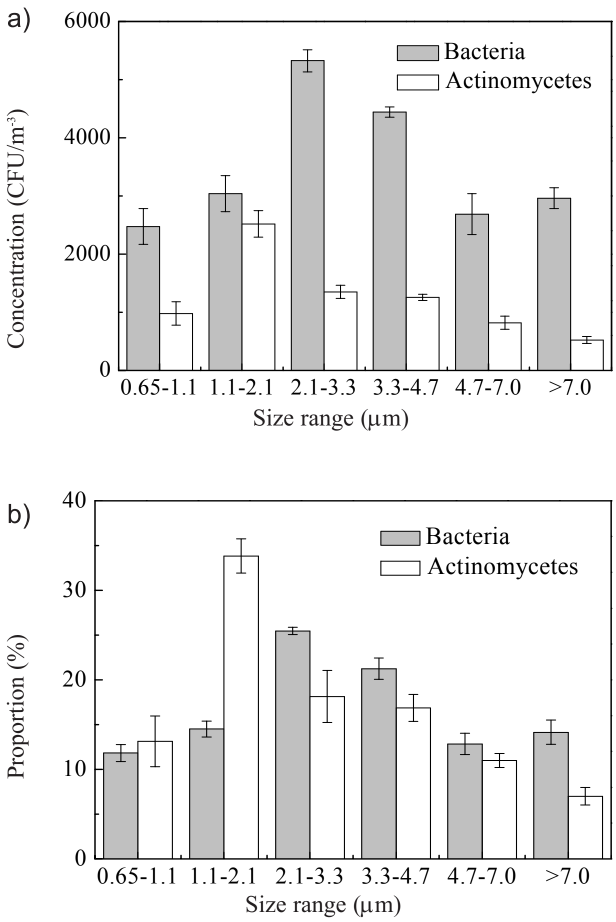

Fig. 2. Size distributions of (a) concentrations and (b) proportions of airborne microorganisms at the wastewater treatment plant.

the 4th (2.1~3.3 μm) and 6th stages (0.65~1.1 μm), respectively, which are consistent with the present results. Moreover, it was interesting that similar distribution patterns were also obtained by several studies on distribution of airborne bacteria in other environments in China, such as commuter buses in northern Taiwan [21] and the Mogao Grottoes of Dunhuang [26]. The reason for this is unknown and further research needs to be performed.

It can be also seen in Fig. 2b that 64.6% of airborne bacteria and 81.9% of airborne actinomycetes were in respirable size range. Although the total concentration of actinomycetes was smaller than that of bacteria in the WWTP, the respirable fraction for actinomycetes was higher than that for bacteria. Therefore, it is necessary to pay more attention to such high respirable fractions for actinomycetes emitted from WWTP with regard to the higher prevalence rates of asthma, pneumonia, and influenza.

To our knowledge, the results related to the respirable fractions of bioaerosols in WWTP are limited. Brandi et al. [8] detected the fraction of respirable bacterial particles with the six-stage Andersen apparatus in a WWTP of Italy and indicated that this fraction ranged from 20-40% of collected bacteria. Since they defined respirable particles as ones with diameter less than 2.1 μm, it is hard to simply compare the present respirable fractions with their results. However, it can be found that the proportion of respirable bacterial particles in the present environment of WWTP was comparable to the results obtained in other environments [19-21, 23], suggesting that the respirable fraction of bacteria might not be dependent on building type and material, geographical and climatic factors, and concentration of atmospheric and indoor particulate aerosol [19].

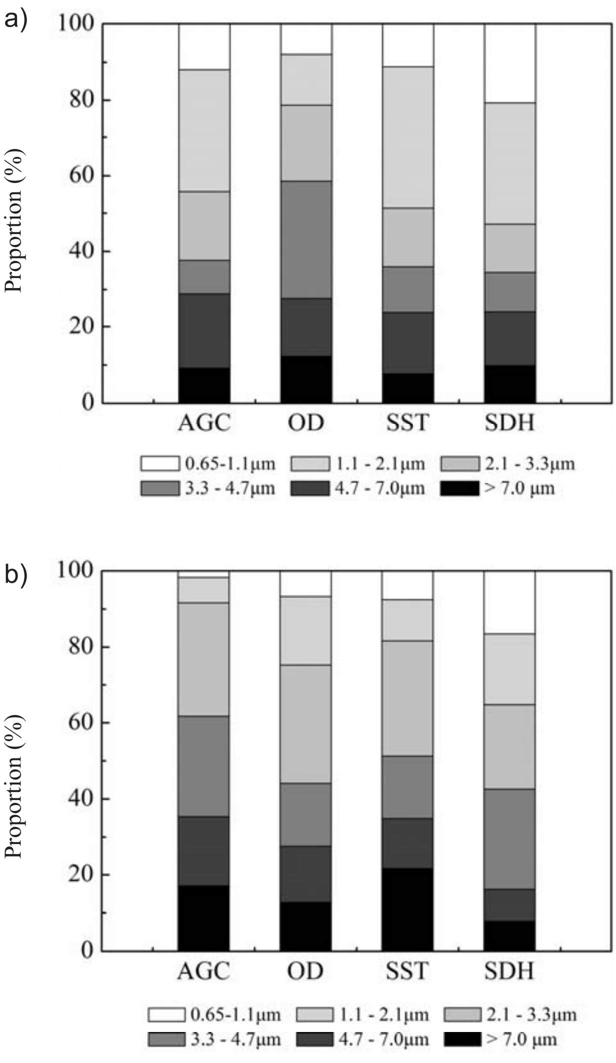

Fig. 3. Size distribution of (a) airborne actinomycetes and (b) airborne bacteria at different wastewater treatment phases.

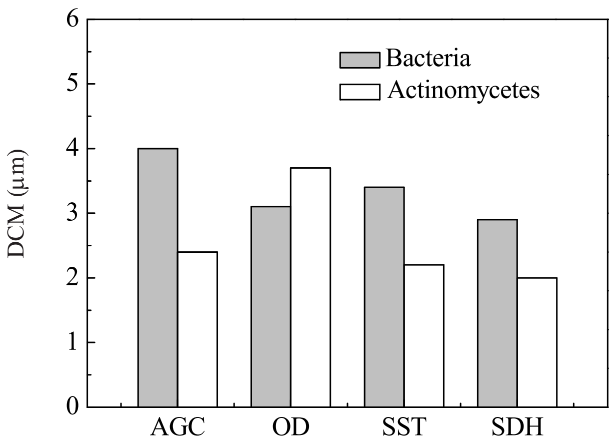

Fig. 4. Comparisons of the count median diameter (CMD) of airborne actinomycetes and bacteria at different wastewater treatment phases.

In order to explore the cause of distribution pattern of microbial aerosols in the WWTP, the size distributions of airborne actinomycetes and bacteria at various wastewater treatment phases were shown in Fig. 3. As seen in Fig. 3a, at each of the AGC, SST, and SDH, the highest proportions of airborne actinomycetes happened on stage 5 (1.1-2.1 μm), with 32.2%, 37.5%, and 32.0%, respectively. Only at the OD did the highest distributions appear on stage 3 (3.3-4.7 μm), with 30.9% proportions. Moreover, the lowest distributions were detected on stage 1 (> 7.0 μm) at all of four sites. It was 9.1%, 11.8%, 8.0%, and 9.8% at the AGC, OD, SST, and SDG, respectively. Similarly, the highest proportions of airborne bacteria were detected on stage 4 (2.1-3.3 μm), with about 30%, 31%, and 30% of total airborne bacteria at the AGC, OD, and SST, respectively, while the lowest values were found on stage 6 (0.65-1.1 μm) with 2%, 7%, and 8%, respectively (shown in Fig. 3b). Obviously, these results from each wastewater treatment phase revealed why the highest concentrations of airborne actinomycetes and bacteria were found in stages 5 and 4, respectively, and why the lowest concentrations of both were detected in stage 1 in overall WWTP.

Fig. 4 illustrated comparisons of the CMD of airborne actinomycetes and bacteria at different wastewater treatment phases. The CMD of airborne actinomycetes at each treatment phase was found to be smaller than that of airborne bacteria except at OD. As a result, it can be expected that the CMD of total airborne actinomycetes was also smaller than that of total airborne bacteria in the WWTP. In fact, the two values were 2.3 and 3.2 μm, respectively.

The lower CMD of total airborne actinomycetes suggested that airborne actinomycetes emitted from WWTPs were more likely to reach the bronchi and lungs of humans, thereby to have a significant effect on public health and urban air quality compared to bacteria. In addition, the detection of bacteria within larger-sized particles implied that airborne bacteria existed primarily in clusters or attached to sludge particles, whereas airborne actinomycetes may exist mainly in single cells or pore patterns.

It can be also found in Fig. 4 that the largest CMD of bacteria and actinomycete aerosols appeared at the AGC and OD, respectively, while the smallest values happened in the SDH. This meant that the CMD of microbial aerosols generated from the AGC and OD were generally larger than that in the SDH, which may be attributed to different activities among these phases of wastewater treatment. 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, which will be further studied in our future work.

Conclusions

1. Compared to background levels, the significantly higher concentrations of airborne culturable actinomycetes and bacteria were detected at each phase of the wastewater treatment process, which indicated that WWTP is not only an emission source of airborne bacteria, but actinomyces.
2. The concentration of airborne actinomycetes was different from place to place at each treatment phase. The largest emission source of actinomycete aerosols occurred at the sludge dewatering house (SDH), with 21-fold exceeding the permissible standards recommended in the literature.
3. Similar to airborne bacteria, the size distribution of airborne actinomycetes was found to present a single-peak distribution pattern in the present WWTP. However, the respirable fraction for actinomycetes (81.9%) was higher than that for bacteria (64.6%). Furthermore, the count median diameter (CMD) of actinomycetes (2.3 μm) was smaller than that of bacteria (3.2 μm) in this study.
4. The size distribution of airborne actinomycetes varied at different phases of the wastewater treatment process. The CMD of microbial aerosols at the aerated grit chamber (AGC) and oxidation ditch (OD) were generally found to be larger than that at SDH due to the different operation mode.

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