Characteristics and formation mechanism of intestinal bacteria particles emitted from aerated wastewater treatment tanks

Yanjie Wang[1,2,3],Lin Li[1,4]*, Song Xue[1,5], Yunping Han[1,4], Kaixiong Yang[1,4]

wangyanjie_2008@126.com, leel@rcees.ac.cn, xuesong@lyun.edu.cn,

yphan@rcees.ac.cn, yang_kaixiong@126.com

1, State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, P. R. China.

2. School of Public Health, Zhengzhou University, Zhengzhou 450001, P. R. China.

3. Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK.

4. National Engineering Laboratory for VOCs Pollution Control Material & Technology, University of Chinese Academy of Sciences, Beijing 101408, P. R. China.

5,Fujian Provincial Colleges and University Engineering Research Center of Solid Waste Resource Utilization, Longyan University, Longyan, P. R. China

*Corresponding author: Lin Li

Tel.: 86-10-62923543

E-mail:leel@rcees.ac.cn

Abstract

Aeration tanks in municipal wastewater treatment plants (WWTPs) are regarded as sources of bioaerosols, often containing particles and microbes. In this study, intestinal bacteria were investigated from biochemical reaction tanks (BRTs) of six municipal WWTPs. It was observed that 86 CFU/m^3 of intestinal bacteria (in average) occurred in the BRTs installed surface aerator, which was higher than those adopted submerged aeration (67 CFU/m^3 in average). 62.72% of fine particles were observed in the BRTs supplied oxygen by submerged aerator, while 75.73% of coarse particles emitted during surface aeration. *Pseudomonas* sp., *Serratia* sp. and *Acinetobacter* sp. were identified as pathogenic bacteria presented in the intestinal bacteria population and most of them existed initially in water or sludge, particularly in water surface. The emission level and particle size distribution were significantly correlated with aeration mode adopted by the WWTPs. The bioaerosols particles emitted from surface aeration process was higher than that from submerged aeration process. Meanwhile, the BRTs with submerged aerators released more fine particles, which can get into the alveoli and represented the potential challenge to human health. Canonical correspondence analysis results exhibited that population of intestinal bacteria had a positive correlation with aeration rate and water quality. As the intestinal bacteria in the bioaerosols emitted from the WWTPs may pose a potential risk to onsite operators, aeration tanks in WWTPs should be paid more attention as a source of intestinal bacterial emissions.

Key words： Formation mechanism, particle size distribution, surface aeration, submerged aeration, WWTPs

44

Nomenclatures

BRTs	Biochemical reaction tanks	WWTPs	Wastewater treatment plants
OAC	Outdoor air control	GNOPB	Gram-negative opportunistic intestinal bacteria
CCA	Canonical correspondence analysis	OTUs	Operational taxonomic units
BIB:	Bacteria in BRT	IBIB	Intestinal bacteria in BRT
BIO	Bacteria in OAC	IBIO	Intestinal bacteria in OAC;
PIBB	Proportion of intestinal bacteria in bacteria in BRT	TBBO	Times of bacteria in BRT on that in OAC
TIBO	Times of intestinal bacteria in BRT on that in OAC	QIIME	Quantitative Insights Into Microbial Ecology
UCHIME	An algorithm for detecting chimeric sequences	RDP	A naive Bayesian classifier
T	Temperature	RH	Relative humidity
SR	Solar radiation	WS	Wind Speed
SH	Sampling height	AF	Airflow
ST	Sampling Time	AV	Sampling volume
SS	Suspended solids	COD	Chemical oxygen demand
AR	Aeration rate	ND	Number of droplets
MC	microbe concentration	UP:	The upper part of observation area (0.09m-0.18 m)
LP	The lower part of observation area(0 m-0.09 m)		
Dg	Aerodynamic diameter	q*	The atomization quantity
Δ log Dg	Logarithm of differences of cut-off diameters for particular stage of 6-stage Andersen	h_e	Aerated water thickness without air
		h_{eu}	Aerated water thickness with air
ΔC	concentration of bacterial aerosol on particular stage of 6-stage Andersen impactor	y*	The starting position of water flow atomization
		u	The droplet ejection speed
C_{total}	Total concentration of bacterial aerosol	β	Water content of section of the breaking points
μ	The wind speed near droplets	y	The distance perpendicular to the center of the stream

1 Introduction

Secondary treatment of wastewater achieves a certain degree of effluent quality via the use of a wastewater treatment plant (WWTP) that features physical separation of settled solids and the biological removal of dissolved and suspended organic compounds. Biological processes offer the advantage of using the organic compounds in the water as a substrate and energy source in metabolic activities. Dissolved oxygen is often provided by the diffusers at the bottom of the water or a rotor aeration system at the water surface to promote the biological process. Bioaerosols can be produced during the aeration process and released into the surrounding atmosphere. Aerosol contamination in sewage treatment plants has been evaluated by Carducci's research group; they found that the aeration tank was a significant source of bioaerosols, with high levels of viral contamination (Carducci et al., 1999). In a study of bioaerosol characteristics generated by a large urban WWTP in the Middle East, the aeration tank had a higher concentration of bacterial emission, with an average of 1973 CFU/m^3 during summer (Sadegh et al., 2015).

There are a variety of means to aerate water, including two broad areas, surface aeration and submerged aeration. Surface aeration devices include a low-speed surface aerator, fountains, floating surface aerators, and paddle wheel aerators (Roberts & Daendliker, 1983). They are typically applied in WWTPs with an oxidation ditch treatment process. Submerged aeration, such as jet aeration and coarse or fine bubble aeration, provides oxygen via air bubble release at the bottom of the aeration tanks (Frank et al., 2009). Bioaerosols emitted from six WWTPs were monitored and compared by researchers in

Spain to evaluate the effect of the aeration mode on aerosol generation. Compared to submerged air diffuser aerators, surface aerators such as horizontal rotors and surficial turbines generated a higher number of bioaerosols (Sánchez-Monedero et al., 2008). A similar phenomenon was observed in Aller's reports (Aller et al., 2005).

Gram-negative opportunistic intestinal bacteria (GNOPB) including *Acinetobacter* sp., *Alcaligenes* sp., *Citrobacter* sp., and *Enterobacter* sp., have been isolated in bioaerosols from the aerial environment of aeration tanks (Zhang et al., 2017). Previous estimates have also suggested that intestinal bacteria such as *Streptomyces* sp. have been emitted from a hospital WWTP (Uhrbrand et al., 2017). Some of the intestinal bacteria in the bioaerosols emitted from WWTPs can cause respiratory diseases and other health effects(Laitinen et al., 1992). *Pseudochrobactrum* sp., *Brevundimonas* sp., *Chryseobacterium* sp., *Micrococcaceae* sp., *Citrobacter* sp., and *Yersinia* sp. which recognised as pathogenic bacteria were also identified from aerosols during the wastewater treatment process (Wang et al., 2019). Inhaled bacteria, particularly Gram-negative bacteria, pose a high risk to human health (Laitinen et al., 1992).

Over 5000 municipal WWTPs had been constructed in China by the end of 2018. Nearly 90% of the WWTPs have implemented treatment processes equipped with surficial aerators or submerged aerators in biochemical reaction tanks (BRTs).With the rapid increase in the size of cities and urban populations in recent years, many WWTPs built in rural areas are now situated near to residential areas. Exposure to bioaerosols can pose a health risk not only to onsite workers at these WWTPs but also to surrounding inhabitants(Gotkowska-Plachta et al., 2013).

As the size of bioaerosols is always between 0.1μm and 100μm, bioaerosols may affect one's health by entering into the human respiratory tract. Most previous studies have focused on the emission level and species of intestinal bacteria in bioaerosols from WWTPs. Their particle size, source analysis, and formation mechanism are rarely addressed. Intestinal bacteria in bioaerosols emitted from aeration tanks were investigated at six WWTPs situated in the Yangtze River deltas, Pearl River Delta region, and the Jing-Jin-Ji region in China. Their emission level, populations, and particle size distribution were assayed, and the formation mechanisms of bioaerosols generated by surface aeration and submerged aeration were also explored by conducting lab-scale simulation experiments. The SourceTracker method was used to identify the intestinal bacteria sources. The Canonical correspondence analysis(CCA) method was applied to analyze the correlation between water quality parameters, processing capacity, and intestinal bacteria emissions. The present study may provide a scientific basis for the effective control of bioaerosols produced during the aeration process at WWTPs.

2 Materials and methods

2.1 Municipal WWTP descriptions

Sampling sites for bioaerosol capture were set up 1.5 m above the aeration tanks of six municipal WWTPs. The six municipal WWTPs were located in the Yangtze River Delta, Pearl River Delta, and Jing-Jin-Ji regions. Their situations were demonstrated in Figure S1. Treatment processes, scales, suspended solids (SS), and water quality were listed in

Table 1. Control samples were taken at the site upwind of each municipal WWTP. Samples were collected during the morning from 8 to 10 a.m. without disturbing the regular operation of the aerators. Meteorological parameters, e.g., temperature (T), relative humidity (RH), wind speed, (WS) and solar radiation (SR), at the given sampling site, were also recorded using portable equipment (Table S1). The air T and RH were determined using a Dewpoint Thermohygrometer (WD-35612, OAKTON, Germany). The wind direction and WS were recorded using a portable anemometer (HD2303, Delta OHM, Padova, Italy) while the SR was measured using a portable irradiance meter (HD2302.0, Delta OHM, Italy).

2.2 Bioaerosol capture

A six-stage viable Andersen Impactor (228–9530 K, SKC Gulf Coast, Inc., Houston, TX, USA) with 400 holes was used to capture bioaerosols at each sampling site as described in a previous report (Wang et al., 2018b). A culture medium was kept under each stage of the sampler. The sampling time was typically 3 min at a speed of 28.3 L/min, and the total impaction volume was 84.9 L (Table S1). Dishes were removed from the sampler when the required volume of air had been drawn. Bacteria and intestinal bacteria were cultured by LB and MacConkey Agar Medium (Hopebio, China), respectively. Results were calculated as the geometric mean of the replicates, expressed as colony-forming units per cubic meter of air (CFU/m^3) or per millilitre of water (CFU/ml). Two replicates were consecutively taken at every sampling site. After each sample was collected, the sampler was sterilised with a solution of 75% ethanol.

2.3 Simulation experiments

A lab-scale device, measuring 600 (length) × 300 (width) × 500 (height) mm^3 and composed of polyvinyl chloride, was set up to explore the mechanism of bioaerosol formation during the surface and submerged aeration processes (figure S2). Both surface aeration and submerged aeration were simulated in this device. For the simulation experiments of bioaerosols generated from surface aeration, a small brush was installed on the water surface of the aeration tank to disturb the water body and supply oxygen. For the submerged aeration, a perforated tube (10 mm in diameter) with one bubble hole (1.0 mm in diameter) serving as the nozzle was fixed at the bottom of the aeration tank to form bubbles. Surface aeration and submerged aeration experiments were alternately conducted to compare the generation mechanism under different aeration processes.

The tank contained a specific volume of wastewater coloured with black ink to observe the coloured bubbles generated by the aeration. The liquid level of the tank was set at 5 mm, just above the nozzle to ensure the neutral buoyancy of the bubbles, such that the bubbles remained attached to the nozzle during the process of production, growing in size, and then bursting out. Rice paper, with a thickness of 0.08 mm and a density of 410.9 kg/m^3, was used to collect droplets and to observe the initial appearance and distribution of the droplets. After collection of the droplets, the papers were removed and flattened and then scanned to convert the droplet information into images. The images were processed using the MATLAB program to obtain information regarding the number and spatial location of the droplets.

2.4 Analysis methods

2.4.1 Microbial population

Illumina MiSeq high-throughput sequencing was applied to analyse the intestinal bacteria population in the bioaerosols. DNA was extracted, purified, and sequenced, as described in previous studies (Wang et al., 2018b). After raw FASTQ files were demultiplexed and quality filtered, operational taxonomic units (OTUs) were clustered with a 97% similarity cut-off, and chimeric sequences were identified and removed. The taxonomy of each 16S rRNA gene sequence was analyzed using a confidence threshold of 70% (Amato et al., 2013; Jiang et al., 2015). Each sample was normalized at the same sequence depth (34,536 reads). The alpha and beta diversity and similarity of the bacterial community's structure were analyzed (Table S2).

2.4.2 Water quality

The parameters total nitrogen (TN), total phosphorus (TP), chemical oxygen demand (COD), and SS were determined according to the Standard Methods (APHA/AWWA/WEF, 1995). Ammonia nitrogen (NH_4^+-N) was determined using a photometric method (Merck kit: 1097130001). The total organic compounds (TOC) in the water were detected using a carbon analyser (TOC-V-CPH Shimadzu, Kyoto, Japan).

2.4.3 SourceTracker

SourceTracker(Knights et al., 2011) was used to profile the potential sources of microbial communities in a set of input samples (bioaerosol samples of the WWTPs).

3 Results and discussion

3.1 Emission level and particle size of intestinal bacteria

3.1.1 Emission level

The concentrations of total bacteria and intestinal bacteria in the bioaerosols were measured in the BRT section of each WWTP as shown in Figure 1. The municipal WWTPs of HB-A and HB-O lied in the Jing-Jin-Ji region, YZ-A and YZ-O were located at the Yangtze River Delta region, and PR-A and PR-O were situated in the Pearl River Delta region (Table1 and Figure S1). The average concentrations of bacteria in the BRT section were 1636 CFU/m^3 at HB-O, 1109CFU/m^3 at HB-A, 848 CFU/m^3 at YZ-O, 554 CFU/m^3 at YZ-A, 530 CFU/m^3 at PR-O, and 389 CFU/m^3 at PR-A, respectively. The numbers of total bacteria detected in the OAC at corresponding sampling site were 375 CFU/m^3 (for HB-O), 259 CFU/m^3 (for HB-A), 158 CFU/m^3 (for YZ-O), 112 CFU/m^3 (for YZ-A), 99 CFU/m^3 (for PR-O), and 51 CFU/m^3 (for PR-A). The concentrations of bacteria in the BRT section were 4 to 7 times as much as OAC (Outdoor air control) in the corresponding WWTPs. The total bacterial emission at the WWTPs in the Jing-Jin-Ji region was higher than that of those in the Yangtze River and Pearl River Delta regions. The airborne bacteria levels showed regional variation, which was consistent with that released from WWTPs in the northern and southern cities of China(Han et al., 2018).

The HB-A, YZ-A, and PR-A WWTPs used the A^2/O treatment process in the BRT section, which can provide oxygen via a submerged aeration process by adopting aeration holes at the bottom. Rotating dishes were installed in the oxidation ditches for oxygen supply at the HB-O, YZ-O, and PR-O WWTPs. This type of aeration process is considered as surface aeration. The concentration of bioaerosols generated from

oxidation ditches using surface aerators（HB-O, YZ-O, and PR-O）was 1005 CFU/m^3 on average, much higher than that emitted from the BRTs using submerged aeration （HB-A, YZ-A, and PR-A）(Table S3). Other researchers observed a similar phenomenon that surface aeration method generated a more significant amount of bioaerosols than air diffuser aerators (Sanchez-Monedero et al., 2008).

The escape of intestinal bacteria was similar to that of total bacteria. When the concentrations of intestinal bacteria found in HB-O, YZ-O and PR-O were 152 CFU/m^3, 72 CFU/m^3 and 35 CFU/m^3, respectively, 127 CFU/m^3, 47 CFU/m^3 and 28 CFU/$m^3$of intestinal bacteria presented in the surrounding air of HB-A, YZ-A and PR-A. In the same region, BRTs equipped with surface aerators produced more intestinal bacteria than those installed submerged aerators. The intestinal bacteria emerged in the OAC were 34 CFU/m^3 at HB-O, 12 CFU/m^3 at YZ-O, 6 CFU/m^3 at PR-O, 26 CFU/m^3 at HB-A, 9 CFU/m^3 at YZ-A, and 3 CFU/m^3 at PR-A, respectively. The concentrations of intestinal bacteria presented in the BRT section were 4 to 9 times as much as those detected at the sampling sites of OAC, indicating most of the intestinal bacteria in the atmosphere of BRT were produced during aeration.

The percentage of intestinal bacteria to total bacteria was 9.29% for HB-A and 11.45% for HB-O in the Jing-Jin-Ji region, 6.48% for YZ-A and 8.49% for YZ-O in the Yangzi River Delta region, and 6.60% for PR-A and 7.20% for PR-O in the Pearl River Delta region. High ratios were found in the WWTPs with surface aeration. The levels of bioaerosols particles in the air of BRT related to the aeration method adopted by WWTP.

3.1.2 Particle size distributions

The particle size distributions of the airborne intestinal bacteria at each sampling site were shown in Figure 2. These particles can be classified as either fine particles or

coarse particles. Fine particles were those smaller than 2.5 μm, while coarse particles were in a range of 2. 5 μm to 10 μm. Inhalation of particles smaller than 10 μm has harmful health effects such as asthma and cardiovascular disease (Urbán et al., 2015; Vestlund et al., 2014). At the sampling sites of HB-O, YZ-O, and PR-O, 76.90% to 80.56% of the bacteria were attached to particles larger than 2.1μm in size with an average concentration of 42 CFU/m^3, while the average concentration of particles smaller than 2.1μm in size (fine particles) was 11 CFU/m^3. At the sampling sites of HB-A, YZ-A and PR-A, more than half of the bacteria were attached to fine particles. The average concentration of the fine and coarse particles was 40 CFU/m^3 and 28 CFU/m^3, respectively. The particle size distributions of airborne bacteria released from WWTPs were similar with previous reports. 72.8% of culturable bacteria emitted from orbal oxidation ditches, a kind of surface aeration, had a particle size larger than 2.1μm (Li et al., 2011). However, high levels of respirable bioaerosol (0.65-3.3 μm in size) emissions were present only in the bubble aeration method(Kim et al., 2012).The size of the bioaerosol particles presented in the WWTPs with submerged aeration tended to be smaller than those equipped with surface aerators. The particle size distribution of bioaerosols was related to the aeration mode adopted by a WWTP.

When exposure to bioaerosols, the health risk was not only related to their concentrations, but also with their particle sizes. Coarse particles (>2.5μm) can penetrate the airways of the thoracic part, and fine particles (<2.5μm) can be directly inhaled and adhere to the respiratory tract and alveolar region (Sarnat et al., 2001). The previous study has reported that an annual exposure to 5 μg/m^3 of fine particles was linked to a 13% increased risk of heart attacks (Stafoggia et al., 2014).Compared with those examined out in the BRTs that using oxidation ditch process, most of the intestinal bacteria collected from WWTPs which utilized A^2/O process were likely to attach on

fine particles which might be more harmful to onsite workers. Besides, fine particles are easily carried by wind and dispersed over considerable distance due to their small size and lightweight. Because of the smaller particle size, bioaerosols produced by submerged aeration, especially those containing pathogenic bacteria, may also endanger the surrounding residents. Appropriate measures, such as covering the aeration tank with caps, were required to reduce bioaerosols diffusion and the risk to WWTPs workers.

3.2 Population of intestinal bacteria and their source tracker

3.2.1 Population

There were 61–67 OTUs in air samples collected from BRTs situated in the Jing-Jin-Ji region, 57–71 OTUs in the Yangtze River Delta region, and 70–78 OTUs in the Pearl River Delta region. The microbial diversity of intestinal bacteria in these WWTPs ranged from 5.22 to 7.39 (Figure 3 and Table S4). Among them, the communities were more abundant (6.24 on average) in the intestinal bacteria released from WWTPs with surface aeration process.

Pseudomonas sp.(41.49% at HB-A, and 58.02% at HB-O), *Clostridium* sp. (5.61% at HB-A, and 5.25% at HB-O) and *Escherichia-Shigella* (3.93% at HB-A, and 13.51% at HB-O) were the main intestinal bacteria detected in the WWTPs located at the Jing-Jin-Ji region. The dominated intestinal bacteria identified from the WWTPs lied in the Yangtze River Delta region were *Pseudomonas* sp.(for YZ-A) and *Enterobacter aerogenes* sp.(for YZ-O), accounting for 90.67% and 99.51% of all intestinal bacteria, respectively. In the Pearl River Delta region, more than 75% of all intestinal bacteria

species presented at PR-A were *Psychrobacter* sp. and *Pseudomonas* sp., while as *Aeromonas* sp. *and Enterobacter aerogenes* sp. accounted for more than 95% of all intestinal bacteria species at PR-O.

Pseudomonas sp. isolated from the WWTPs in the three regions is a kind of rod-shaped, gram-negative bacteria. *P. aeruginosa* is an opportunistic human pathogen which might be associated with human respiratory diseases (Itah & Essien, 2005). *Shigella* sp.*, e.g. Escherichia-Shigella* generally invade the epithelial lining of the colon, causing severe inflammation and cell death. This inflammation resulted in diarrhoea and even dysentery(Mounier et al., 1992).

The population of intestinal bacteria in air were related with the biomass in water, water quality (e.g. SS, COD), operating parameters (e.g. aeration mode, aeration rate), as well as meteorological condition (e.g. Temperature, relative humidity and illumination intensity). From the results of the Canonical Correlation Analysis(CCA) shown in Figure 4, the microbe concentration（MC）in water had a positive correlation with the population of airborne intestinal bacteria. The average concentration of intestinal bacteria in the oxidation ditch water was 8.20×10^6 CFU/ml for the Jing-Jin-Ji region, 5.18×10^6 CFU/ml for the Yangtze River Delta region, and 4.56×10^6 CFU/ml for the Pearl River Delta region (Table1). A large number of intestinal bacteria could transfer from the liquid phase to the air might have been because more microorganisms emerged in the oxidation ditch during the aeration time. A high percentage of *Pseudomonas* sp.(50.94%) was found in the water of the BRTs at HB-A, which likely contributed to the high level of *Pseudomonas* sp.(41.49%) in the surrounding air. Similar phenomena

were observed in WWTPs in other regions. The population of intestinal bacteria in the water differed for each WWTP, the species of intestinal bacteria in the air varied accordingly. The diversity of the excess sludge composition in the WWTPs belonged to different regions could result in a regional variation in the bioaerosols population (Han et al., 2018).

The water quality, such as SS and COD exhibited a positive relationship with the intestinal bacterial population in the air. A similar trend also occurred between the aeration rate (AR) and the community of intestinal bacteria. A positive correlation existed between RH and population of intestinal bacteria when WS and SR were detrimental to biomass in the air. The water quality and aeration rate were the main factors in the population of intestinal bacteria in the air.

3.2.2 Sources of intestinal bacteria

The sources of intestinal bacteria in the air of the BRTs were shown in Figure 5. In the BRTs installed with submerged aerators, the intestinal bacteria detected from the air 1.5 m above the water surface were initially been present in the OAC (5.62%-9.04%), influent water (5.12%-8.53%), water (16.45%-28.58%) and active sludge (3.63%-14.51%) in the BRT, while the sources of the other intestinal bacteria were unknown (54.21%-63.23%). The unknown sources were likely the surrounding soil and plants. For the BRTs with surface aeration process, the average proportions of intestinal bacteria were 9.22 % from OAC, 7.06 % from influent water, 22.98 % from water and 8.85 % from active sludge in BRT sections, respectively.

311 Wastewater, sludge, and OAC were the primary sources of intestinal bacteria detected
312 in the surrounding air of the BRTs. In the present study, all of the BRTs considered for
313 WWTPs bioaerosols investigation were built outdoors. The compositions of the
314 bioaerosols surrounding the BRTs were affected by the components in the outer
315 atmosphere, particularly the air from the upwind direction. The OAC contributed an
316 average of 8.43% of intestinal bacteria in the air.

317 A total of 26.02% of the intestinal bacteria found was generated from wastewater in the
318 BRT with submerged aeration process. Sludge (11.76%) was also an essential source of
319 intestinal bacteria found in the BRT surrounding air. During the aeration process,
320 aeration facilities produce bubbles at the bottom of the BRTs. Bubbles rise through the
321 water and eventually burst when they reach the surface as the surficial tension is
322 insufficient, producing a large number of film drops in the air. As some sludge can be
323 carried from the bottom to the surface of the water during the aeration process, the
324 activated sludge, inorganic particles, and other components in the droplets are released
325 and aerosolized in the atmosphere. Therefore, intestinal bacteria presented initially in
326 water and sludge could be detected in the bioaerosols.

327 For BRT sections with surface aeration, 19.93% and 5.94% of the intestinal bacteria
328 were generated on average from wastewater and sludge in the BRT. Rotating dishes
329 were adopted in WWTPs that utilized the oxidation ditch treatment process for aeration,
330 mixing and propelling water flow. The water in the oxidation ditches contained a large
331 number of microorganisms. The microorganisms would transfer from the water to the
332 atmosphere during rotation of the dishes. Therefore, intestinal bacteria isolated from

gas phase were originally in the water and sludge, particularly at the water surface.

It could be illustrated that water and sludge in BRTs were the main contributors of intestinal bacteria, with 25.87% in average for submerged aeration and 37.78% on average for surface aeration. Compared with that emitted from WWTPs utilized submerged aeration, intestinal bacteria diffused from BRTs with surface aerators were more likely to come from water and active sludge. Their contribution rates to bioaerosols in the air were closely related to the mode of aeration adopted by the WWTPs.

3.3 Formation mechanisms

3.3.1 Surface aeration

The bioaerosol formation mechanisms were explored using simulation experiments. In the simulation experiments of surface aeration, the deposition of droplets were observed in a fixed area with 0.20 m from the rotating brush in the horizon and 0.18 m from the water surface in vertical. When the speed of the rotating brush turned from 10 rpm to 50 rpm, the number of droplets deposited on observation area increased from 3217 to 16850 within 1 min (Figure 2 and Figure S3). Nearly 60% of the droplets presented on the upper part of the observation area (0.09–0.18 m from water surface), while 43.16% of them dripped at the lower part of the observation area (0–0.09 m from water surface).32.36% of the droplets in the observation area had a particle size larger than 2 mm.

During the surface aeration process, the rotating dishes in BRTs agitated water surface

violently and threw water into the air. The lifted droplets moved as the parabolic curve in the air, mixed with air, and formed atomisation zones on the water surface. Small droplets might lose water and aerosolized in the air to form bioaerosols. The process of bioaerosols formation during surface aeration mainly included three steps: (1) Droplets containing microorganisms were transferred from the aqueous phase to ambient air. (2) The droplets thrown into the air were dehydrated and atomized to form bioaerosols. (3)The disturbance of liquid level will lead to the generation of bioaerosols. The air involved in the water formed bioaerosols by the shaking of the water body caused by the liquid falling. The level and particle size of bioaerosols produced relied on the atomization quantity which affected by diameter and speed of the rotating dishes and the amount, velocity and angle of droplet raised by the dishes (Lasheras et al., 1998). The atomization quantities produced in each rotating speed could be calculated by formula 1(Liu et al., 2006; Sheng et al., 2006). The speed of rotating dishes installed in investigated WWTP was 50 rpm. The calculated atomization quantity in the simulated experiment was 6.3×10^{-5} m^3/s at a rotating speed of 50 rpm.

$$q^* = u\int_{y^*}^{1/2h_{eu}} \beta d_y = 1.6726\mu \frac{h_e}{h_{eu}} \int_{y^*}^{1/2h_{eu}} \exp(-\frac{8y^2}{h_{eu}^2})\, d_y \quad (1)$$

where,

q^* is the atomization quantity(m^3/s),

u is the droplet ejection speed(m/s),

h_{eu} denotes aerated water thickness with air (m),

h_e denotes aerated water thickness without air(m),

y* is the starting position of water atomization(m),

y is the distance perpendicular to the center of the stream(m),

β is the water content of section of the breaking points(m^3),

μ is the wind speed near droplets (m/s).

3.3.2 Submerged aeration

In the simulation experiments of submerged aeration, film drops were observed in a fixed area with 0.01 m from the bursting bubble in the horizon and 0.18 m from water surface. There were 1290 of film drops generated when ten bubbles burst. More film drops would appear with the increase of the number of broken bubbles (Figure 6 and Figure S3). The film drops presented in the lower part of the observation area had a percentage of 80.64%, while the percentage dropped to 19.36% in the upper part. Most of the film drops (87.25%) formed by bubbles bursting had particle size less than 2.0 mm in diameters. Submerged aeration produced smaller particles than that caused by surface aeration, which was consistent with the survey of WWTPs mentioned above equipped with surface or submerged aeration.

Aeration facilities produced bubbles at the water bottom during the submerged aeration process. Bubbles rose through the water layer and eventually burst when they reached the water surface, projecting a large number of film drops into the air. The birth of film drops can be described by Rayleigh instability theory (Spiel, 1998). The process of film drop generation could be divided into three stages. During stage 1, a film cap, which can be considered as a thin curved liquid film, was formed. During stage 2, a collapsing

bubble cavity appeared at the film cap, after which it became increasingly thinner and propagated collecting the film's mass into a ring shape as it progressed. This process was enabled by the surface tension as the toroidal ring provided the force required to sustain the centripetal acceleration. During stage 3, when the surface tension was insufficient to maintain the centripetal acceleration at the toroidal ring, rings break to create countless film drops (Lhuissier & Villermaux, 2012; Resch & Afeti, 1991; Spiel, 1998).

The particle size distribution and the number of bioaerosols formed during submerged aeration were related to the speed of droplets formation, the number and size of droplets. Increasing aeration intensity would result in more droplets (Wang et al., 2018a). The bioaerosols produced after bubble breakdown were related to the diameter, velocity and location of the film droplets when they were formed which affected by the surface tension of water, sewage density and the volume and size before the bubble burst. Their relationship could be described by bubble breakdown model and the formation of film droplets can also be estimated by the same model(Lhuissier & Villermaux, 2012; Resch & Afeti, 1991; Spiel, 1998). Characteristics of bioaerosol particles generated during surface or submerged aeration were determined by their respective mechanisms, formation processes and influencing factors.

The characteristics of aerosols produced by submerged aeration and surface aeration were significantly different due to different mechanisms, formation processes and influencing factors.

4. Conclusion

- The emission level and particle size distribution of bioaerosols varied with the aeration mode adopted by wastewater treatment tanks. Compared with that generated by submerged aerators, a higher concentration of intestinal bacteria with larger particle size in bioaerosols presented during surface aeration.

- *Pseudomonas* sp., *Serratia* sp. and *Acinetobacter* sp. were identified from intestinal bacteria particles as pathogenic bacteria. Water quality and aeration rate were related to the microbial population of bioaerosols. Source tracker revealed that water and active sludge in BRTs were the main sources of intestinal bacteria in the air. They contributed more when the surface aerators were utilized by WWTP.

- The disturbance of water body caused by rotating dishes agitating or bubble burst during aeration processes lead to splash the water into the air. The splashing water is dispersed into fine droplets and become bioaerosols after aerosolization. The characteristics of aerosols produced by submerged aeration and surface aeration are significantly different due to different mechanisms, formation processes and influencing factors.

- It is necessary to establish an effective method to assess the health risks of exposure to intestinal bacteria, especially those larger than 2.5μm in size. Safety protections, such as cover water surface in BRTs, should be undertaken in order to cut off the transmission channel and reduce the concentration of intestinal

bacteria in the surrounding air.

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Figure Captions

Figure 1. Variability of intestinal bacteria concentration and their proportion in total bacteria in each municipal WWTP.

Figure 2. Size distribution of the intestinal bacteria emitted from BRTs (a: surface aeration process ; b: submerged aeration process). (D_g: aerodynamic diameter; ΔC: concentration of bacterial aerosol on particular stage of 6-stage Andersen impactor; C_{total}: total concentration of bacterial aerosol; $\Delta \log D_g$: logarithm of differences of cut-off diameters for particular stage of 6-stage Andersen Impactor.)

Figure 3. Phylogenetic tree of intestinal bacteria in each municipal WWTPs(a:HB-A; b:HB-O; c:YZ-A; d: YZ-O; e: PR-A; f: PR-O).

Figure 4. Canonical correspondence analysis of airborne intestinal bacteria population with respect to factors in BRT section.

Figure 5. Sources of intestinal bacteria in bioaerosols.

Figure 6. The number of drops from surface and submerged aeration simulation experiments.

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