

1 **Characteristics and formation mechanism of intestinal**
2 **bacteria particles emitted from aerated wastewater treatment**
3 **tanks**

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22 **Abstract**

23 Aeration tanks in municipal wastewater treatment plants (WWTPs) are regarded as
24 sources of bioaerosols, often containing particles and microbes. In this study, intestinal
25 bacteria were investigated from biochemical reaction tanks (BRTs) of six municipal
26 WWTPs. It was observed that 86 CFU/m³ of intestinal bacteria (in average) occurred
27 in the BRTs installed surface aerator, which was higher than those adopted submerged
28 aeration (67 CFU/m³ in average). 62.72% of fine particles were observed in the BRTs
29 supplied oxygen by submerged aerator, while 75.73% of coarse particles emitted during
30 surface aeration. *Pseudomonas* sp., *Serratia* sp. and *Acinetobacter* sp. were identified
31 as pathogenic bacteria presented in the intestinal bacteria population and most of them
32 existed initially in water or sludge, particularly in water surface. The emission level and
33 particle size distribution were significantly correlated with aeration mode adopted by
34 the WWTPs. The bioaerosols particles emitted from surface aeration process was higher
35 than that from submerged aeration process. Meanwhile, the BRTs with submerged
36 aerators released more fine particles, which can get into the alveoli and represented the
37 potential challenge to human health. Canonical correspondence analysis results
38 exhibited that population of intestinal bacteria had a positive correlation with aeration
39 rate and water quality. As the intestinal bacteria in the bioaerosols emitted from the
40 WWTPs may pose a potential risk to onsite operators, aeration tanks in WWTPs should
41 be paid more attention as a source of intestinal bacterial emissions.

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

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)	q*	The atomization quantity
Dg	Aerodynamic diameter	h _e	Aerated water thickness without air
$\Delta \log Dg$	Logarithm of differences of cut-off diameters for particular stage of 6-stage Andersen	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
C _{total}	Total concentration of bacterial aerosol	u	The droplet ejection speed
μ	The wind speed near droplets	β	Water content of section of the breaking points
		y	The distance perpendicular to the center of the stream

45 **1 Introduction**

46 Secondary treatment of wastewater achieves a certain degree of effluent quality via the
47 use of a wastewater treatment plant (WWTP) that features physical separation of settled
48 solids and the biological removal of dissolved and suspended organic compounds.
49 Biological processes offer the advantage of using the organic compounds in the water
50 as a substrate and energy source in metabolic activities. Dissolved oxygen is often
51 provided by the diffusers at the bottom of the water or a rotor aeration system at the
52 water surface to promote the biological process. Bioaerosols can be produced during
53 the aeration process and released into the surrounding atmosphere. Aerosol
54 contamination in sewage treatment plants has been evaluated by Carducci's research
55 group; they found that the aeration tank was a significant source of bioaerosols, with
56 high levels of viral contamination (Carducci et al., 1999). In a study of bioaerosol
57 characteristics generated by a large urban WWTP in the Middle East, the aeration tank
58 had a higher concentration of bacterial emission, with an average of 1973 CFU/m³
59 during summer (Sadegh et al., 2015).

60 There are a variety of means to aerate water, including two broad areas, surface aeration
61 and submerged aeration. Surface aeration devices include a low-speed surface aerator,
62 fountains, floating surface aerators, and paddle wheel aerators (Roberts & Daendliker,
63 1983). They are typically applied in WWTPs with an oxidation ditch treatment process.
64 Submerged aeration, such as jet aeration and coarse or fine bubble aeration, provides
65 oxygen via air bubble release at the bottom of the aeration tanks (Frank et al., 2009).
66 Bioaerosols emitted from six WWTPs were monitored and compared by researchers in

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

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

82 Over 5000 municipal WWTPs had been constructed in China by the end of 2018. Nearly
83 90% of the WWTPs have implemented treatment processes equipped with surficial
84 aerators or submerged aerators in biochemical reaction tanks (BRTs).With the rapid
85 increase in the size of cities and urban populations in recent years, many WWTPs built
86 in rural areas are now situated near to residential areas. Exposure to bioaerosols can
87 pose a health risk not only to onsite workers at these WWTPs but also to surrounding
88 inhabitants(Gotkowska-Plachta et al., 2013).

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

104 **2 Materials and methods**

105 **2.1 Municipal WWTP descriptions**

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

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

119 **2.2 Bioaerosol capture**

120 A six-stage viable Andersen Impactor (228–9530 K, SKC Gulf Coast, Inc., Houston,
121 TX, USA) with 400 holes was used to capture bioaerosols at each sampling site as
122 described in a previous report (Wang et al., 2018b). A culture medium was kept under
123 each stage of the sampler. The sampling time was typically 3 min at a speed of 28.3
124 L/min, and the total impaction volume was 84.9 L (Table S1). Dishes were removed
125 from the sampler when the required volume of air had been drawn. Bacteria and
126 intestinal bacteria were cultured by LB and MacConkey Agar Medium (Hopebio,
127 China), respectively. Results were calculated as the geometric mean of the replicates,
128 expressed as colony-forming units per cubic meter of air (CFU/m³) or per millilitre of
129 water (CFU/ml). Two replicates were consecutively taken at every sampling site. After
130 each sample was collected, the sampler was sterilised with a solution of 75% ethanol.

131 **2.3 Simulation experiments**

132 A lab-scale device, measuring 600 (length) × 300 (width) × 500 (height) mm³ and
133 composed of polyvinyl chloride, was set up to explore the mechanism of bioaerosol
134 formation during the surface and submerged aeration processes (figure S2). Both
135 surface aeration and submerged aeration were simulated in this device. For the
136 simulation experiments of bioaerosols generated from surface aeration, a small brush
137 was installed on the water surface of the aeration tank to disturb the water body and
138 supply oxygen. For the submerged aeration, a perforated tube (10 mm in diameter) with
139 one bubble hole (1.0 mm in diameter) serving as the nozzle was fixed at the bottom of
140 the aeration tank to form bubbles. Surface aeration and submerged aeration experiments
141 were alternately conducted to compare the generation mechanism under different
142 aeration processes.

143 The tank contained a specific volume of wastewater coloured with black ink to observe
144 the coloured bubbles generated by the aeration. The liquid level of the tank was set at 5
145 mm, just above the nozzle to ensure the neutral buoyancy of the bubbles, such that the
146 bubbles remained attached to the nozzle during the process of production, growing in
147 size, and then bursting out. Rice paper, with a thickness of 0.08 mm and a density of
148 410.9 kg/m³, was used to collect droplets and to observe the initial appearance and
149 distribution of the droplets. After collection of the droplets, the papers were removed
150 and flattened and then scanned to convert the droplet information into images. The
151 images were processed using the MATLAB program to obtain information regarding
152 the number and spatial location of the droplets.

153 **2.4 Analysis methods**

154 **2.4.1 Microbial population**

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

164 **2.4.2 Water quality**

165 The parameters total nitrogen (TN), total phosphorus (TP), chemical oxygen demand
166 (COD), and SS were determined according to the Standard Methods
167 (APHA/AWWA/WEF, 1995). Ammonia nitrogen ($\text{NH}_4^+\text{-N}$) was determined using a
168 photometric method (Merck kit: 1097130001). The total organic compounds (TOC) in
169 the water were detected using a carbon analyser (TOC-V-CPH Shimadzu, Kyoto, Japan).

170 **2.4.3 SourceTracker**

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

173 **3 Results and discussion**

174 **3.1 Emission level and particle size of intestinal bacteria**

175 **3.1.1 Emission level**

176 The concentrations of total bacteria and intestinal bacteria in the bioaerosols were
177 measured in the BRT section of each WWTP as shown in Figure 1. The municipal
178 WWTPs of HB-A and HB-O lied in the Jing-Jin-Ji region, YZ-A and YZ-O were
179 located at the Yangtze River Delta region, and PR-A and PR-O were situated in the
180 Pearl River Delta region (Table1 and Figure S1). The average concentrations of bacteria
181 in the BRT section were 1636 CFU/m³ at HB-O, 1109CFU/m³ at HB-A, 848 CFU/m³
182 at YZ-O, 554 CFU/m³ at YZ-A, 530 CFU/m³ at PR-O, and 389 CFU/m³ at PR-A,
183 respectively. The numbers of total bacteria detected in the OAC at corresponding
184 sampling site were 375 CFU/m³ (for HB-O), 259 CFU/m³ (for HB-A), 158 CFU/m³
185 (for YZ-O), 112 CFU/m³ (for YZ-A), 99 CFU/m³ (for PR-O), and 51 CFU/m³ (for PR-
186 A). The concentrations of bacteria in the BRT section were 4 to 7 times as much as
187 OAC (Outdoor air control) in the corresponding WWTPs. The total bacterial emission
188 at the WWTPs in the Jing-Jin-Ji region was higher than that of those in the Yangtze
189 River and Pearl River Delta regions. The airborne bacteria levels showed regional
190 variation, which was consistent with that released from WWTPs in the northern and
191 southern cities of China(Han et al., 2018).

192 The HB-A, YZ-A, and PR-A WWTPs used the A²/O treatment process in the BRT
193 section, which can provide oxygen via a submerged aeration process by adopting
194 aeration holes at the bottom. Rotating dishes were installed in the oxidation ditches for
195 oxygen supply at the HB-O, YZ-O, and PR-O WWTPs. This type of aeration process
196 is considered as surface aeration. The concentration of bioaerosols generated from

197 oxidation ditches using surface aerators (HB-O, YZ-O, and PR-O) was 1005 CFU/m³
198 on average, much higher than that emitted from the BRTs using submerged aeration
199 (HB-A, YZ-A, and PR-A) (Table S3). Other researchers observed a similar
200 phenomenon that surface aeration method generated a more significant amount of
201 bioaerosols than air diffuser aerators (Sanchez-Monedero et al., 2008).

202 The escape of intestinal bacteria was similar to that of total bacteria. When the
203 concentrations of intestinal bacteria found in HB-O, YZ-O and PR-O were 152 CFU/m³,
204 72 CFU/m³ and 35 CFU/m³, respectively, 127 CFU/m³, 47 CFU/m³ and 28 CFU/m³ of
205 intestinal bacteria presented in the surrounding air of HB-A, YZ-A and PR-A. In the
206 same region, BRTs equipped with surface aerators produced more intestinal bacteria
207 than those installed submerged aerators. The intestinal bacteria emerged in the OAC
208 were 34 CFU/m³ at HB-O, 12 CFU/m³ at YZ-O, 6 CFU/m³ at PR-O, 26 CFU/m³ at HB-
209 A, 9 CFU/m³ at YZ-A, and 3 CFU/m³ at PR-A, respectively. The concentrations of
210 intestinal bacteria presented in the BRT section were 4 to 9 times as much as those
211 detected at the sampling sites of OAC, indicating most of the intestinal bacteria in the
212 atmosphere of BRT were produced during aeration.

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

218 **3.1.2 Particle size distributions**

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

221 coarse particles. Fine particles were those smaller than 2.5 μm , while coarse particles
222 were in a range of 2.5 μm to 10 μm . Inhalation of particles smaller than 10 μm has
223 harmful health effects such as asthma and cardiovascular disease (Urbán et al., 2015;
224 Vestlund et al., 2014). At the sampling sites of HB-O, YZ-O, and PR-O, 76.90% to
225 80.56% of the bacteria were attached to particles larger than 2.1 μm in size with an
226 average concentration of 42 CFU/ m^3 , while the average concentration of particles
227 smaller than 2.1 μm in size (fine particles) was 11 CFU/ m^3 . At the sampling sites of HB-
228 A, YZ-A and PR-A, more than half of the bacteria were attached to fine particles. The
229 average concentration of the fine and coarse particles was 40 CFU/ m^3 and 28 CFU/ m^3 ,
230 respectively. The particle size distributions of airborne bacteria released from WWTPs
231 were similar with previous reports. 72.8% of culturable bacteria emitted from orbal
232 oxidation ditches, a kind of surface aeration, had a particle size larger than 2.1 μm (Li
233 et al., 2011). However, high levels of respirable bioaerosol (0.65-3.3 μm in size)
234 emissions were present only in the bubble aeration method (Kim et al., 2012). The size
235 of the bioaerosol particles presented in the WWTPs with submerged aeration tended to
236 be smaller than those equipped with surface aerators. The particle size distribution of
237 bioaerosols was related to the aeration mode adopted by a WWTP.

238 When exposure to bioaerosols, the health risk was not only related to their
239 concentrations, but also with their particle sizes. Coarse particles (>2.5 μm) can
240 penetrate the airways of the thoracic part, and fine particles (<2.5 μm) can be directly
241 inhaled and adhere to the respiratory tract and alveolar region (Sarnat et al., 2001). The
242 previous study has reported that an annual exposure to 5 $\mu\text{g}/\text{m}^3$ of fine particles was
243 linked to a 13% increased risk of heart attacks (Stafoggia et al., 2014). Compared with
244 those examined out in the BRTs that using oxidation ditch process, most of the intestinal
245 bacteria collected from WWTPs which utilized A²/O process were likely to attach on

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

253 **3.2 Population of intestinal bacteria and their source tracker**

254 **3.2.1 Population**

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

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

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

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

277 The population of intestinal bacteria in air were related with the biomass in water, water
278 quality (e.g. SS, COD), operating parameters (e.g. aeration mode, aeration rate), as well
279 as meteorological condition (e.g. Temperature, relative humidity and illumination
280 intensity). From the results of the Canonical Correlation Analysis(CCA) shown in
281 Figure 4, the microbe concentration (MC) in water had a positive correlation with the
282 population of airborne intestinal bacteria. The average concentration of intestinal
283 bacteria in the oxidation ditch water was 8.20×10^6 CFU/ml for the Jing-Jin-Ji region,
284 5.18×10^6 CFU/ml for the Yangtze River Delta region, and 4.56×10^6 CFU/ml for the
285 Pearl River Delta region (Table1). A large number of intestinal bacteria could transfer
286 from the liquid phase to the air might have been because more microorganisms emerged
287 in the oxidation ditch during the aeration time. A high percentage of *Pseudomonas*
288 sp.(50.94%) was found in the water of the BRTs at HB-A, which likely contributed to
289 the high level of *Pseudomonas* sp.(41.49%) in the surrounding air. Similar phenomena

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

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

301 **3.2.2 Sources of intestinal bacteria**

302 The sources of intestinal bacteria in the air of the BRTs were shown in Figure 5. In the
303 BRTs installed with submerged aerators, the intestinal bacteria detected from the air 1.5
304 m above the water surface were initially been present in the OAC (5.62%-9.04%),
305 influent water (5.12%-8.53%), water (16.45%-28.58%) and active sludge (3.63%-
306 14.51%) in the BRT, while the sources of the other intestinal bacteria were unknown
307 (54.21%-63.23%). The unknown sources were likely the surrounding soil and plants.
308 For the BRTs with surface aeration process, the average proportions of intestinal
309 bacteria were 9.22 % from OAC, 7.06 % from influent water, 22.98 % from water and
310 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

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

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

341 **3.3 Formation mechanisms**

342 3.3.1 Surface aeration

343 The bioaerosol formation mechanisms were explored using simulation experiments. In
344 the simulation experiments of surface aeration, the deposition of droplets were observed
345 in a fixed area with 0.20 m from the rotating brush in the horizon and 0.18 m from the
346 water surface in vertical. When the speed of the rotating brush turned from 10 rpm to
347 50 rpm, the number of droplets deposited on observation area increased from 3217 to
348 16850 within 1 min (Figure 2 and Figure S3). Nearly 60% of the droplets presented on
349 the upper part of the observation area (0.09–0.18 m from water surface), while 43.16%
350 of them dripped at the lower part of the observation area (0–0.09 m from water
351 surface).32.36% of the droplets in the observation area had a particle size larger than 2
352 mm.

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

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

$$369 \quad q^* = u \int_{y^*}^{1/2 h_{eu}} \beta d_y = 1.6726 \mu \frac{h_e}{h_{eu}} \int_{y^*}^{1/2 h_{eu}} \exp\left(-\frac{8y^2}{h_{eu}^2}\right) d_y \quad (1)$$

370 where,

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

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

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

374 h_e denotes aerated water thickness without air(m),

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

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

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

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

379 3.3.2 Submerged aeration

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

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

396 bubble cavity appeared at the film cap, after which it became increasingly thinner and
397 propagated collecting the film's mass into a ring shape as it progressed. This process
398 was enabled by the surface tension as the toroidal ring provided the force required to
399 sustain the centripetal acceleration. During stage 3, when the surface tension was
400 insufficient to maintain the centripetal acceleration at the toroidal ring, rings break to
401 create countless film drops (Lhuissier & Villermaux, 2012; Resch & Afeti, 1991; Spiel,
402 1998).

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

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

417 4. Conclusion

- 418 • The emission level and particle size distribution of bioaerosols varied with the
419 aeration mode adopted by wastewater treatment tanks. Compared with that
420 generated by submerged aerators, a higher concentration of intestinal bacteria
421 with larger particle size in bioaerosols presented during surface aeration.
- 422 • *Pseudomonas* sp., *Serratia* sp. and *Acinetobacter* sp. were identified from
423 intestinal bacteria particles as pathogenic bacteria. Water quality and aeration
424 rate were related to the microbial population of bioaerosols. Source tracker
425 revealed that water and active sludge in BRTs were the main sources of intestinal
426 bacteria in the air. They contributed more when the surface aerators were
427 utilized by WWTP.
- 428 • The disturbance of water body caused by rotating dishes agitating or bubble
429 burst during aeration processes lead to splash the water into the air. The
430 splashing water is dispersed into fine droplets and become bioaerosols after
431 aerosolization. The characteristics of aerosols produced by submerged aeration
432 and surface aeration are significantly different due to different mechanisms,
433 formation processes and influencing factors.
- 434 • It is necessary to establish an effective method to assess the health risks of
435 exposure to intestinal bacteria, especially those larger than 2.5 μ m in size. Safety
436 protections, such as cover water surface in BRTs, should be undertaken in order
437 to cut off the transmission channel and reduce the concentration of intestinal

438 bacteria in the surrounding air.

439 **Acknowledgments**

440 The authors thank sincerely to Editage services center and Professor Oliver Wild for
441 the English revision of the manuscript.

442 **Formatting of funding sources**

443 This work was supported by the National Natural Science Foundation of China (No.
444 51878650).

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447 **Figure Captions**

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

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

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

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

459 **Figure 5.** Sources of intestinal bacteria in bioaerosols.

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

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