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# Reduction and characterization of bioaerosols in a wastewater treatment station via ventilation

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## ABSTRACT

Bioaerosols from wastewater treatment processes are a significant subgroup of atmospheric aerosols. In the present study, airborne microorganisms generated from a wastewater treatment station (WWTS) that uses an oxidation ditch process were diminished by ventilation. Conventional sampling and detection methods combined with cloning/sequencing techniques were applied to determine the groups, concentrations, size distributions, and species diversity of airborne microorganisms before and after ventilation. There were  $3021 \pm 537$  CFU/m<sup>3</sup> of airborne bacteria and  $926 \pm 132$  CFU/m<sup>3</sup> of airborne fungi present in the WWTS bioaerosol. Results showed that the ventilation reduced airborne microorganisms significantly compared to the air in the WWTS. Over 60% of airborne bacteria and airborne fungi could be reduced after 4 hr of air exchange. The highest removal (92.1% for airborne bacteria and 89.1% for fungi) was achieved for 0.65–1.1 μm sized particles. The bioaerosol particles over 4.7 μm were also reduced effectively. Large particles tended to be lost by gravitational settling and small particles were generally carried away, which led to the relatively easy reduction of bioaerosol particles 0.65–1.1 μm and over 4.7 μm in size. An obvious variation occurred in the structure of the bacterial communities when ventilation was applied to control the airborne microorganisms in enclosed spaces.

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## Introduction

Bioaerosols present in wastewater treatment plants (WWTP) have gained increasing attention in recent years due to their close correlation with air pollution and possible cause of adverse health effects for plant workers (Fracchia et al., 2006). Many studies have evaluated the biological risks of aerosols by determining the concentrations of viable microorganisms, using different sampling and detection methods (Fannin et al., 1985; Carducci et al., 2000; Bauera et al., 2002; Pascual et al., 2003; Karra and Katsivela, 2007; Stellacci et al., 2010). Previous reports of municipal WWTP bioaerosols have shown that high levels of culturable airborne bacteria and fungi were present in the indoor facilities and the aeration system (Brandt et al., 2000; Sánchez-Monedero et al.,

2008; Li et al., 2011). Raw wastewater contains a large diversity of microorganisms, such as viruses, bacteria, fungi, and protozoans, which may be aerosolized during mechanical agitation and water aeration. Aeration systems are considered the main sources of bioaerosols in WWTP. High numbers of airborne microorganisms have been observed in indoor facilities, possibly explained by internal walls obstructing the dispersal of bioaerosols as well as insufficient ventilation and reduced die-off rates from limited solar radiation.

Many countries are attempting to regulate the impact of bioaerosols and have proposed a number of measures for their control (Hung et al., 2010). Air filtration and UV germicidal irradiation (UVGI) are two air purification systems routinely used for removing or inactivating microorganisms (Riley and Nardell,

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1989; Green and Scarpino, 2002; Kujundzic et al., 2007). Air filtration systems must be changed periodically and require routine maintenance. Compared with air filtration, UVGI generally provides effective control at a lower cost, with easier installation and maintenance. Both systems, however, initially require adequate ventilation.

Ventilation removes unpleasant smells and excessive moisture, introduces outside air into internal air circulation, prevents air stagnation, and is one of the most important factors in maintaining acceptable indoor air quality. Ventilation dilutes the concentration of microorganisms in the air via air exchange, which is a convenient and economic method of reducing airborne microorganisms. Building ventilation can be divided into mechanical/forced and natural types.

Understanding the microbial characteristics of bioaerosols will help develop effective measures to control their emission. Previous research has investigated the concentrations, particle size distributions, and species diversity of airborne microorganisms at different stages in WWTPs (Fannin et al., 1985; Buttner et al., 2001; Pillai and Ricke, 2002; Grisoli et al., 2009), and their hazards have also been identified (Palmer et al., 1995; Carducci et al., 2000). However, little is known regarding the characteristics, e.g., particle size characteristics and microbial population structure, of airborne bioaerosols removed from the air by ventilation. Bioaerosol monitoring requires appropriate analysis of air samples and detection of microbial wastewater species. In addition to the culture method, molecular biology techniques provide more widely applicable tools in microbial assay (Sawyer et al., 1994; Maron et al., 2005; An et al., 2006).

In the present study, airborne microorganisms generated from a wastewater treatment station (WWTS) that uses the oxidation ditch process were diminished by ventilation. Conventional sampling and detection methods combined with cloning/sequencing techniques were applied to determine the groups, concentrations, size distributions, and species diversity of airborne microorganisms before and after ventilation. The objective was to explore the variation in bioaerosol levels and microbial structures to find a suitable method to reduce airborne microorganisms generated from internal wastewater treatment facilities.

## 1. Materials and methods

### 1.1. Station description and sampling locations

The present study on bioaerosol emissions in a WWTS that uses a vertical oxidation ditch was conducted in September 2012 in Beijing, China. Oxidation ditches have been used in small communities as a cost-effective secondary treatment from the 1960s (Denton, 1997). Compared with the conventional oxidation ditch with intrachannel clarifier, the integrated oxidation ditch with a vertical circle reduces land area by about 50% and energy consumption by 40%. Its characteristics are compact configuration, simple operation and easy maintenance (Xia and Liu, 2004). This station serves a residential community with a population of 1500, and has a daily capacity of 100 m<sup>3</sup>. The oxidation ditch is located indoors for maintaining suitable temperatures in winter. Rotating brushes with a speed of 50 r/min were installed in the oxidation ditch to aerate the water. The average chemical oxygen demand (COD) and NH<sub>4</sub>-N in the influent sewage were 356 and 43.8 mg/L, respectively. The removal efficiencies were 91.1% for COD and 98.2% for NH<sub>4</sub>-N after 10 hr of hydraulic retention.

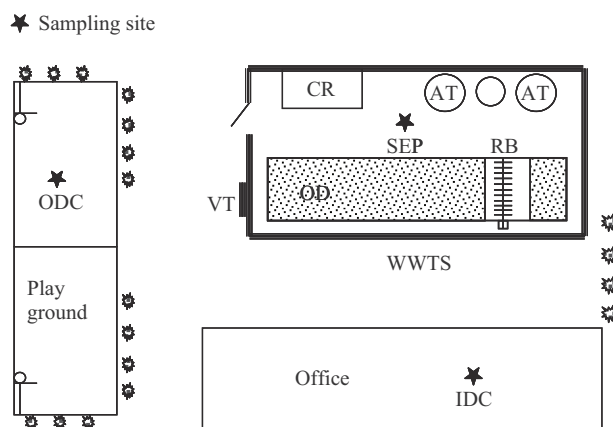
Sampling sites were established at the center of the WWTS (SEP), a clean room of the office building (IDC) and outside

upwind (ODC) in the present study (Fig. 1 and Table 1). Microbial samples for microbiological assays were collected between 10:00 am and 11:30 am within one day. During sampling, temperature, relative humidity (RH), and wind speed were also measured (Table 1). A Dewpoint Thermo hygrometer (WD-35612, OAKTON, Germany) was used to determine RH and temperature. The wind speed was recorded by a portable anemometer (HD2303, Delta OHM, Italy). A ventilator (FA-50, Shanghai, China) was used for air exchange and its flow rate was 50 m<sup>3</sup>/min.

### 1.2. Airborne microorganism capture

A Six-stage Viable Andersen Cascade Impactor (228-9530 K, SKC Gulf Coast Inc., USA), which is a bioaerosol and microbial particle sizing sampler designed to measure the concentration and particle size distribution of aerobic bacteria and fungi in intramural or ambient air, was used to capture airborne microorganisms on-site. The sampler is constructed from aluminum and consists of a series of six consecutive stages. Each stage contains a plate perforated by 400 holes, which are of constant size for a given stage, but decrease for each succeeding stage. Airborne particles are separated into six fractions, and the aerodynamic cut-size diameters in the six stages are: >7.0 μm (stage 1), 4.7–7.0 μm (stage 2), 3.3–4.7 μm (stage 3), 2.1–3.3 μm (stage 4), 1.1–2.1 μm (stage 5), and 0.65–1.1 μm (stage 6), respectively. Directly below each stage is a glass Petri dish containing a suitable medium. The stages are held together by three spring clamps, and are gasketed with O-ring seals. Air is drawn through the sampler by means of a pump, at a rate of 28.3 L/min.

All inside surfaces were sterile until sampling. Sampling time was typically 2–3 min and impaction volume was 56.6–84.9 L. Once the required volume of air had been drawn through, the dishes were removed from the sampler, covered, inverted, and incubated. Three replicates were taken consecutively at every sampling site. After each sample was collected, the sampler was sterilized with 75% ethanol solution. Airborne bacteria were incubated in Nutrient agar (BR, Aoboxing Biotech,



**Fig. 1 – Schematic diagram of sampling site locations in the wastewater treatment station. WWTS: wastewater treatment station; OD: oxidation ditch; RB: rotating brushes; CR: control room; VT: ventilator; ODC: outdoor control; IDC: indoor control; AT: advanced treatment.**

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