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# Bacterial population and chemicals in bioaerosols from indoor environment: Sludge dewatering houses in nine municipal wastewater treatment plants

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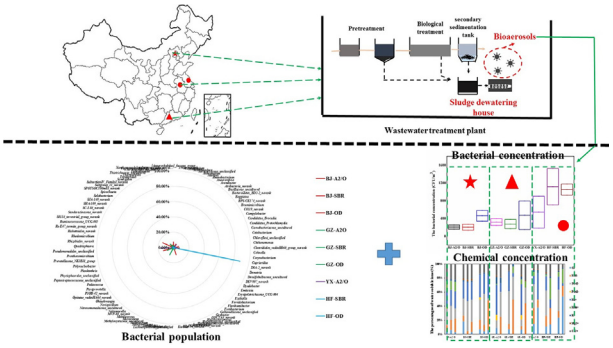
## HIGHLIGHTS

- Chemical and bacterial properties in sludge dewatering house were analyzed.
- Levels of airborne bacteria and chemicals showed regional variations.
- Bacterial population in bioaerosols also presented significant regional disparity.
- Common potential pathogens were detected in bioaerosols from all SDHs.
- RH and temperature were major parameters on bioaerosols to survive in SDHs.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Municipal wastewater treatment plants (MWTPs) are regarded as sources of airborne microorganisms. Sludge dewatering house (SDH) is one of the most serious indoor bioaerosol pollution treatment sectors in MWTPs. In this study, properties of bioaerosols from SDHs of nine MWTPs were investigated in China. Results suggested that bioaerosols were generated mainly from the mixed liquor and will be promoted by the mechanical motion of belts of dewatering devices. They will accumulate in the SDHs due to the treatment devices placed inside. Levels of airborne bacteria and chemicals showed regional variations. In Hefei and Yixing, the emissions of total suspended particles (TSP) and airborne bacteria were higher than those in Beijing and Guangzhou. Results of bacterial population showed that bacterial species in bioaerosols from SDHs also presented significant regional disparity; these regional disparities were closely related with the source of bioaerosols in SDHs. Among these identified bacterial species, some common potential pathogens were detected in all SDHs, such as *Aeromonas caviae*, *Flavobacterium* sp., and *Staphylococcus lentus*. Relative humidity (RH) and temperature were the major parameters on bioaerosols to survive. As shown in this study, SDHs in wastewater treatment plants should be provided considerable attention for being an emission source of indoor bioaerosols.



## 1. Introduction

Bioaerosols (short for biological aerosols) are natural or artificial particles of biological (microbial, plant, or animal) origin suspended in the air. Bioaerosol particles can be suspended in air as single cells or aggregates of microorganisms that are as small as 1–10 μm in size

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(Pastuszka et al., 2000). Bioaerosols are potentially related to various human health effects (Burge, 1990; Wouters et al., 2000) and indoor environments provide unique exposure situations; thus, concerns about indoor bioaerosols have increased over the last decade. Indoor bioaerosols are bioaerosols from indoor environments. For nonresidential buildings, some specific indoor environments, such as hospitals, wastewater treatment plants (WWTPs), composting facilities, and certain biotechnical laboratories, contain bioaerosol sources related to their particular environmental characteristics (Pastuszka et al., 2000; Bauer et al., 2002; Li and Hou, 2003; Sánchez-Monedero et al., 2003; Sánchez-Monedero et al., 2008).

Bioaerosol emissions from WWTPs were widely investigated in the previous reports (Han et al., 2013; Masclaux et al., 2014). Species and concentrations of bioaerosols differed from place to place, depending on the kind of wastewater treated, the process selected, and the meteorological parameters (Peccia et al., 2001; Szyłak-Szydłowski et al., 2016). One of the main sources of bioaerosols at WWTPs are sludge dewatering houses (SDH) (Bauer et al., 2002).

Bioaerosols cause certain human diseases, such as tuberculosis, Legionnaire's disease, and different forms of bacterial pneumonia, coccidioidomycosis, influenza, measles, and gastrointestinal illness (Burge, 1990; Peccia and Hernandez, 2006). The ability of bioaerosols to cause human disease depends on their chemical composition, biological characteristics, quantity of bioaerosols inhaled, and their size distribution.

Some concentration limits for the total number of bioaerosol particles, such as 1000 colony-forming units (CFUs)/$m^3$ (National Institute for Occupational Safety and Health) and 1000 CFUs/$m^3$ (American Conference of Governmental Industrial Hygienists) with the cultivable count for total bacteria not exceeding 500 CFUs/$m^3$, are recommended by different agencies and organizations because of the confirmed and potential adverse health effects associated with indoor bioaerosols (Kalogerakis et al., 2005). In previous research on indoor bioaerosols in residential environments, microorganisms were quantified by conventional culture-based techniques, wherein CFUs on selective media were counted. Given that not all microbes can be cultured, many were undetected before the development of DNA-based tools. Louis Pasteur was the first to research microbes and their activity within the air and nonculture-based methods, such as immunoassays and molecular biological tests; moreover, optical and electrical methods have been developed over the past few decades (Smets et al., 2016). Major culture-independent identification and quantification methods adopted in previous bioaerosol studies include polymerase chain reaction (Li et al., 2011), fluorescent in situ hybridization (Griffin, 2007), and immunoassays (i.e., enzyme-linked immunosorbent assay) (Reponen et al., 2011). High-throughput sequencing of DNA was achieved by massive parallelization of the sequencing (e.g., over a billion fragments were sequenced simultaneously on the Illumina platform). Differences in error rate, throughput, and cost are the main factors that distinguish the systems. High-throughput sequencing is widely applied to study the microbial characteristics from environments, including soil (Hong et al., 2015), wastewater (Du et al., 2017), atmosphere (Cao et al., 2014), and compost (Hou et al., 2017). However, few studies have reported the application of such technique in analyzing the population of bioaerosols emitted from WWTPs.

In recent years, with the rapid economic growth and strict discharge standard, the MWTP number has rapidly increased to 3500 by the end of 2016 in China. Biological processes are broadly used in MWTPs. With increased treatment rates and treatment capacity of municipal sewage treatment plants, the production of sewage sludge with 80% moisture content from WWTPs has reached up to 27.86 million tons. In all activated sludge plants, once the wastewater receives sufficient treatment, excess mixed liquor, which is the combination of wastewater and biological mass, is discharged into settling tanks, and water content of mixed liquor will be reduced before further disposal. Belt filter presses (BFPs) are widely applied in the MWTPs of China as a separate mechanical process for sludge dewatering. After dewatering, sludge may be handled as solids containing 50% to 75% water. Besides moisture, organic matter, and inorganic salts, the excess mixed liquor contains bacteria, fungi (and spores and cell fragments of fungi), viruses, microbial toxins, and pathogens, which pose threats to human health and the environment when released into the air during dewatering. Furthermore, most of the dewatering devices are placed inside, and few studies have become available in this typical indoor bioaerosol emission.

In the present study, an investigation was conducted to efficiently estimate indoor bioaerosols present in SDH environments in MWTPs. Airborne particles were collected from SDHs of nine municipal WWTPs. Moreover, chemicals and microbial populations were analyzed. The correlation between meteorological parameters and the emissions of airborne particles were also observed. Objectives of this study were as follows: (i) to apply Illumina MiSeq high-throughput sequencing technique to identify the bacterial population in bioaerosols from SDHs; (ii) analyze the relationship among bioaerosol populations, concentrations, and other parameters, such as temperature, and relative humidity (RH) in SDH at the WWTP; and (iii) determine the presence of potential pathogens in bioaerosols generated during sludge dewatering in WWTPs located at different regions.

## 2. Materials and methods

### 2.1. Observation locations and sewage plants description

Air samples were collected from 9 sludge dewatering houses which respectively belong to 9 municipal wastewater treatment plants (MWTPs). The 9 MWTPs were located in Beijing (116.44, 39.84; 116.52, 39.97; 116.55, 39.90), Guangzhou (113.23, 23.13; 112.92, 22.96; 114.11, 22.79), Hefei (117.20, 31.85; 117.22, 31.72) and Yixing (119.86, 31.35), respectively. Their situations and overviews were exhibited in Fig. 1 and listed in Table 1. Samples were taken through the day from 8 a.m. to 6 p.m. without disturbing the normal operation procedure of the activated sludge treatment processes.

Each of the sampling point was set up in the center of the sludge dewatering house and 1.5 m above the ground. A control sample of clean outdoor air was taken upwind from each MWTP. During air sampling, select meteorological parameters such as direction and speed of wind, air temperature, and relative humidity (RH) at the given sampling site were recorded with the portable equipment (Table 2). RH and temperature were determined by a Dewpoint Thermohygrometer (WD-35612, OAKTON, Germany), while wind speed (WS) and solar radiation (SR) were measured by a portable anemometer (HD2303, Delta OHM, Italy) and irradiance meter (HD2302.0, Delta OHM, Italy), respectively.

### 2.2. Bioaerosols capture

Bioaerosols were collected by a total suspended particles sampler (TH-150C, Wuhan Tianhong Inc., China). Gases were gathered on 90 mm quartz micro fiber filter (Whatman QM-A) via a suction pump at a flow rate of 100 L/min. All membranes for particles capture were sterilized in advance in an autoclave for 120 mins. Prior to sterilize, the quartz micro fiber filters were heated in a Muffle furnace (Nabertherm, LT/9/11/P330, Germany) at 500 °C for 4.5 h to remove the organic compounds. The aerosol particles were continuously collected for 4 h at each site. Once the required volume of air had been drawn through, the membrane was removed and sealed to prevent contamination.

The membranes were weighted by Mettler Toledo AL204 microbalance after constant temperature (25 °C) and humidity (50%) treatment for 24 h before and after sampling, the microbalance was calibrated using standard weight. The weight difference before and after sampling was the weight of the total suspended particles (TSP).

Each membrane was cut into pieces, mixed with 50 mL of sterile distilled water and then homogenized for 30 min using a magnetic stirrer.