Ecotoxicology and Environmental Safety 154 (2018) 162–170

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Intestinal bacteria in bioaerosols and factors affecting their survival in two oxidation ditch process municipal wastewater treatment plants located in different regions

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ARTICLE INFO

Keywords:
MWTP
Bioaerosols
Intestinal bacteria
Chemicals
Total suspended particulates
Soluble ions

ABSTRACT

Samples from two oxidation ditch process municipal wastewater treatment plants (MWTPs) (HJK and GXQ) in two regions of China were analysed for bacteria, particles, total organic carbon, and water-soluble ions in bioaerosols. Diversity and potential pathogen populations were evaluated by high-throughput sequencing. Bioaerosol sources, factors affecting intestinal bacterial survival, and the relationship between bioaerosols and water were analysed by Source tracker and partial least squares-discriminant, principal component, and canonical correspondence analyses. Culturable bacteria concentrations were 110–846 and 27–579 CFU/m^3 at HJK and GXQ, respectively. Intestinal bacteria constituted 6–33% of bacteria. Biochemical reaction tank, sludge dewatering house (SDH), and fine screen samples showed the greatest contribution to bioaerosol contamination. *Enterobacter aerogenes* was the main intestinal bacteria (> 99.5%) in HJK and detected at each sampling site. *Enterobacter aerogenes* (98.67% in SDH), *Aeromonas* sp. (76.3% in biochemical reaction tank), and *Acinetobacter baumannii* (99.89% in fine screens) were the main intestinal bacteria in GXQ. Total suspended particulate masses in SDH were 229.46 and 141.6 μg/m^3 in HJK and GXQ, respectively. Percentages of insoluble compounds in total suspended particulates decreased as height increased. The main soluble ions in bioaerosols were Ca^{2+}, Na^+, Cl^-, and SO_4^{2-}, which ranged from 3.8 to 27.55 μg/m^3 in the MWTPs. Water was a main source of intestinal bacteria in bioaerosols from the MWTPs. Bioaerosols in HJK but not in GXQ were closely related. Relative humidity and some ions positively influenced intestinal bacteria in bioaerosols, while wind speed and solar illumination had a negative influence.

1. Introduction

With increased treatment rates and number of municipal wastewater treatment plants (MWTPs), the treatment capacity reached 1.66 × 10^8 m^3/day by the end of 2013 in China (Gotkowska-Plachta et al., 2013). Biological processes are widely used in MWTPs. Remarkable amounts of bioaerosols including airborne bacteria and fungi are dispersed during sewage waste treatment. Bioaerosols are particles of biological origin suspended in the air.

The dispersal of bioaerosols differs in various parts of MWTPs depending on the type of wastewater treated, process selected, and meteorological parameters (Han et al., 2012a). The highest emission of microorganisms typically occurs from aeration tanks in which oxygen is supplied by mechanical agitation of sludge and in units such as bar screens, pump stations, grit chambers, and sludge storage sites (Filipkowska et al., 2000; Niazi et al., 2015). Relatively higher biomass of bioaerosol (more than 10^3 CFU/m^3) may be produced by oxidation ditch process MWTPs compared to fine aeration techniques (10^2 CFU/m^3) (Sánchez-Monedero et al., 2008); most MWTPs in China employ oxidation ditch process (Zhang et al., 2012).

Molecular biology methods provide widely applicable tools in determination of microbial population. High-throughput sequencing technique developed in the late 1990s was one of the effective DNA-decoding tools (Ronaghi et al., 1996). It has helped to characterize genetic structure and community diversity of microorganisms in complex environments due to its low cost and ability in generating massive amounts of genetic variation data (Huang et al., 2014; Young et al., 2014; Serrano-Silva and Calderon-Ezquerro, 2017). Bioaerosols emitted from MWTPs can contain various pathogenic microbes, such as viruses, fungi, and bacteria, particularly intestinal bacteria from the

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https://doi.org/10.1016/j.ecoenv.2018.02.041
Received 28 August 2017; Received in revised form 8 February 2018; Accepted 12 February 2018
Available online 22 February 2018
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Enterobacteriaceae species, which are capable of causing infection through inhalation, ingestion, and contact with skin (Gotkowska-Plachta et al., 2013). Some detrimental human health impacts such as respiratory diseases, allergies and skin rashes, and tuberculosis may also related to bioaerosols (Peccia and Hernandez, 2006; Kolesnikov et al., 2017). *Streptococcus pneumoniae* and *Shigella* found in bioaerosols of MWTPs are pathogens that can cause community-acquired pneumonia and diarrhoea (Smets et al., 2016). Some serotypes of *Escherichia coli* can cause serious cases of food poisoning in their hosts (Korzeniewska et al., 2013). The composition of bioaerosol emitted from MWTPs differs from that of other bioaerosols from the air and soil. They may contain not only microorganism, but also small water droplets and activated sludge, particles, and ions from sewage and sludge. These components may influence the survival of microorganisms. Ions and organics can provide nutrition to microorganisms and water plays in a role in protection from damage caused by ultraviolet light.

Bioaerosols generated during wastewater treatment processes may contain microorganism which present in sewage (Karra and Katsivela, 2007). The bacterial community in wastewater undergoes dynamic changes with changing wastewater quality (Qian et al., 2012). Temperature, total phosphorus (TP), NH_3-N, and chemical oxygen demand influence the structure of the bacterial community (Qian et al., 2012). China has a very large area (Wei, 1993). There are vast differences in wastewater quality between distant locations because the lifestyle of the population, industrialization degree, geography, and climate differs (Sun et al., 2016). MWTPs in different regions of China exhibit different bacterial communities in sewage and bioaerosols (Sun et al., 2016).

In this study, samples were collected from two typical oxidation ditch process MWTPs located in different regions of China. Samples were collected at various stages of wastewater treatment (including fine screens (FS), biochemical reaction tank (BRT), secondary settling tank (ST)) and sludge treatment operations (sludge dewatering house (SDH). The main constitutes of the bioaerosol including bacteria, particles, total organic carbon (TOC), and water-soluble ions (SO_4^{2-}, NO_3^-, Cl^-, PO_4^{3-}, NO_2^- NH_4+, Ca^{2+}, K^+, Mg^{2+}, Na^+) were analysed. Elements in wastewater treatment plants were identified to determine which sites have the greatest effect on bioaerosol contamination. High-throughput sequencing technique was used to determine the diversity and population of potential pathogens in bioaerosols in this study. The source of bioaerosols, factors affecting intestinal bacterial survival, and relationship between bioaerosols and water in intestinal bacterial populations were analysed.

2. Materials and methods

2.1. Overview of wastewater treatment plants and sampling sites

Air sampling sites were set up at two MWTPs (HJK and GXQ) to investigate the emission of bioaerosols. The sampling sites included water and sludge treatment stages such as FS, BRT, ST, and SDH. Outdoor control sites were designed upwind 80 m from the oxidation ditch. These two MWTPs were located respectively in Hefei (117.22°E,31.82°N) and Guangzhou (113.28°E,23.13°N) city as shown in Fig. S1 and Table S1.

2.2. Sample collection and preparation

At the treatment stage of BRT, the samples were collected at 0.1 m, 1.5 m, and 3 m above the water surface. At other sampling sites, samplers were placed on a platform 1.5 m above ground level.

2.2.1. Total suspended particulate collection

Medium Flow Samplers (TH-150, Wuhan, China) were used to collect the total suspended particulates (TSPs) in the air of each MWTP. A Medium Flow Sampler was a type of impingement sampler that is portable, small, and easy to carry and is used for highly accurate monitoring. A glass fibre membrane was applied to the medium for sample deposition. Each glass fibre membrane had a 99.90% particle rejection coefficient and was 90 mm in diameter. Before and after sampling, membranes were dried in a $CaCl_2$ desiccator for 48 h and weighed for gravimetric determination of TSPs mass using an electronic balance with a detection limit of 0.1 mg (AL204, Mettler Toledo, Columbus, OH, USA). The membrane holders were cleaned with 75% ethanol before use. The Medium Flow Samplers were used to collect samples continuously for 4 h at a flow rate of 100 L/min. After sampling, the membranes were stored in a bacteria-free environment at − 20 °C.

2.2.2. Culturable airborne bacteria collection

A one-stage viable Andersen Impactor (228–9530 K, SKC Gulf Coast, Inc., Houston, TX, USA) with 400 holes was used to capture culturable airborne bacteria and intestinal bacteria. A dish containing the medium was placed in the impactor. An air stream with a flow rate of 28.3 L/min was drawn through the impactor by a pump. The sampling time for each sampling site was 2 min. After sampling, dishes were placed in the refrigerator until being transported to the laboratory for analysis. Samples at each sampling point were collected three times. Before and after each sampling, 75% ethanol was used to sterilize the impactor (Ding et al., 2016). Airborne bacteria were cultivated in Luria-Bertani medium at 35 °C for 48 h. Intestinal pathogenic bacteria were incubated in MacConkey Agar medium 35 °C for 48 h. MacConkey Agar was used to isolate and identify intestinal pathogenic bacteria such as *E. coli* and *Salmonella*, as it contains bile acid, which benefits their growth.

A positive-hole correction method was used to correct the number of colony counts (Macher, 1989). The results were calculated as the geometric mean of the replicates and expressed as colony-forming units per cubic meter of air (CFU/m^3) (formula (1)).

$$C = \frac{N \times 1000}{t \times F} \quad (1)$$

where C is airborne bacterial concentrations in CFU/m^3, N is the total number of bacterial colonies in CFU, t is sampling time in minutes, and F is gas flow during sampling in L/min.

During air sampling, the temperature and relative humidity were recorded by a Dewpoint Thermohygrometer (WD-35612, OAKTON, Vernon Hills, IL, USA). Wind speed and irradiance were measured with a portable anemometer (HD2303, Delta OHM, Padova, Italy) and irradiance meter (HD2302.0, DeltaOHM), respectively. Sampling time and meteorological conditions in the two MWTPs are described in Table 1.

2.3. Analysis method

2.3.1. Chemicals analysis

Each sample membrane was cut and extracted with 50 mL ultrapure water (18.2 MΩ, Millipore, Billerica, MA, USA) in an ultrasonic bath for 20 min at room temperature (25 °C), and then the extraction liquid was filtered through 0.22-μm filters. The membranes with residue were dried to obtain insoluble substances, while the filtrate was used for soluble matter analysis.

A carbon analyser (TOC-V-CPH Shimadzu, Kyoto, Japan) was used to detect TOC. Concentrations of SO_4^{2-}, NO_3^-, Cl^-, PO_4^{3-}, and NO_2^- anions in each sample were determined with an ion chromatogram analyser (ICS-3000, Dionex, Sunnyvale, CA, USA). Concentrations of NH_4^+, Ca^{2+}, K^+, Mg^{2+}, and Na^+ cations in each sample were determined with an ion chromatogram analyser (IC plus 883, ion chromatography system, Metrohm, Herisau, Switzerland).

The chromatography apparatus included a column oven, conductivity detector, manual injector, and chromatography workstation (Metrohm); AS19 Column and Metrosep C 4150/4.0 separation column; eluent: 20 mM NaOH (anions), 2.0 mM HNO_3 (cations); column temperature: 30 °C; flow-rate: 1.0 mL/min; inject volume: 10 L. The detection limit of the method was less than 0.05 mg/L for anions and cations.

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