Water Research 112 (2017) 110–119

Contents lists available at ScienceDirect

Water Research

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

Assessment of airborne bacteria and noroviruses in air emission from a new highly-advanced hospital wastewater treatment plant

K. Uhrbrand [a, b, *], A.C. Schultz [b], A.J. Koivisto [a], U. Nielsen [c], A.M. Madsen [a]

[a] The National Research Centre for the Working Environment, Lersø Parkallé 105, 2100 Copenhagen Ø, Denmark
[b] National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, 2860 Søborg, Denmark
[c] DHI, Agern Allé 5, 2970 Hørsholm, Denmark

ARTICLE INFO

Article history:
Received 23 August 2016
Received in revised form
20 December 2016
Accepted 22 January 2017
Available online 25 January 2017

Keywords:
Air sampling
Bioaerosols
Microbial diversity
Norovirus
Wastewater

ABSTRACT

Exposure to bioaerosols can pose a health risk to workers at wastewater treatment plants (WWTPs) and to habitants of their surroundings. The main objective of this study was to examine the presence of harmful microorganisms in the air emission from a new type of hospital WWTP employing advanced wastewater treatment technologies. Air particle measurements and sampling of inhalable bacteria, endotoxin and noroviruses (NoVs) were performed indoor at the WWTP and outside at the WWTP ventilation air exhaust, downwind of the air exhaust, and upwind of the WWTP. No significant differences were seen in particle and endotoxin concentrations between locations. Bacterial concentrations were comparable or significantly lower in the exhaust air than inside the WWTP and in the upwind reference. Bacterial isolates were identified using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry. In total, 35 different bacterial genera and 64 bacterial species were identified in the air samples. Significantly higher genus and species richness was found with an Andersen Cascade Impactor compared with filter-based sampling. No pathogenic bacteria were found in the exhaust air. *Streptomyces* was the only bacterium found in the air both inside the WWTP and at the air emission, but not in the upwind reference. NoV genomes were detected in the air inside the WWTP and at the air exhaust, albeit in low concentrations. As only traces of NoV genomes could be detected in the exhaust air they are unlikely to pose a health risk to surroundings. Hence, we assess the risk of airborne exposure to pathogenic bacteria and NoVs from the WWTP air emission to surroundings to be negligible. However, as a slightly higher NoV concentration was detected inside the WWTP, we cannot exclude the possibility that exposure to airborne NoVs can pose a health risk to susceptible to workers inside the WWTP, although the risk may be low.

1. Introduction

Wastewater contains high amounts of microorganisms, such as pathogenic bacteria and viruses, which can be aerosolized in different stages of the wastewater and sludge treatment process (Sanchez-Monedero et al., 2008). Inhalation of bioaerosols generated during wastewater treatment may therefore pose a health hazard to workers at wastewater treatments plants (WWTPs) or to habitants of their surroundings (Sanchez-Monedero et al., 2008; Van Hooste et al., 2010). Several studies have reported an increased prevalence of gastrointestinal (Lundholm and Rylander, 1983; Rylander, 1999; Thorn et al., 2002) and respiratory symptoms (Thorn et al., 2002) among wastewater workers. The precise cause of symptoms is unknown, but exposure to airborne pathogenic enteric bacteria and viruses, e.g. noroviruses (NoVs), has been suggested as a source of the gastrointestinal illness (Masclaux et al., 2014; Uhrbrand et al., 2011). Mucosal inflammation caused by inhalation of endotoxin from Gram-negative bacteria has also been suggested as the cause of an increased incidence of diarrhea, airway symptoms, and fatigue reported among wastewater workers (Lundholm and Rylander, 1983; Rylander, 1999). Others have been unable to determine such an association between endotoxin exposure and symptoms (Douwes et al., 2001; Melbostad et al., 1994), suggesting other biological agents to be the culprit, and one study demonstrated a correlation between high exposure levels to rod-shaped bacteria and total bacteria and respiratory and

* Corresponding author. The National Research Centre for the Working Environment, Lersø Parkallé 105, 2100 Copenhagen Ø, Denmark.
E-mail address: kau@nrcwe.dk (K. Uhrbrand).

http://dx.doi.org/10.1016/j.watres.2017.01.046
0043-1354/

flu-like symptoms among wastewater workers (Melbostad et al., 1994).

Several studies have been performed to evaluate exposure to bioaerosols at WWTPs by determining the concentration of microorganisms using different sampling and detection methods (Li et al., 2013; Masclaux et al., 2014; Uhrbrand et al., 2011). The various sampling methods have different advantages and disadvantages and the suitability of a method depends on the purpose of sampling and the detection method employed, e.g. cultivation of viable microorganisms or molecular detection of genomic material. The feasibility of using different air samplers also relies on factors such as sampling environment, sampling time, airflow and relative humidity. Some samplers, such as the widely used Andersen Cascade Impactor (ACI) that collects microorganisms by direct impaction on an agar medium, are prone to overloading and thus only appropriate for short-time sampling in environments that are not heavily contaminated with bioaerosols (Thorne et al., 1992). For sampling in environments with high levels of bioaerosols filter-based samplers are better suited due to the possibility of diluting the sample after collection. This also allows longer sampling times and filter-based samplers have previous been used to characterize exposure during an entire working day (Durand et al., 2002; Madsen, 2006b; Uhrbrand et al., 2011). Nevertheless, prolonged sampling and high airflow may reduce viability of microorganisms due to cell damage or desiccation (Stewart et al., 1995; Wang et al., 2001).

The degree of human exposure to airborne microorganisms at WWTPs may vary depending upon the type and capacity of a plant, performed activities and meteorological conditions (Fracchia et al., 2006). Consequently, extrapolation of exposure data obtained at one type of WWTP to another may not be appropriate, especially if WWTP technologies and processes differ markedly from previously studied WWTPs. Conventionally wastewater treatment takes place in large open basins and relies on mechanical, biological and chemical means of treatment. However, in May 2014 operations of a new technologically advanced on-site pilot hospital WWTP in Denmark commenced. This WWTP differs from conventional WWTPs by consisting of a membrane bioreactor (MBR) for biological treatment followed by a combination of polishing technologies such as granular activated carbon treatment, ozone and UV treatment. In addition, all processes are encapsulated. Nonetheless, as the new WWTP is located close to the hospital in a residential area it is essential to determine that airborne pathogens are not released from the WWTP. Our objective was therefore to examine the WWTP air emission for the presence of potential harmful bacteria, NoVs (as a model for environmentally persistent enteric viruses) and endotoxin. Secondary objective was to examine the exposure to bioaerosols inside the WWTP that could represent an occupational risk. A final aim was to compare the diversity of viable bacteria obtained using ACI and filter-based sampling.

2. Methods

2.1. Sampling locations

Sampling was conducted at the new full-scale pilot WWTP at Herlev Hospital in the Capital Region of Denmark. The WWTP is located on the hospital premises with approximately 50 m to nearest residential area. The WWTP treats between 250 and 550 m^3 of hospital wastewater daily. The WWTP is a private-public project, where new combinations of technologies are tested to demonstrate how hospital wastewater cost-efficiently can be treated to obtain an effluent water quality allowing discharge directly into a nearby stream and reuse as technical or recreational water. The plant design is based around a membrane biological reactor system (MBR), which consists of biological process tanks, where activated sludge is added, followed by a micro-filtration membrane (pore size 0.2 μm) for retention of biomass and physical bacterial removal. Post-treatment processes consist of granular activated carbon (9.3 m^3), ozone (3.4 mg O_3/mg DOC) and UV light (45 mJ/cm^2). All treatment processes are encapsulated, and air emissions are treated using photoionization, UV light and a catalyst before being released to the surroundings via a ventilation air exhaust on the roof of the WWTP (Grundfos, 2014). The layout of the WWTP is shown in Fig. 1.

2.2. Sampling procedures

Sampling was carried out on May 27 and June 23, 2015, at Herlev Hospital WWTP. On both occasions stationary sampling were conducted 1.5 m above ground level at the following positions at the WWTP: pretreatment unit (indoor), bagging station (indoor), wastewater outlet (indoor), ventilation air exhaust on top of the WWTP roof (outdoor), downwind of air exhaust—approximately 9 m from ventilation air exhaust (outdoor) (Fig. 1). To ensure that the measured bioaerosols originated from the WWTP reference measurements were taken upwind from the WWTP.

Particle concentrations in air were measured with an optical particle sizer (Grimm; Grimm Aerosoltechnik, Model 1109, Ainring, Germany) in 31 channels between 0.25 and 32 μm in intervals of 6 s.

Inhalable Gesamtstaubprobenahme samplers (GSPs; CIS, BGI Inc., Waltham; MA, USA) mounted with 37 mm polycarbonate filters (PC; pore size 1.0 μm, Maine Manufacturing, Sanford, USA) or gelatin filters (GEL; pore size 3.0 μm, SKC Inc, PA, USA) were used to collect bacteria. PC filters also were used to collect endotoxin. For collection of NoVs GSPs with MAGNA nylon filters (NY; pore size 1.2 μm, Maine Manufacturing) were used. Sampling with GSPs, mounted with the various filters, was carried out side-by-side at a flow rate of 3.5 lpm throughout an average sampling period of 409 min, mimicking a working day. In addition, short-time sampling of bacteria was conducted with GSPs using a 30 min sampling period. On each sampling a total of 24, 6 and 6 samples were collected with GSPs for bacterial, NoV and endotoxin analysis, respectively.

Bacteria were also sampled using a Six-stage Viable Andersen Cascade Impactor (ACI; N6, Thermo Fisher Scientific Inc. Waltham, MA, USA) at all locations except downwind from air exhaust. Sampling was performed with a flow rate of 28.3 lpm for 5 and 10 min on Nutrient agar (NA; Oxoid, Basingstoke, England) with actidione (cyclohaximide; 50 mg/l) and for 30 min on SSI Enteric medium (SSI; SSI Diagnostica, Hillerød, Denmark), corresponding to a total of 15 samples (×6 size fractions) collected with ACI on each sampling.

At the WWTP air exhaust, where NoV concentration was presumed to be low due to air being treated prior to release, a high sample flow rate Dekati® Gravimetric Impactor (DGI; model DGI-1571, Dekati Ltd., Tampere, Finland) with 47 mm Nuclepore Track-Etched polycarbonate membranes (PC, pore size 1 μm, GE Healthcare, Brøndby, Denmark) was used for NoV collection. Thereby, allowing sampling of a much larger volume of air as well as size classification of NoVs in the air emission. One sample was collected with DGI on each sampling with an average flow rate and sampling period of 61.5 lpm and 487 min, respectively.

All samplers were setup side-by-side at the different sampling locations and operated at the same time. As quality assurance flow rates of all samplers were monitored and adjusted continuously during sampling and negative filters and agar samples were used as contamination controls.

Finally, to determine the approximate levels of NoVs present in the wastewater during the period of air sampling water samples