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Microbial selectivity of UV treatment on antibiotic-resistant heterotrophic bacteria in secondary effluents of a municipal wastewater treatment plant

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ABSTRACT

Little is known about the microbial selectivity of UV treatment for antibiotic resistant bacteria, and the results of limited studies are conflicting. To understand the effect of UV disinfection on antibiotic resistant bacteria, both total heterotrophic bacteria and antibiotic resistant bacteria (including cephalexin-, ciprofloxacin-, erythromycin-, gentamicin-, vancomycin-, sulfadiazine-, rifampicin-, tetracycline- and chloramphenicol-resistant bacteria) were examined in secondary effluent samples from a municipal wastewater treatment plant. Bacteria resistant to both erythromycin and tetracycline were chosen as the representative of multiple-antibiotic-resistant bacteria and their characteristics after UV treatment were also investigated.

UV disinfection results in effective inactivation for total heterotrophic bacteria, as well as all antibiotic resistant bacteria. After UV treatment at a fluence of 5 mJ/cm^2, the log reductions of nine types of antibiotic resistant bacteria varied from 1.0 ± 0.1 to 2.4 ± 0.1. Bacteria resistant to both erythromycin and tetracycline had a similar fluence response as did total heterotrophic bacteria. The findings suggest that UV disinfection could eliminate antibiotic resistance in wastewater treatment effluents and thus ensure public health security.

Our experimental results indicated that UV disinfection led to enrichment of bacteria with resistance to sulfadiazine, vancomycin, rifampicin, tetracycline and chloramphenicol, while the proportions of cephalexin-, erythromycin-, gentamicin- and ciprofloxacin-resistant bacteria in the wastewater decreased. This reveals the microbial selectivity of UV disinfection for antibiotic resistant bacteria.

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1. Introduction

Bacterial resistance to antibiotic pharmaceuticals has been considered as a major public health problem on a worldwide scale. Many kinds of antibiotic resistant bacteria (ARB) have been detected in various environments (Hu et al., 2008; Huang et al., 2012; Threedeach et al., 2012). ARB might spread their resistance to related human pathogenic microorganisms through numerous routes and then weaken the anti-infectious effect of antibiotics. A global strategy has been proposed by the World Health Organization to contain antibiotic resistance regarding its potential threat to both public health and the environment (Pruden et al., 2006).

Because of the high microbial biomass and abundant nutrients, as well as various antimicrobial agents, wastewater represents a favorable habitat for both the survival of ARB and the transfer of antibiotic resistance, spreading resistant bacteria into subsequent aquatic and terrestrial environments (Iversen et al., 2004; Bouki et al., 2013). Various ARB, including multiple antibiotic resistant (MAR) bacteria, have been detected in a large number of wastewater treatment plants (WWTPs) (Guardabassi et al., 2002; Da Costa et al., 2006; Luczkiewicz et al., 2010). And *Escherichia coli* and *Enterococci* are considered as the principal MAR species (Castiglioni et al., 2008; Luczkiewicz et al., 2010). Although most ARB can be partially removed through common wastewater treatment processes (Guardabassi et al., 2002; Da Costa et al., 2006), there are still large numbers that survive in the effluent, since WWTPs are not designed for the removal of these new pollutants (Pruden et al., 2006).

UV treatment is commonly used as a disinfection process in drinking water and wastewater treatment facilities to promote effluent security after traditional treatment processes. UV disinfection usually contributes to effective inactivation of most bacteria, with microorganism inactivations from about 1 to 6 logs under typical fluences (Sommer et al., 1998; Maya et al., 2003; Hijnen et al., 2006). However, few attempts have been made in previous studies to evaluate the UV disinfection efficiency of ARB, and conflicting results exist. For example, Conner-Kerr et al. (1998) revealed that UV disinfection could inactivate 99.9% of the methicillin-resistant strain of *Staphylococcus aureus* or vancomycin-resistant *Enterococcus faecalis* in vitro at a fluence of 77 mJ/cm^2. Similar effectiveness of UV disinfection was also observed by Macauley et al. (2006), where they found that a fluence of 220 mJ/cm^2 could cause a 3.4–4.2 log reduction of total bacteria as well as ARB in swine wastewater. However, Munir et al. (2011) investigated the inactivation effect of UV disinfection devices installed in several WWTPs of Michigan. They found that UV disinfection did not contribute to any significant reduction of tetracycline- and sulfonamide-resistant bacteria.

Furthermore, the changes in characteristics of various ARB, when exposed to UV disinfection, have been little discussed previously. Meckes (1982) indicated that, although UV disinfected the wastewater effluent effectively, the percentage of the total coliform population resistant to tetracycline or chloramphenicol increased significantly. A recent study indicated that UV treatment caused no change in *E. coli* strains' resistance to amoxicillin and sulfamethoxazole, while the treatment affected resistance of the lower resistance strain to ciprofloxacin (Rizzo et al., 2013). However, according to Templeton et al. (2009), Guo et al. (2012) and Huang et al. (2013), there were no statistically significant inactivation differences between tetracycline (or ampicillin)-resistant *E. coli* and antibiotic-sensitive *E. coli* after UV treatment. Some antibiotic-resistant organisms have been reported to exhibit resistance to chlorine (Murray et al., 1984; Huang et al., 2011), weakening the inactivation effect of chlorination. Whether similar resistance also exists during UV disinfection is still unknown. There is limited evidence showing the selection of ARB after UV disinfection.

Thus, the aim of this bench-scale study was to examine the effect of UV disinfection on antibiotic-resistant heterotrophic bacteria in the secondary effluents of a municipal WWTP in China, and to assist in estimating microbial health risks from ARB. Therefore, the inactivation of bacteria resistant to nine types of antibiotics (cephalexin, ciprofloxacin, chloramphenicol, erythromycin, gentamicin, rifampicin, sulfadiazine, tetracycline and vancomycin) was studied after UV disinfection for a series of fluences. Then, the changes in proportions of ARB in the microbial communities of the secondary effluents were analyzed to indicate UV selectivity of ARB in the wastewater. Finally, the characteristics of MAR bacteria after UV disinfection were also investigated, and bacteria resistant to erythromycin and tetracycline were selected as representative species.

2. Materials and methods

2.1. *Water samples*

Wastewater samples were collected from the effluent after biological aerated filter in a municipal WWTP in Shanghai, China. The plant treatment process is shown in Fig. 1, and the wastewater qualities are given in Table 1. After being collected in four sterile polyethylene bottles, the water samples were immediately stored on ice and transported to the lab for processing (within 2 h).

2.2. *Disinfection procedures*

A specially-designed low-pressure (LP) collimated beam apparatus was employed to conduct the UV disinfection process. This apparatus contains a low-pressure (120 W, 30% UV-C, TL 120W/01, Philips) mercury UV lamp. The irradiance at the center of the beam at the water surface was about 0.12 mW/cm^2. UV exposure tests were carried out according to the standard collimated beam test protocol, as described by Bolton and Linden (2003). The disinfection procedure is described as follows: 40 mL water samples contained in Petri dishes (diameter: 90 mm) were put under the collimating tube, and then gently stirred throughout the UV exposure time. The irradiance values were fixed throughout the experiment, and the fluences were controlled by changing the exposure times. All samples were exposed at room temperature (25 ± 2 °C). In the disinfection process, wastewater samples were exposed to fluences of 5, 10, 20, 50 and 80 mJ/cm^2, respectively. Fluences