Disinfection of treated wastewater as an essential purification step for safe urban reuse: a comparative pilot study of UV- and ClO₂-disinfection systems for urban reuse applications in China

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ABSTRACT

Disinfection of wastewater is vital in addressing the potential health risks of urban water reuse. To compare the applicability of wastewater disinfection methods other than chlorine, identical pilot plants that used ultraviolet (UV) irradiation and chlorine dioxide (ClO₂) dosing were installed at municipal wastewater treatment plants in Shanghai, China and Darmstadt, Germany. The investigation included public health and environmental aspects associated with the two disinfection methods. The results of the pilot-scale studies suggest that, in order to comply with Chinese water quality standards for urban water reuse, it is advisable to have a tertiary treatment before wastewater disinfection. Both methods were able to achieve a 4 log₁₀ reduction in both total coliforms and Escherichia coli (E. coli). There was no evidence for regrowth of E. coli. However, after an initial 3 log₁₀ reduction, HPC increased within 48 h by more than 10-fold after UV irradiation as well as after low doses of ClO₂. An increase in acute toxicity was detected after dosing with ClO₂ but not after UV irradiation.

Key words | chlorine dioxide, disinfection, regrowth, UV radiation, water reuse

INTRODUCTION

Rapidly expanding cities, escalating water scarcity and a deterioration in freshwater quality are driving forces for the establishment of safe water reuse facilities. With a total of 2,050 m³ of internal renewable water resources per capita (cap) per year (y), China possesses significantly less than the world average of 7,000 m³ cap⁻¹ y⁻¹ (FAO 2011). The inhomogeneous spatial distribution and the seasonal variability in water quantity exacerbate problems of water scarcity (Zeng et al. 2008). To alleviate water scarcity problems, the Chinese government has adopted various approaches to promote wastewater treatment and reuse since the 1980s (Funamizu et al. 2008). Wastewater contains a variety of bacteria, viruses, and parasites, some of which can cause severe diseases in humans. Disinfection of treated wastewater protects the public from waterborne diseases and therefore plays a fundamental role in water reuse. As public health concerns are among the main constraints for reuse applications (Huertas et al. 2008), effective disinfection of reclaimed water is vital for consumer acceptance. A joint research project involving Tongji University, Shanghai, China, and Technische Universität Darmstadt, Germany, aimed at improving the microbiological quality of the effluent from municipal wastewater treatment plants and evaluating water quality with respect to urban reuse. China has comprehensive regulations regarding the microbiological quality of wastewater effluents; however, it has few disinfection systems in operation. For water reuse, more stringent regulations that define microbiological standards according to the intended reuse purpose are applicable. The maximum acceptable concentration of
Several concerns associated with the use of chlorine products for water disinfection, in particular the formation of an array of toxic disinfection by-products (DBPs) and resistance of some pathogens, have contributed to predictions that the end of the chlorine era is approaching (Cabaj et al. 2012). To compare disinfection methods for treated wastewater other than chlorine, two identical pilot plants (each with a treatment capacity of 2 m³/h) that used ultraviolet (UV) irradiation and chlorine dioxide (ClO₂) dosing were installed at municipal wastewater treatment plants in Shanghai and Darmstadt. Unlike chlorine, ClO₂ does not react with ammonia, nor does it form trihalomethanes (THMs) (Richardson et al. 2007). ClO₂ is also considerably more effective than chlorine at inactivating viruses (Junli et al. 1997). Disinfectant residuals can be retained in the treated water after both chlorine and ClO₂ disinfection; these help to maintain microbial water quality in water distribution systems. UV irradiation effectively removes a wide range of pathogens, including chlorine-resistant protozoa (Johnson et al. 2005). At the applied wavelength of 254 nm, no unwanted DBPs are created (Lyon et al. 2012).

Bacteria may be able to repair damage caused by UV irradiation via molecular mechanisms such as photoreactivation and dark repair systems (Jungfer et al. 2007). Furthermore, some bacteria are able to induce cellular protective processes in response to oxidative stress (such as ClO₂ or chlorine disinfection) or UV irradiation and can survive in a viable but non-culturable state which, under favourable conditions, may be reversible within 24–48 h (Gião et al. 2009; Bodet et al. 2012). Furthermore, high organic and nutrient loads of the treated effluents may lead to regrowth of microorganisms after disinfection (Jiembá et al. 2010). To monitor the stability of the disinfection process, indicator organisms were microbiologically quantified with culture-based methods for stagnation times up to 14 days. With the analytical techniques currently available, it is not possible to detect all DBPs that may emerge following disinfection (Wang et al. 2007). Bacterial bioluminescence assays have proved to be useful tools for measuring baseline toxicity arising from DBPs and residual disinfectants, because they allow the quantification of effects caused by chemicals with a common mode of toxic action (Escher et al. 2009; Watson et al. 2012). In the present study, the inhibition of light emission from Vibrio fischeri (V. fischeri) photobacteria was measured to estimate the acute toxic effects of the treated effluents.

The current study includes public health and environmental aspects of UV and ClO₂ disinfection systems applied to treated wastewater for urban reuse applications in China.

**METHODS**

**Treatment processes**

In Shanghai, the influent for the disinfection processes was secondary-treated municipal wastewater. The process chain consisted of a fine screen, a primary sedimentation tank, a moving bed biofilm reactor and a secondary sedimentation tank. In Darmstadt, the influent for the disinfection processes was tertiary-treated wastewater, with the process chain consisting of the activated sludge process (ASP), including nitrification, denitrification, and phosphorus removal, followed by microsieving (Figure 1). The disinfection pilot plants in Shanghai and in Darmstadt were identical. The hydraulic capacity of each continuous-flow disinfection system was 1 m³ h⁻¹. ClO₂ was formed on-site by reacting sodium chlorite (NaClO₂) with hydrochloric acid (HCl), using a ClO₂ reactor (ProMaqua, Heidelberg, Germany), followed by residence in a baffled contacting tank. The contact time was 25 min, verified by tracer tests and numerical modelling, and the applied dosage ranged from 1 to 10 mg L⁻¹ ClO₂. The UV system (Umex GmbH, Dresden, Germany) comprised two exchangeable low pressure mercury lamps of 80 and 120 W, which emitted monochromatic (254 nm) radiation. The lamps were installed in a cylindrical thin-film reactor 100 cm long and with a layer thickness of 1.3 cm. The radiation dose (fluence) could be selected and controlled over a range of 50–600 J m⁻² by adjusting the water flow as a function of the UV intensity measured online. All system components conformed to type-tested devices in accordance
to the German standard DVGW W 294 (DVGW 2006), which includes the verification of UV dose by biodosimetry.

Analytical methods

Chemical oxygen demand (COD) and ammonia were determined with commercial photochemical test kits (LCK 414 and LCK 304) on a DR 2800 spectrophotometer (Hach-Lange GmbH, Düsseldorf, Germany). The concentrations of ClO₂ in the water samples were measured by the N,N-diethyl-p-phenylenediamine (DPD) method specified in the Standard Methods (APHA et al. 1998). Turbidity measurements were conducted in accordance with ISO 7027 (ISO 1999a) on a NEPHLA laboratory turbidity photometer (Hach-Lange GmbH, Düsseldorf, Germany) with results expressed in nephelometric turbidity units (NTU). UV absorbance at a wavelength of 254 nm (UVA₂₅₄) was determined with a Zeiss spectrophotometer PMQ 3 (Carl Zeiss GmbH, Jena, Germany). For total suspended solids (TSS) measurements, the samples were filtered through 0.45 μm membrane filters (Pall, Supor™-450) and dried at 105 °C in accordance with German standard method DIN 38409-2 (DIN 1987). Chlorite was analysed by ion chromatography using a DX500 equipped with an AS9-HC column (Dionex, Sunnyvale, CA) according to ISO 10304-4 (ISO 1997b). Adsorbable organically bound halogens (AOX) measurements were performed in acidified samples, in accordance with ISO 9562 (ISO 2004), after adsorption onto activated carbon using a TOX-10 analyzer (Abimed, Langenfeld, Germany). THMs were quantified with a HP 5890 II gas chromatograph/electron capture detector (GC/ECD) (Hewlett Packard, Palo Alto, CA, USA), according to ISO 10301 (ISO 1997a). For the estimation of the overall toxicological impact of disinfected wastewater, a bioluminescence assay using V. fischeri (strain NRRL-B-11177) luminescent bacteria was employed. The inhibitory effect on the light emission of luminescent bacteria was measured according to ISO 11348-2 (ISO 2007).

All water samples for microbiological analysis were processed within 6 h of collection. The analysis procedures were carried out aseptically. Disinfection efficacy was assessed using cultivation tests for quantifying E. coli, total coliforms, enterococci and somatic coliphages (as viral indicators). Colilert-18™ defined substrate tests were employed for determining E. coli and total coliforms and Enterolert™ defined substrate tests were employed for enterococci determination (IDEXX, Westbrook, ME, USA). Colilert-18™ tests were carried out in accordance with ISO 9308-2 (ISO 2012). Enterolert is approved by the US Environmental Protection Agency for enumerating enterococci in wastewater (USEPA 2007). For both tests, 97-well QuantiTray™ 2000 and QuantiTray™ sealer were used as prescribed by the supplier. Results were expressed in terms of most probable number (MPN) per 100 mL. Somatic coliphages were
detected using the double agar layer plaque assay method described in ISO 10705–2 (ISO 2000). *E. coli* strain WG5 (nalidixic acid-resistant) was employed as the host strain for the quantification of somatic coliphages. Results were reported as plaque forming units (PFU) per mL. Furthermore, the stability of the disinfection performance was assessed microbiologically with culture-based methods during stagnation times of up to 14 days. Samples of disinfected effluents and a reference sample (non-disinfected ASP effluent) were placed in a climate chamber for stagnation in the dark for 14 days at 25 °C. Samples were analysed immediately after the designated disinfectant contact time as well as after 3, 8, 24, 72 h (3 days), 144 h (6 days), 240 h (10 days) and 336 h (14 days) stagnation time. *Pseudomonas aeruginosa* (*P. aeruginosa*) counts were determined with Pseudalert™ defined substrate tests (IDEXX), as prescribed by the supplier. Culturable heterotrophic microorganisms were enumerated by colony counts (heterotrophic plate count (HPC)) after inoculation on tryptone yeast extract agar for 44 h at 36 °C and 68 h at 22 °C, according to ISO 6222 (ISO 1999b).

**RESULTS AND DISCUSSION**

**Water quality before disinfection**

In Shanghai, the water quality after secondary treatment and before disinfection complied with the Chinese IB discharge standard for pollutants in municipal wastewater treatment plants (MEPC 2002b), except for coliform bacteria. In Darmstadt, the effluent after tertiary treatment met the standards of the European Urban Wastewater Directive 91/271/EEC (CEU 1991). Table 1 summarises and compares the characteristics of treated effluent in Darmstadt and Shanghai before disinfection.

| Wastewater characteristics of influents to disinfection pilot plants in Shanghai and Darmstadt |
|----------------------------------|--------------------|-----------------|----------------|----------------|----------------|-----------------|-----------------|-----------------|-----------------|
| Influent to disinfection systems (average values) |
| COD [mg L⁻¹] | TSS [mg L⁻¹] | UVA₂₅₄ [1/m] | Turbidity [NTU] | NH₄-N [mg L⁻¹] | Temp. [°C] | pH [-] | Total coliforms [log₁₀ CFU/100 mL] |
| Shanghai | 52 | 19 | 15.5 | 19 | 6 | 29 | 7.6 | 6.5 |
| Darmstadt | 30 | <5 | 20.2 | 1.3 | 0.2 | 17 | 7.3 | 4.3 |

**Inactivation of indicator organisms**

The Chinese urban reuse standard (MEPC 2002a) stipulates that total coliform counts should be below 3 CFU/mL. Figures 2 and 3 display the reduction in total coliforms following UV irradiation and ClO₂ dosing in the pilot plants. After UV irradiation, the tailing effect, by which large particles shield microorganisms from UV light, thus preventing any further reduction in bacterial numbers with increasing UV dose, was observed in both pilot plants, but at different magnitudes. At the Shanghai plant, doses higher than 40 J m⁻² resulted in concentrations of total coliforms between 100 and 2,500 MPN/100 mL in the effluent. In Darmstadt, doses higher than 60 J m⁻² resulted in total coliform concentrations of up to 10 MPN/100 mL in the effluent. Additional UV disinfection experiments in Darmstadt with doses of 400 J m⁻² resulted in total coliform concentrations below detection limits in all analysed samples (*n* = 24). Previous studies have reported that particle-associated bacteria are able to survive UV disinfection, due to shielding mechanisms, UV absorption, and scattering of UV radiation by particles (Wu et al. 2005; Madge & Jensen 2006). According to Emerick *et al*. (1999), particles smaller than a plant-specific critical size do not seem to be able to shield coliform bacteria from UV radiation. Madge & Jensen (2006) demonstrated that the UV disinfection rate was affected by particle size distribution of the treated wastewater. Much faster UV disinfection rates were achieved when treated wastewater only contained particles smaller than 5 μm, in comparison to the treatment of wastewater containing particles larger than 20 μm (Madge & Jensen 2006). In agreement with the cited studies, the differences in the lowest concentrations of total coliforms between the pilot plants in Shanghai and Darmstadt are probably due to differences in the water quality of the treated effluents (higher TSS and turbidity values...
recorded in Shanghai (Table 1), absence of particles smaller than 10 μm in the wastewater disinfected in Darmstadt). ClO2 doses higher than 4.5 mg L⁻¹ in Darmstadt and higher than 9 mg L⁻¹ in Shanghai were able to reduce total coliforms to below detection limits (Figure 3). The dose-response relationship between total coliforms and the ClO2 dose was not clearly log-linear, probably because of fluctuations in the water quality of the wastewater treatment plant effluents. In addition to the ClO2 dose, disinfection efficacy depends on the organic matter content and particle concentration/turbidity of the treated effluent (Leong et al. 1997), which are considerably higher in the treated effluents from the pilot plant in Shanghai (Table 1). These findings are consistent with results from a similar study by Alcalde et al. (2007), in which the ClO2 dosage of 5 mg L⁻¹ for treated wastewater with TSS and total organic carbon (TOC) concentrations of 3.6 and 5.2 mg L⁻¹ was needed to decrease faecal coliforms below detection limits. In the same study, a ClO2-dosage of 8 mg L⁻¹ was needed to reduce faecal coliforms below detection limits in treated wastewater with considerably higher concentrations of TSS and TOC (6.7 and 14.5 mg L⁻¹).

Besides total coliforms, E. coli (n = 39) and, to a smaller extent, enterococci (n = 29) and somatic coliphages (n = 19) were also enumerated following UV irradiation and ClO2 dosing at the pilot plant in Darmstadt (data not presented graphically). E. coli is the parameter referred to by numerous international reuse standards when verifying microbial water quality. The enumeration of enterococci was included in this study because they demonstrate a higher resistance than coliforms to several disinfection methods (Laplace et al. 1997; Blatchley et al. 2005). Because E. coli, total coliforms, and enterococci have limited use as indicators for enteric viral pathogens, somatic coliphages were also enumerated, as indicator organisms for enteric viral pathogens and to validate the efficacy of the disinfection processes. The initial numbers of microbes were comparable for all four indicator organisms and were in the range of 10,000–100,000 MPN/100 mL for E. coli, total coliforms, and enterococci and 10,000–100,000 PFU/100 mL for somatic coliphages. There were no major differences between ClO2 disinfection with an average dose of 2.4 mg L⁻¹ and UV disinfection with an average dose of 150 J m⁻² in terms of the level of sensitivity of any of the indicator organisms. E. coli and total coliforms showed the greatest average reductions following both UV irradiation (4.3 and 4.6 log₁₀ reduction) and ClO2 dosing (4.1 and 4.2 log₁₀ reduction), whereas enterococci showed slightly lower average reductions (4.1 log₁₀ reduction following UV disinfection and 3.5 log₁₀ following ClO2 disinfection). Somatic coliphages exhibited the lowest reductions (2.4 log₁₀ reduction following UV disinfection and 2.1 log₁₀ reductions following ClO2 disinfection). Although, because of the small sample size, caution is needed, the present findings are similar to the results of a study by Alcalde et al. (2012), in which a dose of 8 mg L⁻¹ of chlorine to treated wastewater led to a 3.4 log₁₀ reduction of faecal coliforms, whereas enterococci were only reduced by 2.5 and somatic coliphages by 0.9 orders of magnitude. Overall, the results confirm previously published results (Meng & Gerba 1996; USEPA 1999; Jacobello & Trussell 2002; Koutchma et al. 2009; Lee & Sobsey 2011), which generally report a higher resistance of viruses.
towards UV inactivation and chemical disinfectants, compared to faecal indicator bacteria.

**Microbial stability**

Immediately after the disinfection processes, the treated effluents from the pilot plant in Darmstadt were placed in a climate chamber for stagnation in the dark for 14 days at 25°C. HPC and concentrations of total coliforms, *E. coli*, and *P. aeruginosa* were determined after various time intervals, to monitor microbial stability. After an initial 3-log_{10} reduction following UV irradiation with a dose of 50 J m^{-2}, a 2-log_{10} increase in HPC was observed within 3 days (Figure 4). Over the following 11 days HPC decreased slightly, in line with the die-off curve of heterotrophic microorganisms in non-disinfected wastewater. A high ClO₂ dose of 7.5 mg L^{-1} resulted in a reduction in HPC to below detection limits (i.e. a more than 5-log_{10} reduction) and residual concentrations of 4.3 mg L^{-1} for ClO₂. After 14 days stagnation time, residual ClO₂ could still be measured, at a concentration of 0.1 mg L^{-1}. No increase in HPC could be detected after disinfection with ClO₂ at a dose of 7.5 mg L^{-1} (Figure 4(a)). Regrowth of total coliforms and *E. coli* was not observed after ClO₂ dosing (Figures 4(b) and (c)). Initial *P. aeruginosa* concentrations of $1.3 \times 10^3$ MPN/100 mL were reduced to below detection limits immediately after disinfection with both UV radiation and ClO₂ (Figure 4(d)). However, an increase in *P. aeruginosa* was observed in the UV-irradiated samples after 3 days and there was a continued increase up to $1.7 \times 10^2$ MPN/100 mL after 14 days of stagnation. *P. aeruginosa* is a bacterial opportunistic pathogen that is involved in a broad range of infections (Cheriaa *et al.* 2012). The reappearance of these bacteria after a period during which they were below detection limits indicates that there are potential health risks associated with UV-irradiated reclaimed water after a period of stagnation. In contrast, no increase in *P. aeruginosa* concentrations was observed during the 14 days of stagnation after disinfection with ClO₂.

At the pilot plant in Shanghai, samples of the treated effluents were disinfected with UV radiation at doses of 40–220 J m^{-2}, and were then either exposed to sunlight for

![Figure 4](https://iwaponline.com/jwrd/article-pdf/3/3/325/377932/325.pdf)
Immediately after UV irradiation, HPC ranged from 30 to 500 CFU/mL. HPC increased by 0.5–1.0 orders of magnitude after exposure to sunlight, irrespective of the applied UV dose. The samples stored in the dark showed a smaller increase in HPC of only 0.2–0.6 orders of magnitude (Figure 5). The results obtained from the samples exposed to sunlight and those stored in the dark differed significantly and suggest that one of the mechanisms responsible for the increase in HPC after UV irradiation is photoreactivation. There are similarities between the results of the current study and those described by Oguma et al. (2002), who reported that a 3 log10 inactivation of E. coli by a low-pressure UV lamp is followed by an increase in E. coli concentration by 1.8 orders of magnitude after 3 h exposure to artificial sunlight. They did not observe an increase in E. coli levels after 3 h of dark storage.

Following ClO2 dosing at 2 and 6 mg L−1 and storage in the dark, HPC increased nearly by 2 orders of magnitude within 48 h (Figure 6). Residual ClO2 concentrations were measured after 48 h stagnation time and were below the limit of quantitation (<0.1 mg L−1). When the doses were increased to 8 and 10 mg L−1, so that disinfectant residuals (0.15 and 0.2 mg L−1) were maintained in the effluent for more than 48 h, no increase in HPC was detected. These findings are supported by a previous study (Narkis et al. 1999), which demonstrated microbial regrowth after ClO2 dosing only if residual disinfectants were removed from the water through the addition of quenching agents.

Effluent toxicity

Acute toxicity, measured as the change in light emission from a pure culture of luminescent bacteria (V. fischeri) in the effluent of the pilot plant in Darmstadt, was determined before and after UV irradiation as well as after dosing with ClO2 and electrolytically produced chlorine gas (Cl2). The analysis of eight samples disinfected by UV radiation (150 J m−2) did not reveal an increase in effluent toxicity. Figure 7 displays the maximum concentration of sample (given here as the volumetric proportion of the sample in dilution water) needed to elicit an inhibitory effect of less than 20% in V. fischeri. (In this study, 6.25–12.5% is defined as the highest toxicity level, 16.7–33.3% as a medium toxicity level and greater than 50% as the non-toxic/lowest toxicity level (after Gellert 2000)). Effluents demonstrated a considerably higher toxicity after dosing with electrolytically produced Cl2 (current density: 28.6 mA cm−2) than after dosing with ClO2 (2.7 mg L−1). Average disinfectant residuals were measured at concentrations of 1.06 mg L−1 total chlorine and 0.12 mg L−1 ClO2. Average chlorite concentrations of 1.88 mg L−1 and no increase in AOX concentrations were detected after dosing with ClO2. Chlorine electrolysis resulted in an increase of AOX from 44 to 522 μgL−1, on average, and average concentrations of THMs of 48 μgL−1. Decreases in total coliform levels were comparable during the toxicity study for ClO2 and Cl2 disinfection (3.2 and 3.3 log10 reduction on average, respectively), whereas UV disinfection resulted in a greater
decrease of total coliform levels (4.5 log10 reduction, on average).

Toxicity measurements at the pilot plant in Shanghai were performed in a similar way, using the change in light output from luminescent *V. fischeri* (Table 2). In this case, the toxicity effects of chlorine were measured after disinfection with sodium hypochlorite. Toxicity is expressed as the percentage of light loss of *V. fischeri*. Data in boldface (Table 2) represent disinfection conditions that resulted in a comparable decrease of HPC (3.2–3.9 orders of magnitude). Similar to the Darmstadt samples, influent and UV-treated samples showed the lowest toxicity, ClO2-treated samples showed higher toxicity, and Cl2-treated samples showed the highest toxicity, while achieving similar reductions in HPC. These results are consistent with those of Svecevicius *et al.* (2005) who, by measuring the toxic effects on aquatic organisms, demonstrated that chlorine dioxide is less toxic and chlorite much less toxic than chlorine (2–4 orders of magnitude). The absence of increased toxicity after UV disinfection is corroborated in a review by Hijnen *et al.* (2006), who reported that low-pressure UV irradiation produces almost no by-products.

**CONCLUSIONS**

Compliance with the Chinese urban water reuse standard (MEPC 2002a), in terms of total coliform levels, was achieved either by tertiary treatment followed by a minimum dose of 4.5 mg L⁻¹ ClO2 or by secondary treatment followed by a
minimum dose of 9 mg L\(^{-1}\) ClO\(_2\). UV fluence of 400 J m\(^{-2}\) after tertiary treatment also resulted in effluents that complied with the Chinese urban water reuse standard.

Disinfection by both UV radiation and ClO\(_2\) were able to effect a 4-log\(_{10}\) reduction in total coliforms and \(E\). coli from secondary- and tertiary-treated effluents immediately after the disinfection process. Lower organic matter content and particulates resulted in more effective disinfection of wastewater for both disinfection methods. Tertiary treatment before wastewater disinfection is generally recommended, to decrease the required dose of ClO\(_2\) or to reduce tailing effects for UV disinfection.

Based on toxicity analysis of the disinfected effluents, measured as the change in light output of a luminescent bacteria (\(V\). fischeri), it can be concluded that UV irradiation does not increase toxicity, and that ClO\(_2\) dosing contributes to a small increase in toxicity, relative to Cl\(_2\) dosing.

The results of the present study indicate potential health risks due to regrowth of opportunistic pathogens after UV irradiation at 50 J m\(^{-2}\). Further regrowth investigations with higher UV fluences are recommended.

Following ClO\(_2\) dosing, regrowth did not occur in the present study if disinfectant residuals were still present in the treated effluents after 48 h stagnation time. To effectively prevent regrowth in reclaimed water storage and distribution systems, it appears that it is necessary to retain disinfectant residuals in the reclaimed water.

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