

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

A. Bischoff, J. H. Fan, P. Cornel, M. Wagner and L. M. Ma

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

A. Bischoff
P. Cornel
M. Wagner
Technische Universität Darmstadt,
Institute IWAR,
Petersenstrasse 13,
64287 Darmstadt,
Germany

J. H. Fan (corresponding author)
L. M. Ma
School of Environmental Science and Technology,
Tongji University,
Shanghai 200092,
China
E-mail: jinhongfan@tongji.edu.cn

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

total coliforms is set at 3 colony forming units (CFU)/L for miscellaneous urban consumption, such as toilet flushing, fire fighting, street and car cleaning, etc. (MEPC 2002a).

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 (Jiembra *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 30–600 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

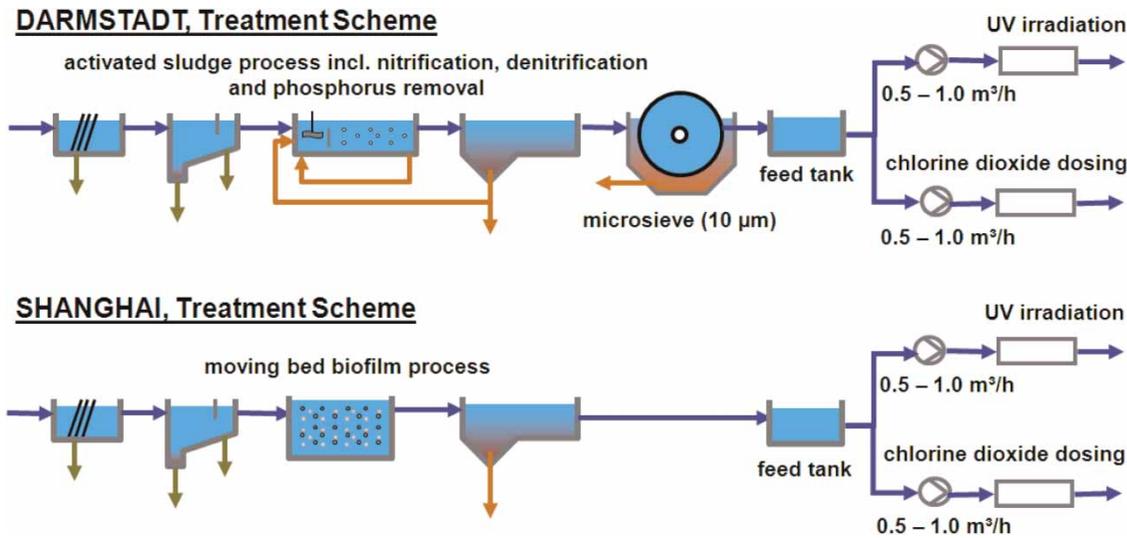


Figure 1 | Flow schemes of the disinfection pilot plants with different pre-treatment techniques in Darmstadt (above) and Shanghai (below).

to the German standard DVGW W 294 (DVGW 2006), which includes the verification of UV dose by biosimetry.

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, SuporTM-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-18TM defined substrate tests were employed for determining *E. coli* and total coliforms and EnterolertTM-defined substrate tests were employed for enterococci determination (IDEXX, Westbrook, ME, USA). Colilert-18TM 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 QuantiTrayTM 2000 and QuantiTrayTM 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.

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

Table 1 | 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]	UVA ₂₅₄ [1/m]	Turbidity [NTU]	NH ₄ -N [mg L ⁻¹]	Temp. [°C]	pH [-]	Total coliforms [log ₁₀ 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

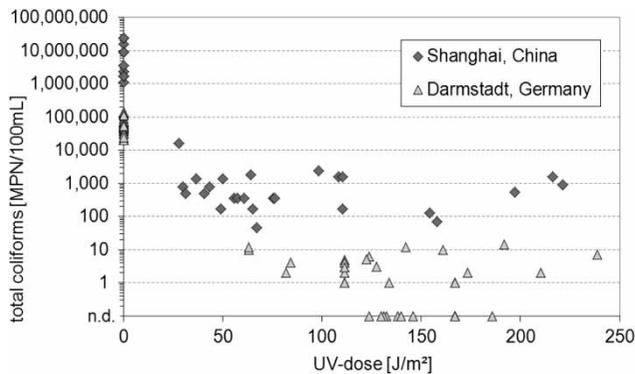


Figure 2 | Concentrations of total coliforms as a function of UV dose.

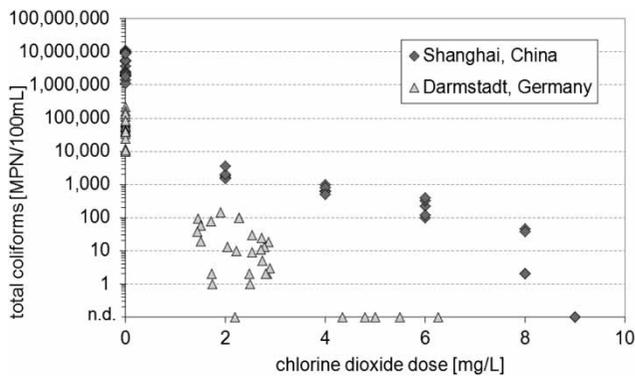


Figure 3 | Concentrations of total coliforms as a function of ClO₂ dose.

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 efficiency depends on the organic matter content and particle concentration/turbidity of the treated effluent (Leong et al. 2008), 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 3 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; Jacangelo & 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-log₁₀ reduction following UV irradiation with a dose of 50 J m⁻², a 2-log₁₀ 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-log₁₀ 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 × 10⁵ 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 × 10² 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

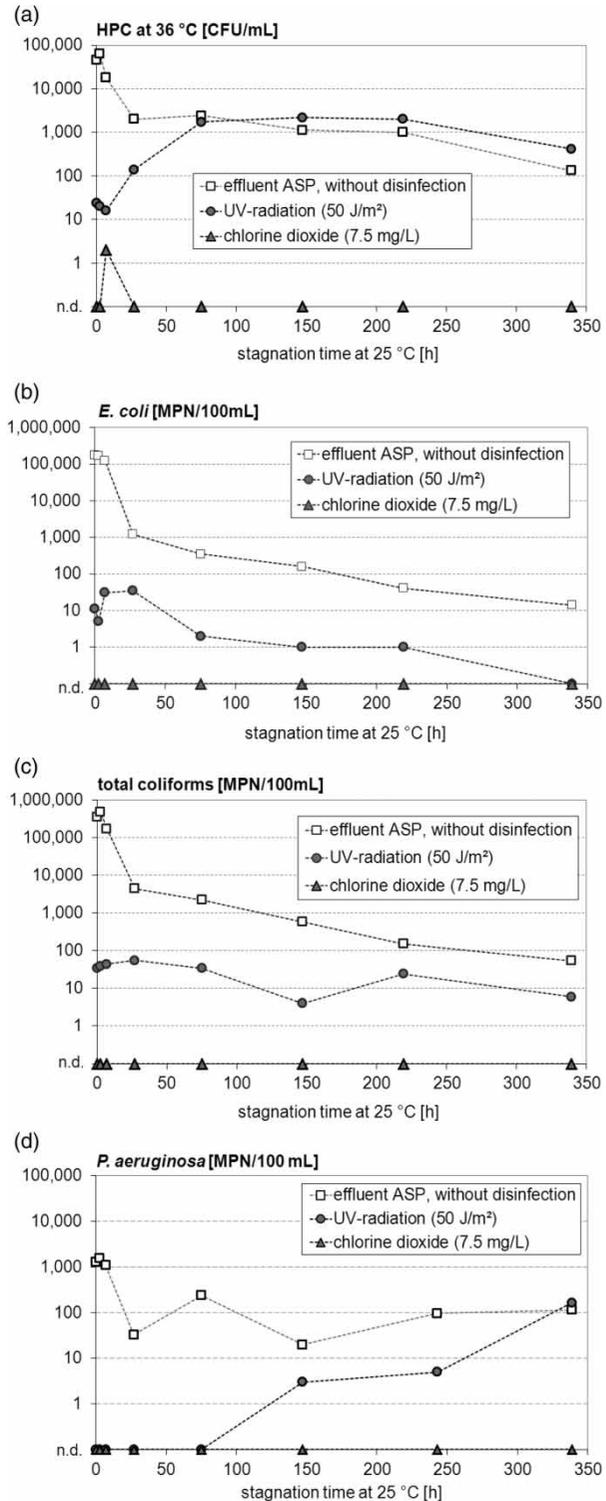


Figure 4 | Impact of stagnation time on HPC (a) and concentrations of *E. coli* (b), total coliforms (c) and *P. aeruginosa* (d) after disinfection of tertiary-treated wastewater at the pilot plant in Darmstadt. Symbols represent measured data and lines connect each data point, to demonstrate the trend.

6 h or stored in the dark for 6 h. Immediately after UV irradiation, HPC ranged from 30 to 500 CFU/mL. HPC increased by 0.3–1.0 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.* 1995), which demonstrated microbial regrowth after ClO₂ dosing only if residual disinfectants were removed from the water through the addition of quenching agents.

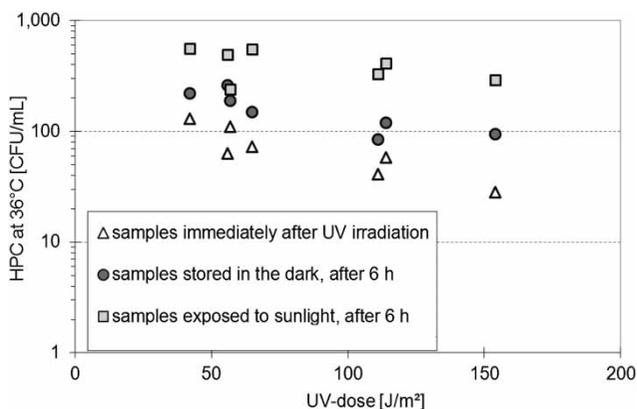


Figure 5 | Impact of UV dosing and storage conditions on HPC at the pilot plant in Shanghai.

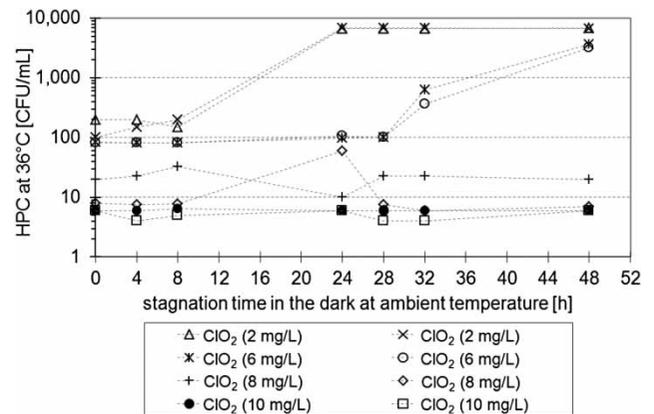


Figure 6 | Impact of ClO₂ dosing and stagnation time on HPC at the pilot plant in Shanghai (duplicate determination). Symbols represent measured data and lines connect each data point to demonstrate the trend.

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 μg L⁻¹, on average, and average concentrations of THMs of 48 μg L⁻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

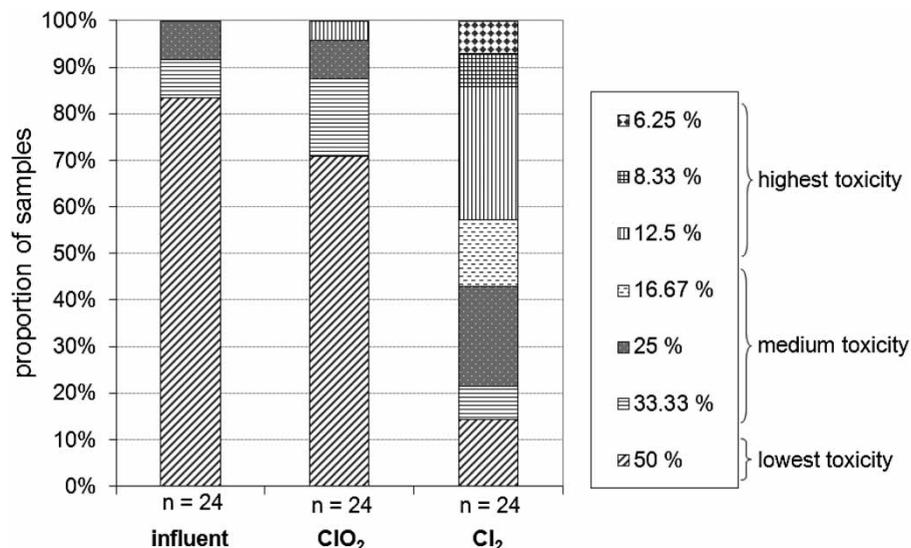


Figure 7 | Toxicity levels of wastewater effluents from the pilot plant in Darmstadt before (left, 'influent') and after ClO₂ and Cl₂ disinfection.

Table 2 | Toxicity levels of wastewater effluents from the pilot plant in Shanghai before and after disinfection

Dosage	Residual concentration	Non-disinfected effluent	UV- disinfected effluent	ClO ₂ - disinfected effluent		Cl ₂ -disinfected effluent	
		-	125 J m ⁻²	5 mg L ⁻¹ 0.7 mg L ⁻¹	9 mg L ⁻¹ 0.1 mg L ⁻¹	12 mg L ⁻¹ 1.5 mg L ⁻¹	20 mg L ⁻¹ 0.5 mg L ⁻¹
Test 1	Light loss [%]	8	13	46	75	46	93
	HPC [CFU/mL]	110,000	64	830	30	910	33
Test 2	Light loss [%]	13	11	17	43	32	98
	HPC [CFU/mL]	180,000	47	650	22	720	26

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 Svecovicus *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 ClO₂. 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 ClO₂ were able to effect a 4-log₁₀ 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 ClO₂ 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 ClO₂ dosing contributes to a small increase in toxicity, relative to Cl₂ 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 ClO₂ 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.

ACKNOWLEDGEMENTS

This research project was funded by the German Federal Ministry for Education and Research (project number: 02WA0764) and by the International Science and Technology Cooperation Fund of Shanghai (project number: 072307030).

REFERENCES

- Alcalde, L., Folch, M. & Tapias, J. C. 2012 Removal and relationships of microbial indicators in a water treatment and reclamation facility. *Journal of Water and Health* 10, 549–556.
- Alcalde, L., Folch, M., Tapias, J. C., Huertas, E., Torrens, A. & Salgot, M. 2007 Wastewater reclamation systems in small communities. *Water Science and Technology* 55 (7), 149–154.
- APHA, AWWA & WEF 1998 *Standard Methods for the Examination of Water and Wastewater*, 20th edition. American Public Health Association (APHA), American Water Works Association (AWWA) & Water Environment Federation (WEF), Washington DC, USA.
- Blatchley III, E. R., Gong, W.-L., Rose, J. B., Huffman, D. E., Otaki, M. & Lisle, J. T. 2005 *Effects of Wastewater Disinfection on Human Health*. WateReuse Foundation, Alexandria, USA.
- Bodet, C., Sahr, T., Dupuy, M., Buchrieser, C. & Hechard, Y. 2012 *Legionella pneumophila* transcriptional response to chlorine treatment. *Water Research* 46, 808–816.
- Cabaj, A., Chen, C., Haider, T., Jimenez, B., O'Halloran, K., Hirschmann, G., Shang, C., Shuval, H., Sommer, R., Tiwari, S. K. & Trussell, R. R. 2012 Disinfection. In: *Global Trends & Challenges in Water Science, Research and Management. A Compendium of Hot Topics and Features from IWA Specialist Groups* (H. Li, ed.). International Water Association (IWA), London, UK.
- CEU 1991 Council Directive 91/271/EEC of the European Parliament and of the Council of 21 May 1991 concerning urban waste water treatment. *Official Journal of the European Communities No. L135/40*, pp. 40–52. Council of the European Union (CEU), Brussels, Belgium.
- Cheriaa, J., Rouabhia, M., Maatallah, M. & Bakhrouf, A. 2012 Phenotypic stress response of *Pseudomonas aeruginosa* following culture in water microcosms. *Journal of Water and Health* 10, 130–139.
- DIN 1987 DIN 38409–2 *Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung; Summarische Wirkungs- und Stoffkenngrößen (Gruppe H); Bestimmung der abfiltrierbaren Stoffe und des Glührückstandes (H 2)*. (German Standard Methods for the Examination of Water, Waste Water and Sludge; Parameters Characterizing Effects and Substances (group H); Determination of Filterable Matter and the Residue on Ignition (H 2)). DIN Deutsches Institut für Normung, Beuth Verlag, Berlin, Germany.
- DVGW 2006 *Arbeitsblatt W 294: UV-Geräte zur Desinfektion in der Wasserversorgung (UV Devices for Disinfection in Drinking Water Supply)*. Deutsche Vereinigung des Gas- und Wasserfaches (German Technical and Scientific Association for Gas and Water) DVGW, Bonn, Germany.
- Emerick, R. W., Loge, F. J., Thompson, D. & Darby, J. L. 1999 Factors influencing ultraviolet disinfection performance: association of coliform bacteria with wastewater particles. *Water Environment Research* 71, 1178–1187.
- Escher, B. I., Bramaz, N. & Ort, C. 2009 Monitoring the treatment efficiency of a full scale ozonation on a sewage treatment plant with a mode-of-action based test battery. *Journal of Environmental Monitoring* 11, 1836–1846.
- FAO Food and Agriculture Organization of the United Nations (FAO) 2011 *AQUASTAT Online Database*. Food and

- Agriculture Organization of the United Nations (FAO), <http://www.fao.org/nr/aquastat>. (Accessed online 1st June 2011).
- Funamizu, N., Huang, X., Chen, G. H., Jiangyong, H. & Visvanathan, C. 2008 Water reuse in Asia. In: *Water Reuse: An International Survey of Current Practice, Issues and Needs* (B. Jimenez & T. Asano, eds). IWA Publishing, London, UK, pp. 142–160.
- Gellert, G. 2000 Beziehungen zwischen ausgewählten chemischen Summenparametern und Toxizität bei Abwässern der chemischen Industrie (Relationships between selected chemical sum parameters and toxicity of wastewater from the chemical industry). *Proceedings of Umweltanalytiktag NRW 2000 - Neue Erkenntnisse auf dem Gebiet der Umweltanalytik*, June, 2000, Duisburg, Germany.
- Gião, M. S., Wilks, S. A., Azevedo, N. F., Vieira, M. J. & Keevil, C. W. 2009 Validation of SYTO 9/Propidium iodide uptake for rapid detection of viable but noncultivable *Legionella pneumophila*. *Environmental Microbiology* **58**, 56–62.
- Hijnen, W. A. M., Beerendonk, E. F. & Medema, G. J. 2006 Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Research* **40**, 3–22.
- Huertas, E., Salgot, M., Hollender, J., Weber, S., Dott, W., Khan, S., Schäfer, A., Messalem, R., Bis, B., Aharoni, A. & Chikurel, H. 2008 Key objectives for water reuse concepts. *Desalination* **218**, 120–131.
- ISO 1997a ISO 10301 Water quality – Determination of Highly Volatile Halogenated Hydrocarbons – Gas-Chromatographic Methods. International Organisation for Standardisation, Geneva, Switzerland.
- ISO 1997b ISO 10304-4 Water Quality – Determination of Dissolved Anions by Liquid Chromatography of Ions – Part 4: Determination of Chlorate, Chloride and Chlorite in Water with low Contamination. International Organisation for Standardisation, Geneva, Switzerland.
- ISO 1999a ISO 7027 Water Quality – Determination of Turbidity. International Organisation for Standardisation, Geneva, Switzerland.
- ISO 1999b ISO 6222 Water Quality – Enumeration of Culturable Micro-Organisms – Colony Count by Inoculation in a Nutrient Agar Culture Medium. International Organisation for Standardisation, Geneva, Switzerland.
- ISO 2000 ISO 10705-2 Water Quality – Detection and Enumeration of Bacteriophages – Part 2: Enumeration of Somatic Coliphages. International Organisation for Standardisation, Geneva, Switzerland.
- ISO 2004 ISO 9562 Water Quality – Determination of Adsorbable Organically Bound Halogens (AOX). International Organisation for Standardisation, Geneva, Switzerland.
- ISO 2007 ISO 11348-2 Water Quality – Determination of the Inhibitory Effect of Water Samples on the Light Emission of *Vibrio Fischeri* (Luminescent bacteria test) – Part 2: Method using Liquid-Dried Bacteria. International Organisation for Standardisation, Geneva, Switzerland.
- ISO 2012 ISO 9308-2 Water Quality – Enumeration of *Escherichia Coli* and Coliform Bacteria – Part 2: Most Probable Number Method. International Organisation for Standardisation, Geneva, Switzerland.
- Jacangelo, J. G. & Trussell, R. R. 2002 International report: water and wastewater disinfection – trends, issues and practices. *Water Supply* **2**, 147–157.
- Jiembra, P. K., Weinrich, L., Cheng, W., Giraldo, E. & LeChevallier, M. W. 2010 *Guidance Document on the Microbial Water Quality and Biostability of Reclaimed Water Following Storage and Distribution*. WaterReuse Foundation, Alexandria, USA.
- Johnson, A. M., Linden, K., Ciociola, K. M., De Leon, R., Widmer, G. & Rochelle, P. A. 2005 UV Inactivation of *Cryptosporidium hominis* as Measured in Cell Culture. *Applied and Environmental Microbiology* **71**, 2800–2802.
- Jungfer, C., Schwartz, T. & Obst, U. 2007 UV-induced dark repair mechanisms in bacteria associated with drinking water. *Water Research* **41**, 188–196.
- Junli, H., Li, W., Nenqi, R., Li, L. X., Fun, S. R. & Guanle, Y. 1997 Disinfection effect of chlorine dioxide on viruses, algae, and animal planktons in water. *Water Research* **31**, 455–460.
- Koutchma, T. N., Forney, L. J. & Moraru, C. I. 2009 *Ultraviolet Light in Food Technology: Principles and Applications*. CRC Press, Boca Raton, USA.
- Laplace, J.-M., Thuault, M., Hartke, A., Boutibonnes, P. & Auffray, Y. 1997 Sodium hypochlorite stress in *Enterococcus faecalis*: influence of antecedent growth conditions and induced proteins. *Current Microbiology* **34**, 284–289.
- Lee, H. S. & Sobsey, M. D. 2011 Survival of prototype strains of somatic coliphage families in environmental waters and when exposed to UV low-pressure monochromatic radiation or heat. *Water Research* **45**, 3723–3734.
- Leong, L. Y. C., Kuo, J. & Tang, C. C. 2008 *Disinfection of Wastewater Effluent – Comparison of Alternative Technologies*. WaterReuse Foundation, Alexandria, USA.
- Lyon, B. A., Dotson, A. D., Linden, K. G. & Weinberg, H. S. 2012 The effect of inorganic precursors on disinfection byproduct formation during UV-chlorine/chloramine drinking water treatment. *Water Research* **46**, 4653–4664.
- Madge, B. A. & Jensen, J. N. 2006 Ultraviolet disinfection of fecal coliform in municipal wastewater. Effects of particle size. *Water Environment Research* **78**, 294–304.
- Meng, Q. S. & Gerba, C. P. 1996 Comparative inactivation of enteric adenoviruses, poliovirus and coliphages by ultraviolet irradiation. *Water Research* **30**, 2665–2668.
- MEPC 2002a Ministry of Environmental Protection of China Water Quality Standard for Urban Miscellaneous Consumption. GB/T 18920-2002. Standards Press of China, Beijing, China.
- MEPC 2002b Ministry of Environmental Protection of China Discharge Standard of Pollutants for Municipal Wastewater Treatment Plant GB18918-2002. Standards Press of China, Beijing, China.
- Narkis, N., Armon, R., Offer, R., Orshansky, F. & Friedland, E. 1995 Effect of suspended solids on wastewater disinfection efficiency by chlorine dioxide. *Water Research* **29**, 227–236.

- Oguma, K., Katayama, H. & Ohgaki, S. 2002 Photoreactivation of *Escherichia coli* after low- or medium-pressure UV disinfection determined by an endonuclease sensitive site assay. *Applied and Environmental Microbiology* **68**, 6029–6035.
- Richardson, S. D., Plewa, M. J., Wagner, E. D., Schoeny, R. & DeMarini, D. M. 2007 Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. *Mutation Research* **636**, 178–242.
- Svecevičius, G., Syvokiene, J., Stasiūnaite, P. & Mickeniene, L. 2005 Acute and chronic toxicity of chlorine dioxide (ClO₂) and chlorite (ClO₂⁻) to rainbow trout (*Oncorhynchus mykiss*). *Environmental Science and Pollution Research* **12**, 302–305.
- USEPA 1999 *Guidance Manual for Alternative Disinfectants and Oxidants*. Office of Water, EPA 815-R-99-014, United States Environmental Protection Agency, Washington DC, USA.
- USEPA 2007 *Guidelines Establishing Test Procedures for the Analysis of Pollutants; Analytical Methods for the Biological Pollutants in Wastewater and Sewage Sludge; Final Rule*. U. S. Federal Register - 40 CFR Parts 136 and 503, Vol. 72(57). United States Environmental Protection Agency, Washington DC, USA.
- Wang, L.-S., Wei, D.-B., Wie, J. & Hua, H.-Y. 2007 Screening and estimating of toxicity formation with photobacterium bioassay during chlorine disinfection of wastewater. *Journal of Hazardous Materials* **141**, 289–294.
- Watson, K., Shaw, G., Leusch, F. D. L. & Knight, N. L. 2012 Chlorine disinfection by-products in wastewater effluent: bioassay-based assessment of toxicological impact. *Water Research* **46**, 6069–6083.
- Wu, Y., Clevenger, T. & Deng, B. 2005 Impacts of goethite particles on UV disinfection of drinking water. *Applied and Environmental Microbiology* **71**, 4140–4143.
- Zeng, S., Chen, J. & Fu, P. 2008 Strategic zoning for urban wastewater reuse in China. *Water Resources Management* **22**, 1297–1309.

First received 25 October 2012; accepted in revised form 10 February 2013. Available online 7 March 2013