The combined performance of UV light and chlorine during reclaimed water disinfection

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Abstract The combined effects of disinfectant agents on the microbiological quality of reclaimed water produced by two full-scale water reclamation plants in Catalonia, Spain, were examined in this work. All the disinfectant treatments tested led to the absence, or near absence, of *E. coli* in 100ml samples of water, with log reductions of more than 3 log u. Hypochlorite reduced the bacterial concentrations. However, ultraviolet light was more effective than hypochlorite at reducing the concentrations of bacteriophages, viruses and pathogenic protozoa such as *Cryptosporidium* spp. We conclude that a combination of these two disinfectant agents is effective in protecting public health, as each agent acts to a different degree against the different groups of microorganisms studied. Further studies should investigate the combined action of disinfectant agents at water reclamation plants with ultraviolet light equipment in more favourable working conditions in order to assess their capacity to inactivate microorganisms.
Keywords: chlorine disinfection; combined disinfection; disinfection assessment; UV light disinfection, water reclamation

INTRODUCTION

Reclaimed water is used as an alternative nonpotable water source for domestic, industrial, agricultural, environmental and recreational purposes. For these purposes, water with low levels of chemical (sulfites, nitrates) or microbiological (bacterial, viruses, protozoan) contaminants does not pose a risk to human health (Asano and Cotruvo, 2004). The contribution of reclaimed water to sustainable water resources is increasing in many areas of the world, including some US States (Asano, 2002) and European areas on the northern shore of the Mediterranean (Angelakis *et al.*, 2001), where this study was performed.

In order to minimize the public health risks associated with exposure to reclaimed water, the main aim of reclamation water treatments, usually known as tertiary treatments, is to reduce the pathogen load. The effectiveness of pathogen removal in wastewater treatments is assessed by routinely monitoring the final effluent for standard bacterial indicators. The removal of coliform bacteria does not adequately reflect the removal of pathogenic viruses and protozoa achieved in disinfection procedures (Tyrrel *et al.*, 1995; Jacangelo *et al.*, 2003; Harwood *et al.*, 2005). Here we attempt to establish whether a combination of disinfection processes (UV light and chlorination) provides better quality reclaimed water than the use of only one disinfection agent (chlorination). The study was undertaken in two full reclamation water plants in Catalonia, Spain. An additional aim of this study was to experimentally determine the treatment and doses of disinfectant agents to be applied to the water produced by two water reclamation plants (WRP) in order to achieve broad spectrum disinfection against bacteria, viruses and protozoa, while minimizing the operating costs of the disinfection system and the subsequent monitoring of its quality.

MATERIALS AND METHODS

Water reclamation plants (WRP) description

The combined performance of UV light and chlorine during full-scale disinfection of reclaimed water was experimentally assessed at two water reclamation plants in the Costa Brava (NE Spain): Castell-Platja d'Aro and Blanes. Both WRPs treat wastewater by a conventional activate sludge system. Tertiary treatment conditions at the WRP in Castell d'Aro (theoretical treatment capacity of 625 m^3/h) consisted of a secondary effluent filtration (pulsed-bed sand filters) followed by the application of UV light (2 banks of 4 medium pressure lamps each) and chlorination (contact time of 45 minutes). At the WRP in Blanes (700 m^3/h) the tertiary treatment consisted of coagulation and flocculation followed by lamellar settlement and filtration (pulsed-bed sand filters); disinfection with UV light (4 banks of 8 lamps, which can be used independently according to disinfection requirements) and chlorination (contact time of 210 minutes). Reclaimed water is used for agriculture and golf course irrigation (WRP Castell d'Aro) and for aquifer recharge of the river Tordera (WRP Blanes). Table 1 shows the routine physicochemical data of the WRP.

Table 1. Physicochemical parameters routinely tested in the water produced by the WRP of Castell d'Aro and Blanes during the disinfection study.

	WRP Castell d'Aro		WRP Blanes	
Parameter	Secondary effluent	Tertiary effluent*	Filtered secondary effluent	Tertiary effluent*
Suspended solids (mg/l)	8.8 ± 1.6	5.0 ± 3.4	2.1 ± 0.5	2.3 ± 1.7
Turbidity, (NTU)	3.8 ± 1.2	2.5 ± 1.4	1.7 ± 0.5	2.0 ± 1.2
Transmittance at 254 nm (%)	46 ± 5	47 ± 16	71 ± 1.5	70 ± 3

*Average of all disinfection treatments tested.

Disinfection experiments

A 2-week experiment was conducted at the WRP of Castell-Platja d' Aro in July of 2005. Secondary (n=9) and tertiary effluent samples from different disinfection treatments were collected for 3 days/week. Disinfection treatments included UV light (n=9); chlorination (10 ppm, n=8); and a combination of UV light and chlorination (5 ppm, n=9). The UV light doses applied during the study had average values of around 25 mJ/cm^2 and transmittance values of ≤ 50%. Chlorination CT-values (mg Cl_2 min/l) were 100 for samples with 5 ppm and 216 for samples with 10 ppm.

A 4-week experiment was conducted at the WRP of Blanes in May 2006. Filtered secondary effluent (n=9) and tertiary effluent samples from different disinfection treatments were collected for 3 days/week. The combination of disinfectants tested were 1 UV module + 1 ppm of hypochlorite (UVCL, n=9); 1 UV module + 2 ppm of hypochlorite (UVCLCL n=9); 2 UV module + 1 ppm of hypochlorite (n=9); and hypochlorite added to attain a total residual chlorine value of 0.6 mg Cl_2/l (0.6CL). Additionally, samples disinfected with one (UV, N=18) or two modules (UVUV, n=9) of UV light only were collected before hypochlorite addition. The specific operating conditions to be tested were established on the Thursday of the previous week and the sampling process was carried out for three consecutive days of the week in question. On each sampling day, a sample of secondary effluent or filtered water was collected to represent the starting conditions, and three samples of reclaimed water were obtained from the treatment being tested. Table 1 shows the routine physicochemical data of the WRP.

Microbiological parameters

Bacterial quantification. Indicator bacteria were quantified by membrane filtration using 47-mm cellulose acetate filters with a nominal pore size of 0.45 μm (Millipore EZ-Pack ™ membrane filters, USA). Total coliform (TC) bacteria were cultured on mEndo LES agar (Difco, Sparks, MD) for 24 h at 37ºC; *E. coli* (EC) were cultured on Chromocult® Coliform Agar (Merck KGaA, Germany) for 24 h at 37 ºC; fecal enterococci (FE) were cultured in Enterococcus agar (Difco, Sparks, MD) for 48 h at 37 ºC and suspected colonies were confirmed on Bile esculin agar (Difco, Sparks, MD) for a minimum of 3 h at 44ºC. Sulfite reducing clostridia (SRC) were cultured on SPS agar medium for 24 h at 44 ºC.

Bacteriophages quantification. Somatic coliphages (ISO 10705-2), RNA F-specific phages (ISO 10705-1), bacteriophages infecting *Bacteroides fragilis* strain RYC 2056 (ISO 10705-4) and bacteriophages infecting *Bacteroides thetaiotaomicron* strain GA-17 (ISO 10705-4) were analyzed according to ISO standards. Tertiary effluents were concentrated (100 to 250 mL) according to the method modified by Mendez *et al.* (2004). Secondary effluents were enumerated directly.

Virus quantification. Enteroviruses were quantified on the BGM cell line (ECAAC 90092601) by the double layer plaque assay, as described by Moce-Llivina *et al.* (2004). Secondary effluents (20 mL) were filtered though 0.22 μm pore size filters (Sterivex GP, Millipore) and analyzed directly. Tertiary effluents (100L) were concentrated through MK cartridges (AMF Corp, Cuno Division, Meriden, USA) and eluted with glycine buffer (9.25M, pH 10.5). A secondary concentration was produced by the organic flocculation procedure with beef extract (3%, BBL, Becton Dikinson), as described by Katzenelson *et al.* (1976). The concentrate (50 mL) was decontaminated and detoxified by filtration through Sterivex GP 0.22μm filters (Moce-Llivina *et al.* 2003). It was then analyzed using the double layer plaque assay method. Enteroviruses were determined in the WRP of Blanes only.

Cryptosporidium quantification. Secondary (20L) and tertiary effluents (50-75L) were concentrated by Envirochek sampling capsules (Pall Gelman, Ann Arbor, USA) and purified by immunomagnetic separation (IMS) with a Dynabeads Anti-*Cryptosporidium* kit (Dynal, Oslo, Norway), as described in Method 1623 (U.S.EPA 1999). One hundred microlitres of the IMS volume (500 μL) were used to determine total and viable oocysts. Vital dye staining (viable oocysts) was performed as described by Campbell *et al.* (1992). Oocysts were visualized with a laser scanning cytometer (Compucyte, Cambridge, UK), as previous described by Montemayor *et al.* (2005). Four hundred microlitres of the IMS were used for the *Cryptosporidium* infectivity assay on the HCT-8 cell line (ECACC 90032006), following the procedure described by Slikfo *et al.* (1997).

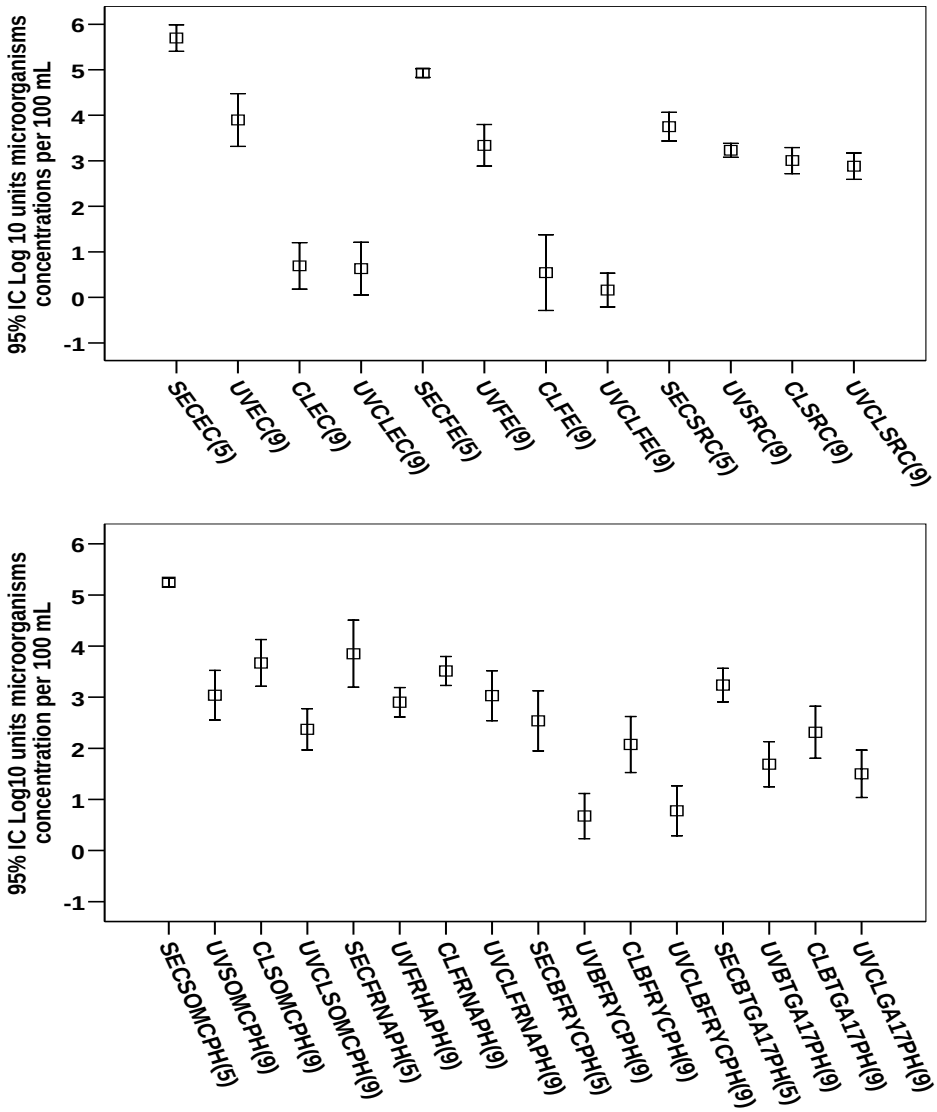
RESULTS AND DISCUSSION

WRP Castell d'Aro experiment

The average inactivation rates for the microorganisms studied in the WRP of Castell d'Aro are shown in Figure 1. Chlorination disinfection (with or without UV light) preferentially inactivates non-spore forming bacteria such as fecal coliforms and enteroccoci, with $\log_{10}$units (log u) inactivation values between 4.4 and 5. Nevertheless, the use of chlorine as the only disinfectant, even at CT values higher than those used in combination with UV light, had practically no effect on the FRNAPH and BFRYCPH bacteriophages (0.34 and 0.46 log u respectively) and was totally ineffective against viable and infectious *Cryptosporidium* oocyst concentrations (0.13, 0.15 and 0.29 log u respectively). In spite of the low transmittances levels in reclaimed water (<50%), UV light treatment had lower inactivation levels for bacterial indicators than when used in combination with chlorination (1.7 log u). However, the inactivation values for all bacteriophages and infectious oocysts were from 2 to 6 times higher for UV light treatment than for chlorination. When a

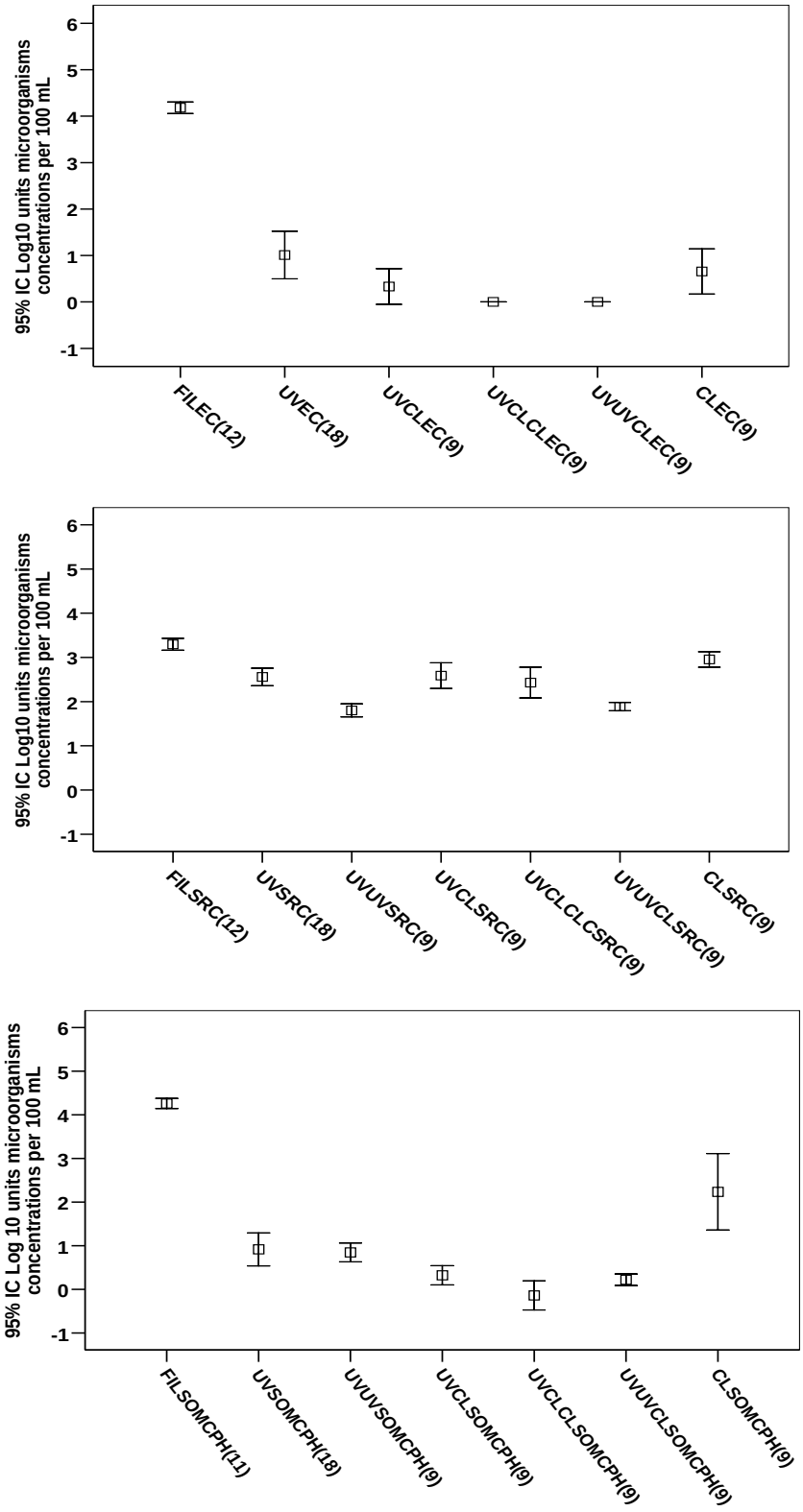
combination of both disinfectant agents was used, the inactivation pattern was similar to that observed for chlorination. Nevertheless, all bacteriophages and infectious *Cryptosporidium* oocysts were reduced at a higher rate. The combination of disinfectants had an added effect on the reduction of somatic coliphages (2.88 log u) and, to a lesser extent, on sulfite-reducing clostridia (0.87 log u) and BTGA17PH (1.74 log u). For the other microorganisms, inactivation by the combination of disinfection processes was equivalent to that observed with the most effective disinfection treatment for each microorganism.

Figure 1. Mean values and 95 % confidence levels of microorganisms concentrations (expressed in logarithmic units) in secondary and disinfected effluents of WRP of Castell d'Aro.



SEC: secondary effluent, UV: UV light disinfection, UVCL: UV light disinfection and chlorination (5 ppm), CL: chlorination (10) ppm, EC: *Escherichia coli*, ufc/100ml; FE: fecal enterococci, ufc/100ml; SRC: sulfite-reducing clostridia spores, ufc/100ml; SOMCPH: Somatic coliphages, ufp/100ml; FRNAPH: RNA F-specific phages ufp/100ml; BFRYCPH: bacteriophages infecting *Bacteroides fragilis* strain RYC 2056 ufp/100ml; BTGA17PH:, bacteriophages infecting *Bacteroides thetaiotaomicron* strain GA-17ufp/100ml.

Figure 2. Mean values and 95% confidence intervals for the concentrations of microorganisms analysed in the different types of water studied in the Blanes WRP.



FIL: filtered water; UV: UV light disinfection with one module; UVUV: UV light disinfection with two modules; UVCL: one UV module and 1 ppm of hypochlorite; UVCLCL: one UV module and 2 ppm of hypochlorite; UVUVCL: two UV modules and 1ppm hypochlorite; CL: residual chlorine 0.6 mg Cl_2/L; EC: *E.coli*, SRC: sulfite reducing clostridia, SOMCPH: somatic coliphages.

WRP Blanes experiment

The physicochemical quality requirements for the water produced by the Blanes reclamation plant are very strict and the tolerance margins are relatively tight, as this water is used for replenishing the aquifer in the lower part of the River Tordera. The concentration of microbes in the conditions prior to disinfection are slightly lower than in a conventional activated sludge WWTP, as the secondary effluent is subjected to an additional filtering process. The geometric means obtained in this study are close to 4 log u in the case of *E. coli*, 3 log u for sulfite-reducing clostridia spores and 4 log u for somatic coliphages.

A 3 log u inactivation of *Escherichia coli* was achieved by all combinations of disinfection agents, resulting in almost undetectable concentrations in the reclaimed water. Chlorine alone was not able to significantly inactivate the spores of sulfite-reducing clostridia (0.35 log u). UV light application resulted in higher removal rates, which were dose-dependant (0.7 – 0.8 log u with one bank and 1.4 – 1.5 log u with two banks). The addition of chlorine after UV light exposure resulted in minor improvements in inactivation efficiency. There was only limited inactivation of somatic coliphages when chlorine was used alone. However, removal efficiencies reached maximum values when UV light was applied in combination with chlorine (≥ 3.9 log u) (Figure 2). *Cryptosporidium spp.* total oocysts were found in all samples tested (all filtered and disinfected samples), but low concentrations of infective oocysts were only found in filtered effluents (1.6/100L) and chlorinated effluents (1.4/100 L). Samples that suffered disinfection process including UV light exposure didn't present infective *Cryptosporidium spp*. oocysts. Regarding enteroviruses, low concentrations were detected in filtered waters (2.3 pfu/100L); nevertheless, they were not found in the 100-litre samples collected from any of the different disinfection processes tested.

From a strictly microbiological perspective, and given the individual log reductions for each of the microorganisms and the overall inactivation values, the most appropriate combination of disinfectant agents is that composed of 2 modules of UV light (approx. dose of 80 mJ/cm^2) and 1 mg Cl_2/l as hypochlorite. This conclusion is based on the total inactivation of *E. coli* and infectious *Cryptosporidium* oocysts; on the greater reduction in the concentrations of sulfite-reducing clostridia spores with respect to other treatments; and on the high reduction in concentrations of somatic coliphages. Additionally, this combination of disinfectant agents can reduce the costs of producing reclaimed water, as high levels of chlorination are not needed and thus the formation of undesired by-products is also prevented.

CONCLUSIONS

The main conclusions of this study are that chlorine is more efficient than small UV light doses at removing non-spore forming bacteria indicators such as *Escherichia coli* and fecal enterococci. However, bacteriophages, enteroviruses and infective *Cryptosporidium* spp. oocysts are more efficiently removed when UV light is used, even at the low doses applied. To ensure broad spectrum disinfection and therefore maximum protection of public health, we propose that disinfection treatment in WRPs should include at least one operating ultraviolet light module and a minimal addition of chlorine. This produces reclaimed water of high enough quality to be reused as non-drinking water for urban areas and as irrigation water for agricultural land and gardens. This operating system not only totally inactivates indicator microorganisms like *E. coli* or somatic coliphages, but also offers maximum protection against infectious *Cryptosporidium* spp oocysts and enteroviruses.

ACKNOWLEDGEMENTS

This study was supported by grants from the Agencia Catalana del Agua (ACA), the Costa Brava Consorcium, REN2002-04035-C03-03 and CTM2005-02513/TECNO from the Ministerio de Ciencia y Tecnología (Spain). A. Costán and M. Montemayor were recipients of fellowships from the Ministerio de Ciencia y Tecnología (BES-2003-0563) and the Generalitat de Cataluña (FI2002-0039).

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