Effect of salinity on medium- and low-pressure UV disinfection of *Vibrio cholerae*

P. Y. Chen, X. N. Chu, L. Liu and J. Y. Hu

ABSTRACT

The problem of biological invasions attributed to ballast water release is an ongoing problem that threatens ecosystems and human health. Ultraviolet (UV) radiation has been increasingly used for ballast water treatment mainly due to the advantages of short contact time and minimized harmful disinfection by products. In this study, the impact of salinity on the inactivation of *Vibrio cholerae* (NCTC 7253) was examined, and comparison of inactivation level and disinfection kinetics after medium-pressure (MP) (1 kW) and low-pressure (LP) (10 W) UV irradiation was made. MP UV exposure resulted in higher inactivation efficacy against *V. cholerae* than LP UV exposure especially at lower UV doses (≤ 3 mJ cm^{-2}) and salinity had a negative impact on both MP and LP UV disinfection, especially at higher UV doses (≥ 3 mJ cm^{-2} for MP and ≥ 4 mJ cm^{-2} for LP). To understand the mechanisms of salinity effect on *V. cholerae*, the enzyme-linked immunosorbent assay (ELISA) was employed to determine the number of cyclobutane pyrimidine dimers (CPDs), one major type of DNA damage. No significant effects of salinity were found at the CPDs level except for 3% artificial seawater after LP UV exposure case. It is imperative that site-specific conditions of salinity be taken into account in the design of UV reactors to treat *V. cholerae* and other species.

Key words | ballast water, medium- and low-pressure UV, salinity, UV disinfection, *Vibrio cholerae*

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INTRODUCTION

Ballast water is water with its suspended matter carried by ships to ensure stability, trim and structural integrity (IMO 2004). Undesirable non-native organisms including epidemic cholera are introduced into ports throughout the world by the release of ballast water, which appears to be the world's largest invasion vector (Ruiz *et al.* 1997). *Vibrio cholerae*, a Gram-negative bacterium and the causative agent of cholera, has caused great concern owing to its toxigenicity and epidemic nature and its ability to adapt and grow in a new environment (Fykse *et al.* 2012). According to Regulation D2 (ballast water performance standard) set by the International Maritime Organization (IMO) in February 2004, ships are to discharge <1 colony forming unit (CFU) per 100 mL ballast water containing toxigenic *V. cholerae*. Only effective treatment of ballast water can bring down the species to innocuous levels. Different technologies exist to treat ballast water in order to reduce the spread of invasive species (Tsolaki & Diamadopoulos 2010), among which ultraviolet (UV) disinfection has been increasingly applied to microbial inactivation in ballast water

(Sutherland *et al.* 2003), mainly due to the advantages of non-toxic by products and low maintenance costs.

Low-pressure (LP) and medium-pressure (MP) mercury lamps are the two UV sources predominantly used in water treatment. LP UV lamps emit monochromatic UV radiation at 254 nm, which is close to the optimum germicidal wavelength of 260 nm (Harm 1980). Compared to LP UV lamps, MP UV lamps emit a wider range of UV wavelengths (from 200 to 400 nm; Masschelein 2002), allowing them to affect biological molecules other than DNA. MP UV lamps have been gaining popularity and are used in a wide range of disinfection applications, mainly due to higher UV radiation intensity and savings in space and capital costs, despite the higher energy consumption and the shorter lifetime of about 4,000 h (Masschelein 2002). Studies on UV inactivation of *V. cholerae* focused mostly on LP UV lamps (Das *et al.* 1981; Wennberg *et al.* 2013), whereas little was known about the efficiency of MP UV disinfection and the comparison of the inactivation characteristics of LP and MP UV lamp.

UV light with the wavelengths from 100 to 400 nm can cause damage to DNA, cell membranes and cytoplasmic proteins (Schwarz 1998). The main types of photoproducts in irradiated DNA are cis-syn cyclobutane pyrimidine dimers (CPDs), pyrimidine (6-4) pyrimidone photoproducts (6-4PPs), spore photoproduct, pyrimidine hydrates and DNA-protein crosslinks (Patrick & Rahn 1976), among which CPDs are the most abundant photoproduct accounting for ~75% of DNA damage (Thoma 1999). CPDs can inactivate microbes through interference with either replication of DNA for the purpose of cellular reproduction or transcription of DNA into RNA for the purpose of making proteins (Harm 1980). Therefore, the relationship between CPDs formation (absorbance at 492 nm) and cell numbers of *V. cholerae* after UV irradiation was evaluated in the present study.

To our knowledge, the effects of different salt concentrations on UV disinfection are not well documented yet, although salinity is one important factor influencing UV performance, as is the case for *Escherichia coli* (Rubio *et al.* 2013) and fecal coliforms (Shang *et al.* 2009). Hence, the aim of the present work was to assess the effects of salinity on UV inactivation of *V. cholerae* as a ballast water treatment indicator microorganism after MP and LP UV disinfection. Both the cellular study and the enzyme-linked immunosorbent assay (ELISA)-based assay were used to investigate the inactivation efficiency.

MATERIALS AND METHODS

Bacteria

V. cholerae NCTC 7253 was purchased from the United Kingdom National Collection of Type Cultures (NCTC). One colony of the *V. cholerae* culture from an agar plate was inoculated into 30 mL of nutrient broth and shaken overnight at 37 °C to prepare an overnight phase. 1 mL of such overnight culture was added to 30 mL of fresh nutrient broth and incubated in a shaker for 4 h at 37 °C to obtain *V. cholerae* at exponential phase. The *V. cholerae* cells were harvested by centrifuging at 4,600 g for 10 min, washed twice with sterile distilled water, and resuspended in 30 mL of sterile distilled water. The suspension was further diluted in sterile distilled water to achieve an initial concentration of approximately 10^6 CFU mL^{-1} for the UV irradiation study.

UV irradiation experiments

UV irradiation was carried out using the Rayox® bench-scale collimated beam apparatus (Model PS1-1-220,

Calgon Carbon Corporation) equipped with an interchangeable MP (1 kW) and LP (10 W) UV lamps. 10 mL of the diluted *V. cholerae* suspension (approximately 10^6 CFU mL^{-1}) was dispensed into a 6 cm diameter sterile plastic Petri dish and exposed to either MP or LP UV radiation. The UV doses investigated ranged from 1 to 5 mJ cm^{-2}, and the average germicidal irradiance values for LP and MP were approximately 0.037 mW cm^{-2} and 0.652 mW cm^{-2}, respectively, calculated taking into account the various factors that can affect the measured UV intensity such as Petri-, water-, sensor- factor, etc., referring to previous studies (Zimmer & Slawson 2002; Guo *et al.* 2010; Zuo *et al.* 2015). Calculations of UV doses were performed as follows: UV dose (mJ cm^{-2}) = irradiance (mW cm^{-2}) × exposure time (s). All bacterial suspensions were stirred throughout the irradiation process. 0.1 mL samples were taken before and after irradiation for enumeration to confirm the expected log reduction.

Salinity experiments

V. cholerae were resuspended in two types of water (artificial seawater (ASW) and natural seawater (NSW)). ASW was prepared as described by Lleò *et al.* (2005), two levels of salinity of which (1% and 3%) were achieved using an Agilent 3200M Multi-Parameter Analyzer (Agilent Technologies Inc., USA) and represent a hyperosmotic environment of natural seawater down to a hypo-osmotic environment of brackish water (Lin *et al.* 2003). Natural seawater was taken from the western coast of Singapore and passed through a 0.45 μm sterile filter (Millipore, Co., USA). Some physicochemical characteristics of these waters are shown in Table 1.

Cultivation assay

From appropriate dilutions of the microorganisms, the total number of *V. cholerae* was examined by spread plate on nutrient agar consisting of nutrient broth 13 g, agar 15 g

Table 1 | Some physicochemical characteristics of the types of water used in the salinity experiments

Suspension solution	Salinity (%)	Conductivity (mS cm^{-1})	TOC (mg L^{-1})	pH	T (°C)
ASW	1	16.5	–	8.09	22.9
	3	44.1	–	7.98	22.9
NSW	3	43.7	3.47	7.88	22.7

ASW, artificial seawater; NSW, natural seawater.

obtained from Sigma-Aldrich Company (Singapore) per litre. Colonies were counted after 24 h incubation at 37 °C and recorded as CFU mL^{-1}.

Enzyme-linked immunosorbent assay

The ELISA assay was used to determine the accumulation of CPDs. Briefly, DNA was extracted from 10 ml of lysed *V. cholerae* cells according to the protocol of DNeasy® Blood & Tissue kit (Qiagen, Germany). DNA concentration was determined with an ND-1000 spectrophotometer (NanoDrop, Fisher Thermo, Wilmington, DE, USA) to measure the absorbance at 260 nm, and the DNA samples were diluted in PBS to a final concentration of 0.2 μg mL^{-1} for ELISA. The CPDs content was measured according to the protocol of a commercial ELISA kit (Clone TDM-2, Cosmo Bio, Tokyo), and qualified with a Sunrise TECAN spectrophotometer (TECAN, Austria GmbH) at 492 nm. Samples were analyzed after UV irradiation at 5 mJ cm^{-2}.

Data analysis

The Log reduction of the test microorganisms was calculated as:

$$Log\ reduction = log\left(\frac{N_i}{N_0}\right) \quad (1)$$

where N_i is the initial concentration of *V. cholerae* before UV disinfection (CFU ml^{-1}), and N_0 is the concentration of *V. cholerae* immediately after UV disinfection (CFU mL^{-1}).

A double first order kinetic model as suggested by Vélez-Colmenares *et al.* (2011) was applied to describe the kinetics of UV disinfection as follows:

$$\frac{N_i}{N_0} = \sigma\exp(-k_1 t) + (1-\sigma)\exp(-k_2 t) \quad (2)$$

where σ is fraction of microorganisms sensitive to UV radiation, $1-\sigma$ is fraction of microorganisms resistant to UV radiation, k_1 is the inactivation rate for sensitive fraction of microorganisms associated with the slope of the first phase of the curves and with the process of inactivation, k_2 is the inactivation rate for resistant fraction of microorganisms related to the slope of the second phase of the curves (tailing), and t is exposure time to UV light (s).

All experiments were conducted three times to ensure reproducibility of the experimental data. Data are presented

in mean ± standard deviation. A t-test by SPSS 16.0 was used to identify differences in Log reduction and absorbance at 492 nm at the significance level of 0.05.

RESULTS AND DISCUSSION

UV inactivation of *V. cholerae*

Figure 1 shows the inactivation of *V. cholerae* following MP and LP UV disinfection in sterile distilled water (the controls). It can be seen that the UV doses between 1 and 5 mJ cm^{-2} resulted in 0.42 to 4.01 (for MP) and 0.52 to 4.16 (for LP) log reduction in distilled water (the controls). Such inactivation behavior was also reported by Das *et al.* (1981) who claimed that the survival curves for all three *V. cholerae* strains (569B, NIH 41 and 154) exhibited no tailing with the k-values ranging from 0.18–0.24 m^2 J^{-1}. It was also found that when the UV dose was lower than 4 mJ cm^{-2}, higher log reduction values were achieved when MP UV radiation was employed, indicating that MP UV disinfection was more efficient than LP UV disinfection. This has been reported previously on *E. coli* (Hu *et al.* 2005), and is likely due to broader wavelength spectrum emitted by MP UV lamps that may cause damage to intercellular biomolecules other than DNA (Kalisvaart 2004). At higher doses ($\geq$4 mJ cm^{-2}), reduction in cell numbers were similar for LP and MP UV exposure, indicating that both types of UV lamps had similar effects on the inactivation of *V. cholerae*. Likely, Guo *et al.* (2009) also found that when the UV dose was 5–10 mJ cm^{-2}, LP UV exposure resulted in higher
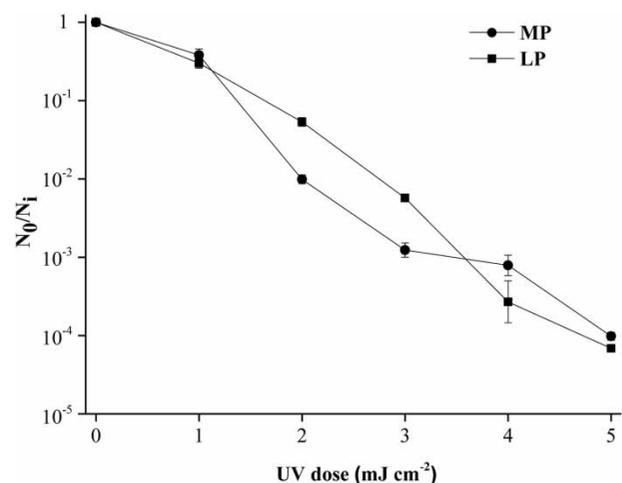
Figure 1 │ UV inactivation of *V. cholerae* by medium-pressure (MP) and low-pressure (LP) UV disinfection with UV dose ranging from 1 to 5 mJ cm^{-2} in sterilized distilled water. Error bars represent standard deviations of three experiments.

Table 2 │ Kinetic parameters of the double first order kinetic model applied to salinity experiments for *V. cholerae*

UV lamp	Suspension solution	Salinity (%)	k_1 (s^{-1})	k_2 (s^{-1})	σ	r^2
LP	DI water	–	1.88	1.88	1.00	0.999
LP	ASW	1	0.0707	0.00342	0.995	0.974
		3	0.0813	4.50E-16	0.996	0.971
LP	NSW	3	0.0793	0.001	0.995	0.99
MP	DI water	–	1.91	1.91	1.00	0.983
MP	ASW	1	1.21	0.100	0.993	0.908
		3	1.61	0.253	0.993	0.995
MP	NSW	3	1.33	0.573	0.900	0.988

DI water, distilled water; LP, low-pressure; MP, medium-pressure; ASW, artificial seawater; NSW, natural seawater.

inactivation level (3 log) against *E. coli* than MP UV exposure, whereas there was no difference in inactivation between LP and MP lamps at UV doses >10 mJ cm^{-2} (3–5 log). Additionally, k_1 was 1.91 (0.188) and 1.88 (0.210) for MP and LP lamps, respectively (Table 2), which showed that inactivation of MP UV disinfection was faster than LP UV disinfection.

Previous studies have shown the sensitivity of *E. coli* to range from 10 to 15 mJ cm^{-2} (MP) (Quek *et al.* 2006) and 5 to 11 mJ cm^{-2} (LP) (Butler *et al.* 1987) for a 4 Log10 inactivation, while for *V. cholerae*, a UV dose of 5 mJ cm^{-2} was needed to result in the same level of reduction, revealing that *V. cholerae* is more sensitive to UV light than *E. coli*, which was in good agreement with previous studies (Hijnen *et al.* 2006; Coohill & Sagripanti 2008). The relative sensitivity of *V. cholerae* was 0.5 to 1.0 for *E. coli* (Coohill & Sagripanti 2008). The k-values varied from 0.506–0.642 cm^2 mJ^{-1} for *E. coli* to 1.341 cm^2 mJ^{-1} for *V. cholerae* (Hijnen *et al.* 2006), demonstrating *V. cholerae* was relatively higher UV sensitive.

Effect of salinity on UV inactivation

The effect of salinity on log removal of *V. cholerae* by two types of UV lamps is shown in Figure 2. It was observed that at 1% or higher, salinity had a negative impact on both MP and LP UV disinfection especially at higher UV doses (≥3 mJ cm^{-2} for MP and ≥4 mJ cm^{-2} for LP). After MP or LP exposure, UV inactivation was significantly suppressed in artificial seawater (ASW) or natural seawater than in sterile distilled water with a lower log reduction and inactivation rate (k_1) shown in Figure 2 and Table 2.

The effect of salinity on UV disinfection is currently under debate. Rubio *et al.* (2013) evaluated the disinfection efficiency of *E. coli* by UV radiation and found that the
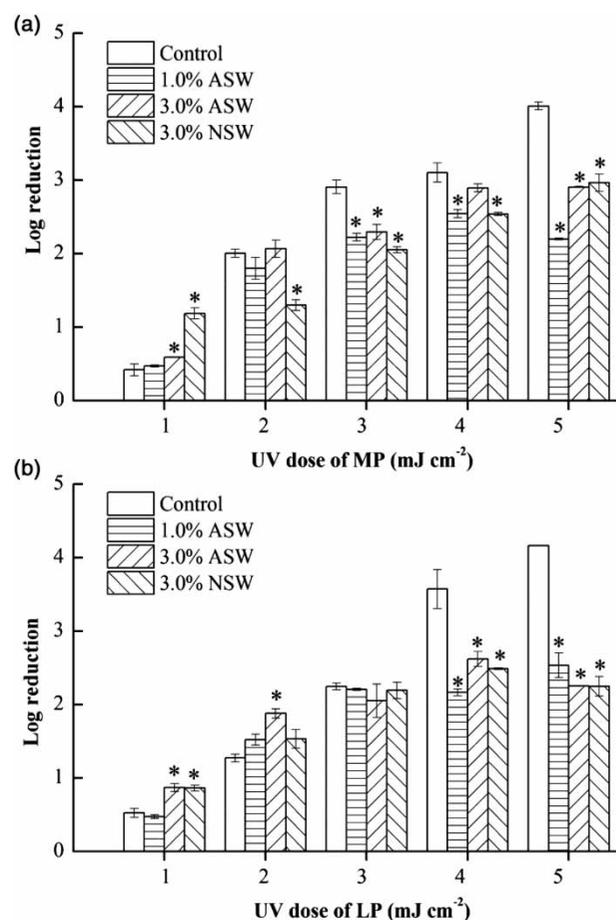

Figure 2 │ The effect of salinity on UV inactivation of *V. cholerae* by (a) medium-pressure (MP) and (b) low-pressure (LP) UV disinfection. Error bars represent standard deviations of three experiments. ASW, artificial seawater; NSW, natural seawater. The * denote values that are significantly different (*P < 0.05) from the control value.

UV inactivation rate decreased when increasing the salt concentration, whereas Shang *et al.* (2009) observed that higher salinity resulted in higher level of inactivation of fecal coliform bacteria at a UV-C dose of 12 mJ cm^{-2}. The

formation of radicals that interact with bacteria such as dissolved molecular oxygen (Legrini *et al.* 1993; Buschmann *et al.* 2005) may contribute to the overall effect of salinity on UV inactivation. Another reason is clustering or adsorption of the micro-organisms to surfaces due to declined repulsive forces at high ionic strength values. Results indicated that salinity had a detrimental effect on inactivation of *V. cholera*e at high UV doses, probably due to the reason that the negative effect on clustering and other interface processes (adsorption) is probably higher. Therefore, at high salinity, UV disinfection performance can be improved by increasing UV doses in actual operation.

DNA damage using ELISA assay

As shown in Figure 3, after LP UV exposure, in 3% ASW significantly less CPDs were formed, which was in accordance
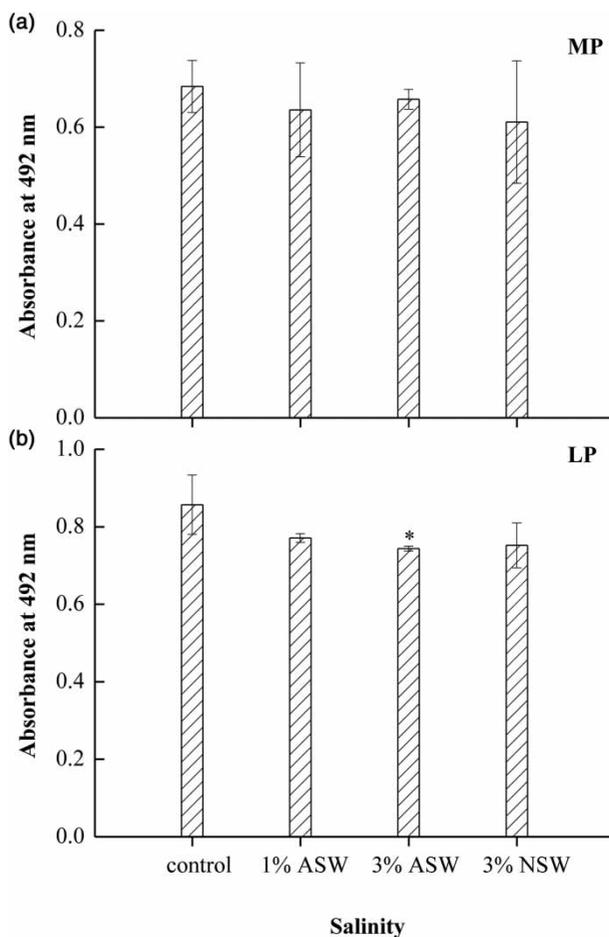

Figure 3 │ Effect of salinity on formation of cyclobutane pyrimidine dimers (CPDs) after (a) medium-pressure (MP) and (b) low-pressure (LP) exposure at 5 mJ cm^{-2}. Absorbance at 492 nm was used as a measure of induced CPDs. Error bars represent standard deviations of three experiments. ASW, artificial seawater; NSW, natural seawater.

with the decreasing net log reduction in cell numbers. In other cases, salinity of 1% or 3% led to a trend of decreased CPDs content, but the decreases were not statistically significant. The cell number reduction may be associated with DNA damage, as is the case for *E. coli* (Eischeid & Linden 2007), *M. terrae* (Bohrerova & Linden 2006) and *A. variabilis* (Sakai *et al.* 2007). Further support is provided by the study of Cairns & MacDougall (1995) showing that the presence of the CPDs could prevent the accurate reading of the genetic code in the microorganisms for important cellular processes such as protein synthesis during growth or nucleic acid replication during cell division, and such mutations ultimately lead to cell death. However, for *V. cholerae*, no significant effects of salinity were found at the CPDs level, which did not agree with those observed in the cellular study. Further studies should be conducted on the quantification of UV-induced pyrimidine dimers in genomic DNA to provide fundamental insight into the relationship between formation of CPDs and log inactivation.

CONCLUSION

In summary, *V. cholerae* was inactivated by either MP or LP UV irradiation, and the use of the MP lamp leads to a higher disinfection efficiency than LP. In general, increased salinity levels hindered MP or LP UV disinfection when UV doses were more than 3 or 4 mJ cm^{-2}, respectively. DNA damage seems not likely to contribute to cell number reduction as to *V. cholerae*. Further research on salinity effects on UV inactivation at high doses (60–120 mJ cm^{-2}; Oemcke *et al.* 2004) is needed to better simulate a ballast water treatment under realistic conditions by increasing the initial microorganism concentration.

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REFERENCES

Bohrerova, Z. & Linden, K. G. 2006 Assessment of DNA damage and repair in *Mycobacterium terrae* after exposure to UV irradiation. *Journal of Applied Microbiology* **101** (5), 995–1001.

Buschmann, J., Canonica, S., Lindauer, U., Hug, S. J. & Sigg, L. 2005 Photoirradiation of dissolved humic acid induces arsenic (III) oxidation. *Environmental Science and Technology* **39** (24), 9541–9546.

Butler, R. C., Lund, V. & Carlson, D. A. 1987 Susceptibility of *Campylobacter jejuni* and *Yersinia enterocolitica* to UV radiation. *Applied and Environmental Microbiology* **53** (2), 375–378.

Cairns, W. & MacDougall, A. 1995 Advances in UV disinfection technology for treatment of low quality wastewater. In: *Proceedings of the Australian Water Works Association 16th Federal Convention*, pp. 631–637.

Coohill, T. P. & Sagripanti, J.-L. 2008 Overview of the inactivation by 254 nm ultraviolet radiation of bacteria with particular relevance to biodefense. *Photochemistry and Photobiology* **84** (5), 1084–1090.

Das, G., Sil, K. & Das, J. 1981 Repair of ultraviolet-light-induced DNA damage in *Vibrio cholerae*. *Biochimica et Biophysica Acta* **655** (3), 413–420.

Eischeid, A. C. & Linden, K. G. 2007 Efficiency of pyrimidine dimer formation in *Escherichia coli* across UV wavelengths. *Journal of Applied Microbiology* **103** (5), 1650–1656.

Fykse, E. M., Nilsen, T., Nielsen, A. D., Tryland, I., Delacroix, S. & Blatny, J. M. 2012 Real-time PCR and NASBA for rapid and sensitive detection of *Vibrio cholerae* in ballast water. *Marine Pollution Bulletin* **64** (2), 200–206.

Guo, M. T., Hu, H. Y., Bolton, J. R. & El-Din, M. G. 2009 Comparison of low- and medium-pressure ultraviolet lamps: photoreactivation of *Escherichia coli* and total coliforms in secondary effluents of municipal wastewater treatment plants. *Water Research* **43** (3), 815–821.

Guo, H. L., Chu, X. N. & Hu, J. Y. 2010 Effect of host cells on low- and medium-pressure UV inactivation of adenoviruses. *Applied and Environmental Microbiology* **76**, 7068–7075.

Harm, W. 1980 *Biological Effects of Ultraviolet Radiation*. Cambridge University Press, New York, NY, USA.

Hijnen, W., Beerendonk, E. & Medema, G. J. 2006 Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Research* **40** (1), 3–22.

Hu, J. Y., Chu, X. N., Quek, P. H., Feng, Y. Y. & Tan, X. L. 2005 Repair and regrowth of *Escherichia coli* after low- and medium-pressure ultraviolet disinfection. *Water Science and Technology: Water Supply* **5** (5), 101–108.

International Maritime Organization 2004 *International Convention for the Control and Management of Ship's Ballast Water and Sediments*. http://www.imo.org/Conventions/mainframe.asp?topic_id=867 (accessed 27 March 2016).

Kalisvaart, B. F. 2004 Re-use of wastewater: preventing the recovery of pathogens by using medium-pressure UV lamp technology. *Water Science and Technology* **50** (6), 337–344.

Legrini, O., Oliveros, E. & Braun, A. M. 1993 Photochemical processes for water treatment. *Chemical Reviews* **93** (2), 671–698.

Lin, Y. M., Chen, C. N. & Lee, T. H. 2003 The expression of gill Na, K-ATPase in milkfish, *Chanos chanos*, acclimated to seawater, brackish water and fresh water. *Comparative Biochemistry and Physiology – Part A: Molecular & Integrative Physiology* **135** (3), 489–497.

Lleò, M. D., Bonato, B., Benedetti, D. & Canepari, P. 2005 Survival of enterococcal species in aquatic environments. *FEMS Microbiology Ecology* **54** (2), 189–196.

Masschelein, W. J. 2002 Available lamp (or burner) technologies. In: *Ultraviolet Light in Water and Wastewater Sanitation* (R. G. Rice, ed.). Lewis Publishers, Boca Raton, FL, USA.

Oemcke, D., Parker, N. & Mountfort, D. 2004 Effect of UV irradiation on viability of micro scale and resistant forms of marine organisms: implications for the treatment of ships' ballast water. *Journal of Marine Environmental Engineering* **7** (3), 153–172.

Patrick, M. & Rahn, R. 1976 Photochemistry and photobiology of nucleic acids: photoproducts. In: *Photochemistry and Photobiology of Nucleic Acids,* Vol. II (S. Y. Wang, ed.). Academic Press, New York, NY, USA, pp. 35–95.

Quek, P. H., Hu, J. Y., Chu, X. N., Feng, Y. Y. & Tan, X. L. 2006 Photoreactivation of *Escherichia coli* following medium-pressure ultraviolet disinfection and its control using chloramination. *Water Science and Technology* **53** (6), 123–129.

Rubio, D., Nebot, E., Casanueva, J. F. & Pulgarin, C. 2013 Comparative effect of simulated solar light, UV, UV/H_2O_2 and photo-Fenton treatment (UV-Vis/H_2O_2/$Fe^{2+,3+}$) in the *Escherichia coli* inactivation in artificial seawater. *Water Research* **47** (16), 6367–6379.

Ruiz, G. M., Carlton, J. T., Grosholz, E. D. & Hines, A. H. 1997 Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. *American Zoologist* **37** (6), 621–632.

Sakai, H., Oguma, K., Katayama, H. & Ohgaki, S. 2007 Effects of low- or medium-pressure ultraviolet lamp irradiation on *Microcystis aeruginosa* and *Anabaena variabilis*. *Water Research* **41** (1), 11–18.

Schwarz, T. 1998 UV light affects cell membrane and cytoplasmic targets. *Journal of Photochemistry and Photobiology B-Biology* **44** (2), 91–96.

Shang, C., Cheung, L. M., Ho, C.-M. & Zeng, M. 2009 Repression of photoreactivation and dark repair of coliform bacteria by TiO_2-modified UV-C disinfection. *Applied Catalysis B-Environmental* **89** (3), 536–542.

Sutherland, T. F., Levings, C. D., Petersen, S. & Hesse, W. W. 2003 Mortality of zooplankton and invertebrate larvae exposed to cyclonic pre-treatment and ultraviolet radiation. *Marine Technology Society Journal* **37** (2), 3–12.

Thoma, F. 1999 Light and dark in chromatin repair: repair of UV-induced DNA lesions by photolyase and nucleotide excision repair. *EMBO Journal* **18** (23), 6585–6598.

Tsolaki, E. & Diamadopoulos, E. 2010 Technologies for ballast water treatment: a review. *Journal of Chemical Technology and Biotechnology* **85** (1), 19–32.

Vélez-Colmenares, J. J., Acevedo, A. & Nebot, E. 2011 Effect of recirculation and initial concentration of microorganisms on the disinfection kinetics of *Escherichia coli*. *Desalination* **280** (1), 20–26.

Wennberg, A. C., Tryland, I., Østensvik, Ø., Secic, I., Monshaugen, M. & Liltved, H. 2013 Effect of water treatment on the growth potential of *Vibrio cholerae* and *Vibrio parahaemolyticus* in seawater. *Marine Environmental Research* **83**, 10–15.

Zimmer, J. L. & Slawson, R. M. 2002 Potential repair of *Escherichia coli* DNA following exposure to UV radiation from both medium- and low-pressure UV sources used in drinking water treatment. *Applied and Environmental Microbiology* **68** (7), 3293–3299.

Zuo, X. J., Chu, X N. & Hu, J. Y. 2015 Effects of water matrix on virus inactivation using common virucidal techniques for condensate urine disinfection. *Chemosphere* **136**, 118–124.