INACTIVATION AND REPAIR OF ENTEROCOCCUS FAECALIS AND VIBRIO CHOLERAE BY UV FOR BALLAST WATER TREATMENT

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DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety, under the supervision of Associate Professor Hu Jiangyong, National University of Singapore, between 12/08/2013 and 12/08/2014.

I have duly acknowledged all the sources of information which have been used in the thesis.

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SUMMARY

Invasive aquatic species discharged through ballast water carried by ships to ensure stability is one of the serious problems posed nowadays in the marine environment. UV disinfection has been increasingly applied to microbial inactivation in ballast water mainly due to the advantages of non-toxic by-products and low maintenance costs. In this study, *Enterococcus faecalis* (DSM 20478) and *Vibrio cholerae* (NCTC 7253) were selected as indicators to investigate the UV susceptibility and repair potential. Results indicated that *V. cholerae* is more sensitive to UV inactivation than *E. faecalis*. For repair, LP UV resulted in a greater level of light repair than MP and the light repair results were all higher than dark repair. The tested three water quality parameters including salinity, turbidity and temperature were found to decrease log removal of UV. Effects of salinity and turbidity on repair were related with different UV sources, but the temperature usually inhabits the repair.

Keywords: ballast water, *Enterococcus faecalis*, *Vibrio cholerae*, UV disinfection, photoreactivation and dark repair

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List of Nomenclature

Abbreviation	Full description
UV	Ultraviolet (irradiation)
E. faecalis	*Enterococcus faecalis*
V. cholerae	*Vibrio cholerae*
CFU	Colony forming unit (s)
LP	Low pressure
MP	Medium pressure
TSB	Tryptone soya broth
CPDs	Cyclobutane pyrimidine dimmers
6-4PP(s)	Pyrimidine (6-4) pyrimidone photoproducts
IMO	International Maritime Organization
L	Light
D	Dark

CHAPTER 1 INTRODUCTION

1.1 Overview of Ballast Water

Ballast water is water with its suspended matter carried by ships to ensure stability, trim and structural integrity (IMO, 2004). When a ship is empty of cargo, it fills its tanks with ballast water to ensure safe navigation. The spread of ballast water is aggravated with the rapid development of marine transportation industry. Some species carried in ballast water may survive the voyage and thrive in their new environment, which may have negative ecological, economic and public health impacts on the receiving environment (Tsolaki and Diamadopoulos, 2010). The introduction of invasive marine species into new environments by ships' ballast water has been identified by the Global Environment Facility (GEF) as one of the four greatest threats to the world's oceans.

1.1.1 Hazards of ballast water: the risk of bioinvasion

Undesirable non-native organisms 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). The spread of ballast water is shown in Fig. 1-1. These invasive species often have the following characteristics: (1) relatively small body size with a planktonic lifestyle; (2) dominant species in the former habitat; (3) high adaptability to the novel environment; (4) biological and ecological characteristics of species such as the reduction of the

metabolic rate and the formation of dormant spores to resist stress (Hallegraeff and Bolch, 1991). They can cause changes in biodiversity, reconstructing of the food web, and directly impact the society and human health by affecting the fisheries and causing health hazards (Anil et al., 2002). It is known that the introduction and spread of alien invasive species has been a serious problem and can cause considerable economic losses to society (Ruiz et al., 2001).

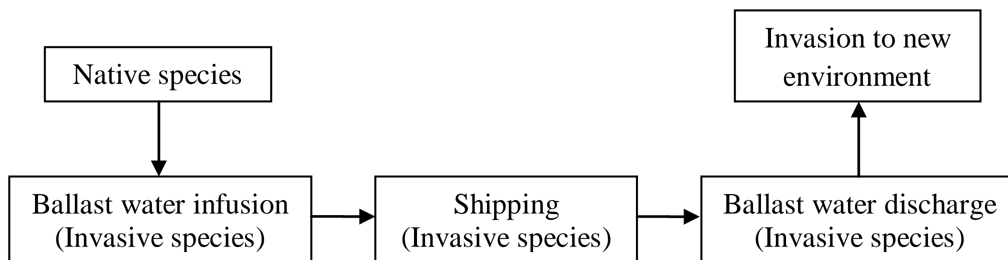

Fig. 1-1 The spread of ballast water

1.1.2 Relevant regulations on ballast water

With the aim of regulating discharges of ballast water and reducing the risk of introducing non-native species from ships' ballast water, Regulation D1 (the ballast water exchange standard) and Regulation D2 (ballast water performance standard) were set by the International Maritime Organization (IMO) in February 2004. The principles of ballast water exchange lie in that the freshwater organisms are unlikely to survive in the open ocean with high salinity level of the water (3% or greater), and fewer organisms (including fewer human pathogens) will be taken up in the open ocean (Cohen et al., 2012). The two most common approaches of ballast water exchange are flow-through exchange and sequential exchange. Ballast water carried by the ship is required by Regulation D1 to be exchanged three times during its voyage in

order to achieve 95% or better volumetric exchange. At present, Regulation D1 has been the main recourse for ships in operation. However, due to an operational inconvenience having both time and financial impact and ineffectively removing salt-tolerant species, Regulation D1 is recognized as an interim measure enforced to minimize the transport of invasive species and more emphasis are placed on Regulation D2 which sets the standard that ballast water treatment systems must meet (Table 1-1). Numeric ballast water discharge standards are established including the limit for viable organisms and human pathogens (including *E. coli*, intestinal *enterococci*, and *V. cholerae*). As shown in Table 1-1, only effective treatment of ballast water can bring down the species to innocuous levels.

Table 1-1 Regulation discharge organisms according to Regulation D-2 Ballast Water Performance Standard

Organism	Regulation of discharge ballast water
Phytoplankton/zooplankton ≥ 50 micrometers	Less than 10 organisms per cubic meter
Phytoplankton/zooplankton < 50 - ≥ 10 micrometers	Less than 10 organisms per milliliter
Toxicogenic *Vibrio cholerae* (O1 and O139)	Less than 1 CFU∗ per 100 mL
Escherichia coli	Less than 250 CFU∗ per 100 mL
Intestinal enterococci	Less than 100 CFU∗ per 100 mL

∗CFU=colony forming unit

1.2 Treatment Technologies of Ballast Water

Many treatment technologies have been emerging, which mainly include mechanical, physical and chemical methods.

1.2.1 Mechanical methods

Filtration

Filtration is frequently used for the pretreatment of ballast water, which is effective to remove large particles and organisms, and footprints are as low as 3.5 m^2 to 18 m^2 for combination systems such as filtration-UV (Lloyd's Report, 2010). The filter equipment is usually simple, small, and easy-operational. However, it would cost much time and energy to wash the clogged filters. Kong et al. (2007) found that micro-hole ceramic filters combined with UV radiation showed high efficiency on removing chlorella (>93%) and bacteria (>87%). Parsons and Harkins (2000) installed a modular system and conducted extended testing with 25, 50 and 100 µm filter screens. The removal efficiency of phytoplankton and zooplankton was from 30% to 90%.

Cyclone or Hydrocyclone

Suspended micro-organisms can be separated from water by centrifugal forces generated from high-speed water flow in ballast water. Compared with screen

filters, hydrocyclones require less pump pressure and allow separation of sediments and other suspended solids to approximately 20 μm. However, some organisms with a similar density to water are always discharged. Therefore, hydrocyclone is also employed a pre-treatment followed by a form of disinfection. Sutherland et al. (2001) investigated the effect of an integrated hydrocyclone plus UV-C ballast water treatment system on the mortality and the results showed that clam, mussel, and oyster larvae exhibited statistically similar mortality thresholds ranging between 96% and 99% at the higher UV doses. Waite et al. (2003) evaluated the effects of the individual primary treatment processes (the 50 μm screen and the hydrocyclone), as well as the overall treatment system (Screen plus UV and hydrocyclone plus UV), and found that a significant reduction in the number of all zooplankton was observed not for the hydrocyclone but for the screen, and the UV treatment unit appeared to be capable of significantly reducing bacterial populations in all cases.

1.2.2 Physical methods

1.2.2.1 Heat-thermal treatment

Harmful organisms can be killed by increasing the temperature of ballast water to cause damage to their original living environment. Heating to a temperature of 35 °C for 20 hours or longer indicated an effective heat treatment (Quilez-Badia et al., 2008). Ballast water can be heated from machinery systems, steam heat rejections and exhaust gases (Balaji and

Yaakob, 2012). Heat treatment is constrained by the heat availability and time for the treatment, which depend on distance of voyage, volume of ballast water and outside temperature. Quilez-Badia et al. (2008) studied the effectiveness of a high-temperature thermal treatment system operating over a short time at 55-80 °C and found that the heat treatment reduced the viable count of zooplankton, phytoplankton and bacteria by 95%, 63-90% and 95% respectively. Novel techniques similar to heat treatment are microwaves (Boldor et al., 2008), ultrasound (Holm et al., 2008) and electric pulse (Hwang et al., 2010).

1.2.2.2 UV radiation

UV disinfection has been increasingly applied to microbial inactivation in ballast water mainly due to the advantages of non-toxic by-products and low maintenance costs. It is noted that UV light with the wavelengths from 100 to 400 nm can cause damage to DNA, cell membranes and cytoplasmic proteins (Schwarz, 1998). The effectiveness of UV treatment relies on the size and the morphology of organisms. UV radiation is frequently used for the disinfection of ballast water (Sutherland et al., 2003; Wright et al., 2007) as well as wastewater (Lindenauer and Darby, 1994), drinking water (Wolfe, 1990) and reclaimed water (Tang et al., 2006). There are several challenges when UV light is used to treat ballast water, considering that the effective UV dosage can be reduced by high flow rates of water and the presence of dissolved organic matter or suspended particulates (First and Drake, 2013).

1.2.3 Chemical methods

1.2.3.1 Chlorination

Chlorination is commonly used in water treatment by use of strong oxidizing power of chlorine. Exposure to chlorine has been demonstrated to have adverse effects on cell functions with the involvement of the cell membrane and perhaps DNA as targets for chlorine damage (Haas and Engelbrecht, 1980). Due to easy operations and low expenses, chlorination is feasible to be used on board without special apparatus to treat ballast water. Zhang et al. (2003) added sodium hypochlorite to treat ballast water, and found that almost all the bacteria (anaerobic bacteria, *vibrio* and *E.coli*) in the seawater can be killed by 20 mg/L available chlorine. The efficiency of chlorination is dependent on nitrification (Lazarova et al., 1999), pH (Armstrong, 1997) and temperature, residual chlorine and reaction time (Tsolaki and Diamadopoulos, 2009). The main disadvantages of chlorination disinfection are the harmful health effects of chlorinated DBPs (e.g. trihalomethanes) and the inability of chlorine to disinfect certain emerging pathogens.

1.2.3.2 Chlorine dioxide (ClO_2)

Chlorine dioxide which readily dissolves in water is commonly used as a disinfectant in drinking water and in various industrial applications. The main advantages of ClO_2 disinfection can be summarized as follows: (1) a wide range of organisms can be well inactivated (Huang et al., 1997); (2) ClO_2 does

not form deleterious halogenated byproducts, as chlorine does; (3) the disinfection efficiency of chlorine dioxide is independent of pH and the presence of ammonia unlike chlorine. Maranda et al. (2013) determine the efficacy of ClO_2 in treating ballast water under real operating conditions, and found that 5 mg/L ClO_2 added to ballast water immediately reduced bacteria, protists, and zooplankton to below detectable limits, but the effect did not persist past a few days for certain organisms. The main concerns with chlorine dioxide are cost and the possible environmental toxicity of by-products.

1.2.3.3 Hydrogen Peroxide (H_2O_2)

Reactive and destructive hydroxyl radicals are liberated by neutral H_2O_2 which can pass through cell membrane easily by diffusion (Lynch and Fridovich, 1978), exhibiting significant oxidative properties to decompose organic matter and effectively kill plankton and bacteria in the ballast water. Despite the fact that H_2O_2 can be produced electrochemically and degradation products of residual H_2O_2 (water and oxygen) after treatment are environmentally friendly, relatively high concentrations of H_2O_2 and long treatment durations are needed to eliminate the vast majority of species (Smit et al., 2008).

1.2.3.4 Ozone

Effective elimination of many kinds of pathogens of concern (bacteria, phytoplankton, zooplankton and viruses) by ozonation has been demonstrated in previous studies (Perrins et al., 2006; Tyrrell et al., 1995). However,

disadvantages of ozonation include lack of a disinfectant residual, biological regrowth problems in distribution systems, high cost, and limited information on the nature and toxicity of its by-products. Oemcke and van Leeuwen (2004) found that ozone did not seem to be a good choice for the control of spore-forming organisms in ballast water and the presence of corroding iron surfaces results in a decrease in ozone residual and subsequently in disinfection.

1.2.3.5 Electrolysis

Considering that seawater contains huge amounts of chloride ions (Cl^-), seawater electrolysis is also efficient in generating a strong disinfectant, chlorine ($HOCl/OCl^-$), and has been shown high inactivation efficiency of the target organisms (Dang et al., 2003; Matousek et al., 2006). The main merit of electrolysis process is effective cost since oxidants can be generated onboard the ship without the need to carry chemicals. However, concern for the corrosion of ballast tanks by generated oxidants should be taken.

1.2.4 Combined methods

Because no one method has yet been proven to remove all organisms from ballast water, more research must be conducted into determining the effectiveness of combining ballast water treatment methods which is presented in Table 1-2.

Table 1-2 A summary of combined treatment techniques

Disinfection methods	Target marine organisms	Operational parameters	The inactivation efficiency	References
Ozone+electrolysis	Bacillus subtilis spores	The rate of inlet ozone dose (1 mg/(L·min)), current density (55.5 mA/cm^2)	No lag and no tailing, an extended inactivation with an increase of the CT (C: residual disinfection concentration; T: contact time)	Jung et al., 2013
US+UV, UV+ H_2O_2	Zooplankton	US (50 and 100% amplitudes)+UV (520 L/h (216 mJ/cm^3));	The treatment efficiency: US +UV>US or UV alone, UV+H_2O_2>UV alone	Viitasalo et al., 2005
Sonication+ H_2O_2, Sonication+Ozone, H_2O_2+Ozone, Sonication+H_2O_2+Ozone	Dunaliella tertiolecta and Artemia salina	exposure time (1, 2, 3, 5, 10, 15 and 20 min),Ultrasonic frequency (1.4 kHz), H_2O_2 and Ozone (100 ppm each)UV (800 L/h (141 mJ/cm^3))+ H_2O_2 (15 and 30 mg/L, 48 h incubation)	The highest mortality levels were achieved with a combination of all three treatments	Gavand et al., 2007
Filtration+UV, Cyclone+UV	Zooplankton, phytoplankton, bacteria and the MS-2 Coliphage	Filtration (40 μm)+UV (304 m^3/h), Cyclone+UV (200 m^3/h)	UV significantly increased the effects of filtration or cyclone alone against zooplankton, and added the UV-alone effects on phytoplankton and bacteria.	Cangelosi et al., 2001

Many treatment processes require some types of primary treatment to remove larger organisms and particulate matter from the water, and thus improve the efficiency of the secondary treatment, as clearly indicated in the literature studies. In most cases a combined process is more efficient than separate treatment and can overcome the limitation of the individual process. It seems that combining technologies in an economical way would be an approach to optimize the ballast management.

1.3 UV Disinfection

1.3.1 UV disinfection mechanism

UV inactivation is thought to occur primarily due to its absorption by nucleic acids and to a lesser extent by proteins and other biologically important molecules. 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 and Rahn, 1976), among which the first two are major types of DNA lesions (Thoma, 1999).

According to the wavelength, the spectrum is further divided into four sub-regions: UV-A (315-400 nm), UV-B (280-315 nm), UV-C (200-280 nm) and Vacuum UV (100-200 nm). UV-A not only has lethal and sublethal effects mainly by exciting photosensitive molecules inside the cell to produce active species (e.g. H_2O_2 and *OH) which damage the genome and other intracellular

molecules, but is essential for photoreactivation (Jagger, 1981). Genomes, proteins and enzymes with unsaturated bonds are known to absorb UV-C and UV-B, which may also result in significant damage to the organisms (Kalisvaart, 2001).

1.3.2 UV radiation sources and UV disinfection systems

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 hours (Masschelein, 2002). The new generation of improved polychromatic MP lamps, a third generation of lamps, was developed by Berson UV-techniek of the Netherlands which combines the high UV efficiency of LP lamps with the multiple germicidal effects of the wide-band output from MP lamps (Kalisvaart, 2001). Apart from mercury lamps, the pulsed UV lamp has previously been shown to be effective against a range of bacteria (Rowan et al. 1999) as well as viruses (Lamont et al., 2007). Typically pulsed UV-light sources generate a broad wavelength spectrum ranging from 100 to 1100 nm consisting of ultraviolet, visible, and infrared

radiation (Krishnamurthy et al., 2007). Further studies need to be conducted to verify the applicability of pulsed UV-light treatment on an industrial scale.

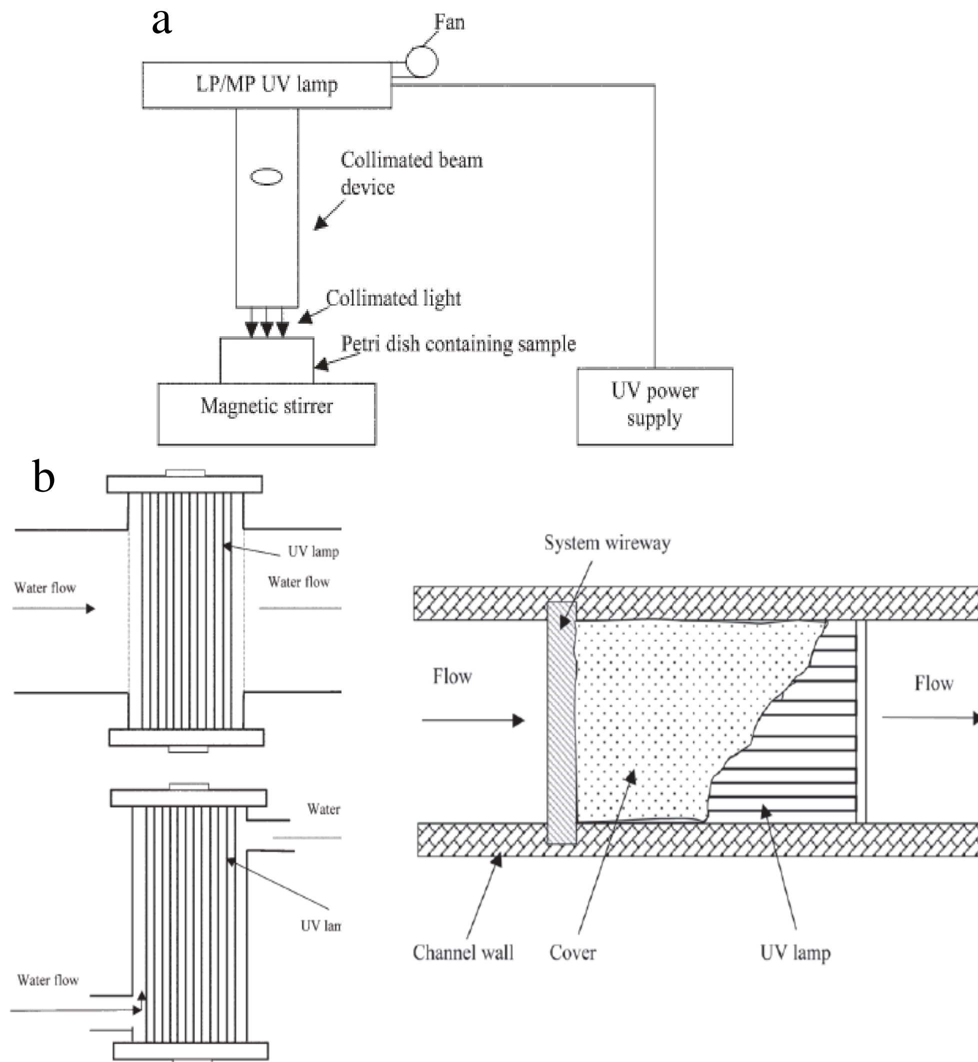

Fig. 1-2 (a) Schematic diagram of a typical bench-scale setup with the collimated beam UV system; (b) Schematic diagram of a flow-through UV system (closed+open) (adapted from Chen et al., 2006)

Generally there are two types of disinfection systems, that is, the collimated beam system for batch disinfection, and the flow-through system in practical applications. A typical setup of the collimated beam apparatus and a flow-through UV system is presented in Fig. 1-2 (Chen et al., 2006). A standardized

bench scale UV testing protocol (Bolton and Linden 2003) is used, in which a Petri dish is used as a completely mixed batch reactor to hold the microorganism suspension in the collimated UV radiation field. The determination of the necessary delivered UV dose for full-scale UV systems is commonly based on laboratory dose-response data from collimated beam tests (Kuo et al., 2003). Both closed and open UV systems can be used as shown in Fig. 1-2b. Closed channel UV system is often used in drinking water disinfection, while open channel UV system is always adopted for wastewater disinfection.

1.3.3 Factors affecting UV disinfection

1.3.3.1 Salinity

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 UV inactivation rate decreased when increasing the solution salt concentration, whereas Shang et al. (2009) observed higher salinity resulted in higher level of inactivation of fecal coliform bacteria by UV both in the presence and in absence of TiO_2. It is known that organic matter and inorganic ions exposed to UV light can not only absorb UV light (Wright and Cairns, 1998) but also form radicals that interact with bacteria (Buschmann et al, 2005). The effect of salinity on UV inactivation is likely dependent on whether the UV light attenuation is greater than the advantages due to radical formation.

1.3.3.2 Turbidity

Turbidity is a regulated, easy to use and widely used particle indicator in water treatment plants (Caron et al., 2007), and affects UV disinfection process in two ways: they may decrease the UV transmittance of the water and affect dose delivery or may shied microorganisms from UV light, thus altering the characteristics of the dose response curve (Laurel et al., 2004). However, inconsistent results have been reported regarding the effect of turbidity on UV disinfection, which may be due to three main factors (the number and size of the particles, the degree of association of microorganisms with particles and the nature of the particles) (Caron et al., 2007).

1.3.3.3 Temperature

UV disinfection is relatively insensitive to temperature change, although there is an increasing trend with the increase of temperature (Severin et al., 1983). However, given the actual case in wastewater treatment processes, extreme temperatures out of the normal operating range of most treatment plants (20 to 40 °C; Abu-ghararah, 1994) may be experienced. A lower UV inactivation level was observed in the freezing treated waterborne microorganisms in general (Gao and Williams, 2013; Williams et al., 2011). Higher inactivation rates and levels were observed at very high temperature (45 or 50 °C), and lower inactivation rates and levels at very low temperature in wastewater (10 or 5 °C) (Abu-ghararah, 1994; Mounaouer and Abdennaceur, 2012).

1.3.3.4 Others

UV disinfection can also be affected by other factors, such as UV transmittance (UVT) or UV absorbance, suspended solids (Murakami et al., 2006; Whitby and Palmateer, 1993), resistances of different microorganisms (Hignen and Medema, 2006) and the initial concentration of microorganisms (Velez-Colmenares et al. 2011).

1.3.4 A comparison between UV and other disinfection technologies

Comparison of commonly used ballast water treatment processes is shown in Table 1-3. Each of these alternative disinfectants has their own advantages and disadvantages. The efficacy of all the disinfectants varies significantly according to the type of pathogens and conditions like pH, temperature and water quality.

1.3.5 Applications of UV disinfection in ballast water treatment

UV is often combined with other disinfection technologies when treating ballast water. Currently, the MP or LP UV unit is included in several ballast water treatment systems finally approved by IMO, such as Pureballast system (Sweden), OxyClean system (Denmark), ClearBallast (Japan). A filter system is often used to enable the following distinfection step to be more efficient.

Table 1-3 Comparison of commonly used ballast water treatment processes

Characteristics	Advantages	Disadvantages	Bactericidal*	Virucidal*	Protozoa removal*	Costs*	Disinfection by-products (DBPs)*
Chlorination	Low cost, mature technology, exists protective residual chlorine, a ability to kill bacterium continually	Ineffective against virus and cysts, toxic DBPs, causes a pungent smell	Middle	Low	Low	Low	High
ClO_2	Low cost, mature technology, persistently killing bacterium, effective in high turbidity waters as it does not combine with organics	Chemically hazardous reagents used	Middle	Low	Low	Middle	Middle
UV	Fast germicidal effect, effective against wide range of micro-organisms	Higher price, lack of residual germicide effect, high requirements for pre-water treatment, poor penetration	Middle	Low	Middle	Middle	None
Ozone	Especially effective at killing micro-organisms	Not effectively killing larger organisms, produces bromate as a by-product. Ozonate generators are required in order to treat large volumes of ballast water. These may be expensive and require sufficient installation space. High security requirements	Middle	Middle	Middle	High	Middle
Membrane filtration	High quality of treated water, absence of bacterial growth and residual toxicity	Relatively high costs	High	High	High	High	None

*Lazarova et al., 1999; Province of Manitoba, 2005.

1.4 Photoreactivation and Dark Repair

DNA repair is a potential drawback of UV disinfection, which is prevalent among many organisms such as bacteria (Goosen and Moolenaar, 2008), cyanobacteria (Levine and Thiel, 1987), plants (Britt, 1996) and can reverse the disinfection effects of UV radiation. Photoreactivation (or called as photorepair) and dark repair are two main mechanisms for the reactivation of UV light-induced damaged organisms.

1.4.1 Photoreactivation mechanism

In order to remove DNA lesions formed by UV, many organisms contain the photolyase enzyme that binds specifically to CPDs (CPD photolyase) or 6-4PPs (6-4 photolyase) and reverses the damage using the energy of light, as is termed as photoreactivation.

The process of photoreactivation includes two steps as shown in Fig. 1-3 (Harm, 1975). First, the pyrimidine dimer combines with a photoreactivating enzyme (PRE) to form PRE-dimer complex, and the rate at which each PRE binds to the pyrimidine dimer is dependent upon temperature, pH and ionic strength. Second, the complex releases PRE and the repaired monomerized dimer under a favorable light wavelength range (310 to 490 nm), and therefore the PRE is free again to combine with another pyrimidine dimer. The reaction occurs very fast in less than a millisecond. Therefore, the extent of photoreactivation is determined by the number of PRE-dimer complex.

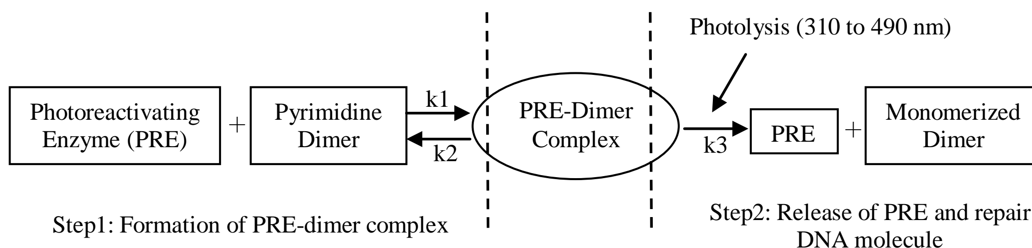

Fig. 1-3 Proposed two steps reaction mechanisom for photoreactivation (adapted from Harm, 1975)

1.4.2 Factors affecting photoreactivation

1.4.2.1 Salinity

The repressive effects of salinity on photoreactivation have been reported in previous studies. Chan and Killick (1995) compared photoreactivation in both synthetic sea water and under isotonic conditions at 15°C after a 95% reduction in initial viable cell count by UV irradiation and found that photoreactivation was slower and a lower maximum recovery was obtained for those cells in a saline environment. The ability of *E.coli* to photoreactivate declines sharply above a 30% of the salinity of synthetic sea water (0.9%) and levels off at 70% of the maximum salinity (2.1%). Additionally, Baron and Bourbigot (1996) also observed that when the salinity of effluent reached an average of 2.4% after 3 h incubation, the photo repair rates of *E. coli* were very small (0.0003 for UV doses above 44 mJ/cm^2) and no repair was observed for *enterococci*, implying that photoreactivation would not pose high risk in marine water environment. Oguma et al. (2013) mimicked the salt condition that UV-treated wastewater is discharged to coastal areas and

studied the effects of different NaCl concentrations on photoreactivation. The results showed that photoreactivation of *E. coli* was significantly suppressed in NaCl solution at 2.4% or higher but not affected in NaCl solution at 1.9% or lower, which demonstrated that photoreactivation was not always suppressed in seawater when the salinity was rather low.

1.4.2.2 Suspended solids (SS)

Increased levels of SS had the effect of reducing the actual UV dose reaching targeted organisms, thus reducing dimer formation and increasing subsequent photoreactivation, and a statistically significant correlation between SS and photoreactivation was demonstrated in previous experiments by Lindenauer and Darby (1994).

1.4.2.3 Temperature

Given that an enzymatic and biological process, photoreactivation can be influenced by temperature, the effects of which are not always consistent for different indicator microorganisms. Kelner (1949) reported that the photoreactivation rate of the actinomycete, *S. griseus* ATC 3326 increased with rise in temperature up to about 50°C. Salcedo et al. (2007) quantified the effect of the temperature on photoreactivation kinetic of three bacterial indicators (total coliforms, faecal coli forms and faecal streptococci) by a logistic model and also found that the extent of photoreactivation was favored by elevated temperatures (5-30 °C). However, Chan and Killick (1995) found

that temperature had no significant effect on the reactivation of a wild strain of *E.coli* in a saline environment although a slight rate increase is evident above 20 °C. Quek and Hu (2008) reported that for both *E. coli* strains (ATCC 11229 and ATCC 15597), photoreactivation levels were higher under near-optimum growth temperatures (23 or 37 °C) than those with too high (50 °C) or too low (4 °C) temperatures, whereas the photoreactivation rates are independent of temperature.

1.4.2.4 Others

The rate and extent of photoreactivation can also be affected by other factors, such as UV lamp type (Oguma et al., 2002; Zimmer and Slawson, 2002), UV dose (Lindenauer and Darby, 1994; Nebot Sanz et al., 2007), light intensity (Quek and Hu, 2008), wavelength of photoreactivating light (Bohrerova and Linden, 2007) and nutrient contents of water (Shang et al., 2009).

1.4.3 Control methods of photoreactivation

Photoreactivation can be controlled by increasing UV intensity and doses, dark treatment between UV irradiation and photoreactivation, and in combination with other disinfection strategies. Liltved and Landfald (1996) observed that to withstand the effect of photoreactivation, required UV doses were higher to achieve the same inactivation level than those in no post-irradiation recovery treatments. Martin and Gehr (2007) kept UV (40 mJ/cm^2) - or UV (20 mJ/cm^2)/peracetic acid (PAA) (2 mg/L)-treated samples in the

dark for three hours, which equals the average time the effluent spended in the outfall tunnel at the Montreal Wastewater Treatment Plant, and discovered that photorepair was significantly lower than the samples immediately exposed to light in both cases. In addition, the effects of photoreactivation may be diminished by use of a combined disinfection scheme, such as UV/chloramination (Mofidi et al., 2002; Quek et al., 2006), UV/ozone (Fang et al., 2014), UV/PAA (Martin and Gehr, 2007) and UV/ TiO_2 (Shang et al., 2009).

1.4.4 Dark repair

Another efficient DNA repair mechanisms in order to counteract the lethal effects of DNA lesions is dark repair, the process in which the inactivated pathogens can be reactivated without a reactivating light. Dark repair experiences much more complex pathways and does not directly reverse DNA damage but replaces the damaged DNA with new and undamaged nucleotides, as is different from photoreactivation (Britt, 1995). The most common in the dark repair is excision repair, two major subpathways of which are base excision repair (BER) and nucleotide excision repair (NER) with the help of a number of glycosylases and polymerases, respectively. For BER, the base is cleaved and removed from the deoxyribose backbone, and then the gap is filled to repair the patch (Sancar, 1996). For NER, a wide range of DNA distorting lesions including CPDs and 6–4PPs are removed. NER is highly conserved in eukaryotes and present in most organisms. Previous studies have shown that dark repair does not occur to a significant extent after UV

disinfection for some microorganisms such as *E. coli* and Cryptosporidium parvum (Oguma et al., 2001; Zimmer and Slawson, 2002). Less attention has been paid to dark repair compared with photoreactivation.

1.5 Research Objectives and Scope

1.5.1 Research Objectives

In this thesis, the inactivation and repair potential of two kinds of indicator microorganisms (*E. faecalis* and *V. cholerae*) following LP and MP UV disinfection will be evaluated and compared. The specific objectives are set out as follows:

1) To assess the effects of salinity, turbidity and temperature on UV inactivation of *E. faecalis* after LP and MP UV disinfection;

2) To evaluate the effects of salinity, turbidity and temperature on the rate and extent of photoreactivation and dark repair of *E. faecalis* after LP and MP UV disinfection;

3) To identify the influence of salinity on UV disinfection of *V. cholerae*.

1.5.2 Scope

1.5.2.1 Bacteria strain

Two kinds of microorganisms, *E. faecalis* and *V. cholerae*, are chosen as indicators concerning human health listed in IMO D2 Standards (Tsolaki and Diamadopoulos, 2010). The IMO regulated value for *E. faecalis* and *V. cholerae* are <100 CFU/100ml and <1 CFU/100ml respectively. *E. faecalis* as a gram-positive bacterium is one of the most common enterococcal strains found in environmental waters (Cabral, 2010), and has the ability to tolerate a variety of harsh conditions such as high salinity (6.5% NaCl) due to the robust physiology (Solheim et al., 2014). *Enterococci* are currently the only fecal indicator bacterial (FIB) recommended by the U.S. EPA for brackish and marine waters, given that they correlate better with human health outcomes than other FIB, such as fecal coliforms or *Escherichia coli* (Byappanahalli et al., 2012). *V. cholerae*, a highly pathogenic, gram negative and highly ubiquitous bacterium, is a useful indicator for the presence of pathogens and significance transmission of pathogens via ballast water.

1.5.2.2 UV disinfection

In this study, both *E. faecalis* and *V. cholerae* were irradiated with LP and MP UV lamps, two most commonly used UV lamps, with the aim of comparing the inactivation and repair characteristics of different UV sources under the same condition.

The influence of environmental conditions (salinity, turbidity and temperature for *E. faecalis*, and salinity for *V. cholerae*) on the inactivation efficiency was examined, and comparison of LP and MP lamps in terms of the final inactivation levels and disinfection kinetics was made. UV inactivation was monitored by the conventional culture method to determine log reduction at cellular level and ELISA at molecular level to determine the number of CPDs, one major type of DNA damage.

1.5.2.3 Photoreactivation and dark repair

In this study, after UV exposure using both LP and MP lamps, the degree of photoreactivation and dark repair in *E. faecalis* was examined under a variety of salinity, turbidity and temperature. Final repair levels were monitored by the cellular study and ELISA-based assay.

A summary of the various phases of the study is shown in Fig. 1-4.

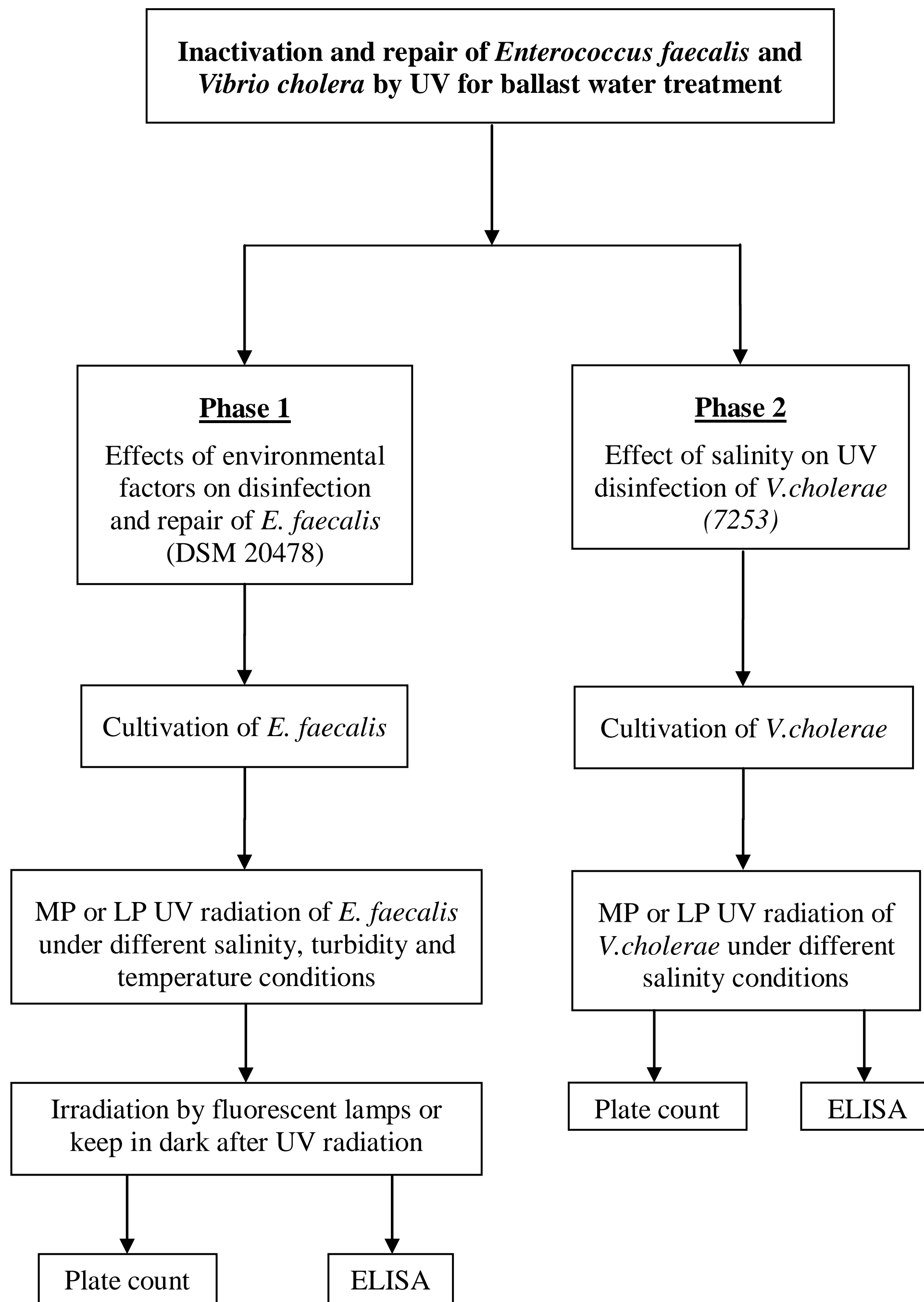

Fig. 1-4 Schematic diagram showing various phases of research study

1.6 Main Innovation Points

1) For *E. faecalis*, first and foremost, to our knowledge, there is little information available concerning the response of *E. faecalis* (DSM 20478) to UV inactivation. Given that responses to environmental stressors such as freezing (Gao and Williams, 2013) seems to be stain dependent, there is a need for elucidating the effects of several factors including temperature, turbidity and salinity on the inactivation of this strain for comparison with the previous work. Besides, many studies have been conducted on water disinfection by LP UV technology, while information on the comparison of the response of *E. faecalis* bacteria to LP and MP UV inactivation is quite scarce, which will be discussed in this study. Last but not least, dark repair was paid attention to as well as photoreaction, mainly considering that long contact time in the dark was a characteristic of ballast transport. However, little was known about the repair levels in the dark yet.

2) For *V. cholerae*, salinity is one important factor influencing UV performance, as is the case for *E. coli* (Rubio et al., 2013) and fecal coliforms (Shang et al., 2009), whereas few studies have investigated the effect of salinity on the inactivation efficiency of *V. cholerae*.

CHAPTER 2 Effect of Salinity, Turbidity and Temperature on Inactivation and Potential Repair of *Enterococcus faecalis* following low- and medium-pressure ultraviolet irradiation

2.1 Background

Aquatic nuisance species including bacteria may be introduced by ballast water discharge, which has become an ongoing problem threatening ecosystems and human health. The international standards of reduction for three indicators concerning human health (*E.coli*, *enterococci* and *V. cholerae*) have been set by the IMO for successful ballast water treatment (IMO 2004). Different technologies exist to treat ballast water in order to reduce the spread of invasive species (Tsolaki and Diamadopoulos 2010), among which UV seems to have a great deal of potential as a viable technology due to simple operation, no chemical storage or handling and no harmful residuals.

For *E. faecalis*, one of the most common species of *enterococci*, which was selected as an indicator microorganism in this study, many studies have been conducted on water disinfection by LP UV technology (Ananou et al., 2010; Kolvunen and Heinonen-Tanski, 2005; Gao and Williams, 2013; Hassen et al., 2000; Venieri et al., 2011), while information on the comparison of the response of *E. faecalis* bacteria to LP and MP UV inactivation is quite scarce.

There are several factors affecting UV inactivation, including salinity (Rubio et al., 2013; Shang et al. 2009), turbidity (Hu et al., 2007) and temperature

(Abu-ghararah, 1994; Mounaouer and Abdennaceur, 2012). Salinity and temperature may vary drastically during the transport and discharge of ship ballast water. Given a different sensitivity to environmental stressors between species (Liu and Zhang, 2006) and within a species (Gao and Williams, 2013), there is a need for elucidating the effects of several factors such as salinity, turbidity and temperature on the inactivation of the strain of *E. faecalis* (DSM 20478) for comparison with the previous work.

DNA repair is a potential drawback of UV disinfection, which is prevalent among many organisms such as bacteria (Goosen and Moolenaar, 2008), cyanobacteria (Levine and Thiel, 1987), plants (Britt, 1996) and can reverse the disinfection effects of UV radiation. Photoreactivation and dark repair are two main mechanisms for the reactivation of UV treated organisms. When discharging treated ballast water to coastal areas, different levels of salinity and turbidity and different temperature may be encountered, the effects of which on the reactivation phase are still little studied. In addition, little was known about the repair levels in the dark yet, although long contact time in the dark was a characteristic of ballast transport.

Hence, the aims of the present work were (1) to assess the effects of salinity, turbidity and temperature on UV inactivation of *E. faecalis* after LP and MP UV disinfection; (2) to evaluate the effects of these three factors on the rate and extent of photoreactivation and dark repair of *E. faecalis* following LP and MP UV disinfection. Both the cellular study and ELISA-based assay were

used to investigate the inactivation efficiency, photoreactivation and dark repair.

2.2 Materials and Methods

2.2.1 Microorganisms

The strain of *E. faecalis* bacterial chosen for this study was DSM 20478 purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ). An overnight phase was prepared by inoculating 1 mL of the frozen stock culture into 30 mL of tryptic soy broth (TSB) and shaking overnight at 37 °C. 1 mL of this overnight culture was then added to 30 mL of fresh TSB and incubated in a shaker for 4 h at 37 °C to obtain a log phase culture. The *E. faecalis* cells were harvested by centrifugation at 3,000g for 10 min, washed twice with sterile distilled water, and resuspended in 30 mL of sterile distilled water. Before UV irradiation was carried out, the suspension was further diluted in sterile distilled water to obtain an initial concentration of approx. 10^8 CFU/mL.

2.2.2 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 LP (10 W) and MP (1 kW) UV lamps (Fig. 2-1). 10 mL of the diluted *E. faecalis* suspension was dispensed into a 6 cm diameter sterile

plastic Petri dish and exposed to either LP or MP UV radiation. The UV doses investigated ranged from 4 to 19 mJ/cm^2 and were determined as previously described by Bolton and Linden (2003) and Zimmer and Slawson (2002). 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, while the rest of the sample was covered and used for photoreactivation and dark repair studies.

Fig. 2-1 Collimated beam apparatus for UV disinfection

(i) Salinity experiments. *E. faecalis* were resuspended in two types of water (artificial seawater (ASW) and natural seawater (NSW)). Use of ASW is compared to use of NSW as a culture media. ASW was prepared as described by Lleo` 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 hyposmotic environment of brackish water (Lin et al.,

2003). Natural seawater with an average salinity of 3% 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 2-1.

Table 2-1 Some physicochemical characteristics of the types of water used in the salinity experiments.

Suspension solution	Salinity (%)	Conductivity (mS/cm)	TOC (ppm)	pH	T (°C)
ASW	1	16.5	0.882	8.09	22.9
	3	44.1	1.50	7.98	22.9
NSW	3	43.7	3.47	7.88	22.7

(ii) Turbidity experiments. Kaolin clay with tendency to swell and its active surface was chosen as the representative of inorganic particles and a potential worst particle for shielding. Generally, the most turbid waters naturally encountered are in the range of 10 to 15 NTU (Waite et al., 2003), whereas the variability in the turbidity of seawater between locations and over time has been reported in previous studies (2 to 30 NTU, Desormeaux et al., 2009; <10 NTU, Lauri et al., 2010). Therefore, UV exposure was performed in three levels of turbidity water (1, 10 and 30 NTU) which were obtained by seeding different amount of kaolin clay to sterile water. Turbidity was measured with HACH 2100N turbidimeter (Hach Co, Loveland, Colo.).

(iii) Temperature experiments. To investigate the effects of temperature on UV inactivation, *E. faecalis* suspensions were irradiated at 4 and 25 °C. Temperature changes were recorded before and after irradiation.

2.2.3 Photoreactivation and dark repair

A UV dose of 16.5 mJ/cm^2 was selected for repair studies for achieving a similar average reduction of *E. faecalis* (about 3.7 Log units) following MP or LP exposure. The Petri dishes containing the irradiated *E. faecalis* suspensions were placed on magnetic stirrers and stirred continuously while being exposed to a light intensity of about 12 kLux for up to 6 h using two 24 W fluorescent lights (National, Matsushita Electrical Industrial Co. Ltd, Japan). The light intensity was measured using a digital luxmeter (Model E2, B. Hagner AB, Sweden) and samples were taken at hourly intervals for bacteria enumeration. The same procedures were adopted for dark repair, except that the Petri dishes were placed on magnetic stirrers in the dark, and were also covered with aluminum foil to prevent accidental exposure of samples to light during sample collection. For salinity and turbidity experiments, the temperature for the repair experiments was maintained at 25 °C, whereas for temperature experiments, irradiated *E. faecalis* suspensions were incubated either in the 4 °C refrigerator or at room temperature (25 °C).

2.2.4 *E. faecalis* enumeration

From appropriate dilutions of the microcosms, the total number of *E. faecalis* was examined by spread plate on Trypticase soy yeast extract medium (TSYEA) consisting of TSB 30 g, agar 15 g, yeast extract 3 g per litre.

Colonies were counted after 36 h incubation at 37 °C and recorded as CFU/mL.

2.2.5 Enzyme-linked immunosorbent assay (ELISA)

The ELISA assay was used to determine the accumulation and repair of CPDs. Briefly, DNA was extracted from 10 mL of lysed *E. faecalis* cells according to DNeasy® Blood & Tissue kit (Qiagen, Germany). DNA was determined by the absorbance at 260 nm and diluted in PBS to a final concentration of 0.2 μg/mL. 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 before and after UV irradiation at 16.5 mJ/cm^2, and at 6 h after irradiation.

2.2.6 Data analysis

The Log reduction of the test microorganisms was calculated as:

$$\text{Log reduction} = \log (N_i/N_0) \qquad (1)$$

where N_i is the initial concentration of *E. faecalis* before UV disinfection (log CFU/mL), and N_0 is the concentration of *E. faecalis* immediately after UV disinfection (log CFU/mL).

The following equation proposed in previous studies (Quek and Hu, 2008a, b) was applied to obtain repair at each hour:

$$\% repair = \frac{N_t - N_0}{N_i - N_0} \qquad (2)$$

where N_t is the concentration of *E. faecalis* at time of exposure, t, after UV irradiation (log CFU/mL).

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:

$$\frac{N_i}{N_0} = \sigma \exp(-k_1 t) + (1-\sigma)\exp(-k_2 t) \qquad (3)$$

where σ is fraction of microorganisms sensitive to UV radiation, 1-σ is fraction of microorganisms resistant to UV radiation, k_1 is the inactivation rate for sensitive fraction of microorganisms, k_2 is the inactivation rate for resistant fraction of microorganisms, and t is exposure time to UV light (s).

All experiments were repeated three times to ensure the validity and reproducibility of the experimental data. Data were presented in mean ± standard deviation. One-way ANOVA with post hoc least significant difference (LSD) was conducted to assess the significance of effects of environmental conditions at the significance level of 0.05.

2.3 Results and Discussion

2.3.1 UV inactivation of *E. faecalis*

Fig. 2-2 shows the inactivation of *E. faecalis* following MP and LP UV disinfection in sterile distilled water (the controls). It can be seen that for both

types of UV radiation, at high UV doses (long exposure to UV light), the inactivation rate decreased and tailed off (tailing effect), which was suspected possibly to be due to shielding or clumping of the bacteria. Such inactivation behavior had previously been reported by Gao and Williams (2013) when they studied the behaviour of various *E. faecalis* strains (ATCC strain 29212 and ATCC strain 51299) to UV inactivation. It was also found that when MP UV radiation was employed, lower UV doses were required to achieve the same log reduction of *E. faecalis*, indicating that MP UV disinfection was more efficient than LP UV disinfection. This has been reported previously for *E.coli* strains (Hu et al., 2005), and is likely due to the more intense radiation and broader wavelength spectrum emitted by MP UV lamps that caused damage to intercellular biomolecules other than DNA (Kalisvaart 2004). Additionally, k_1 was 0.509 and 0.0229 for MP and LP lamps respectively, which showed that inactivation rate of MP UV disinfection was faster than LP UV disinfection.

Previous studies have shown the sensitivity of *E.coli* ranged from 10 to 15 mJ/cm^2 (MP) (Quek et al., 2006) and 5 to 11 mJ/cm^2 (LP) (Butler et al., 1987; Hollaender, 1942) for a 4 Log kill, while for *E. faecalis*, a higher UV dose more than 19 mJ/cm^2 was needed to result in the same level of reduction, revealing that *E. faecalis* has lower UV sensitivity compared to *E.coli*. It is possible that the high concentration of peptidoglycan, teichoic acids, polysaccharides, and peptidoglycolipids, in the cell composition of *E. faecalis* which act as a protective coating (Gomes et al., 2009) resulted in high UV resistance.

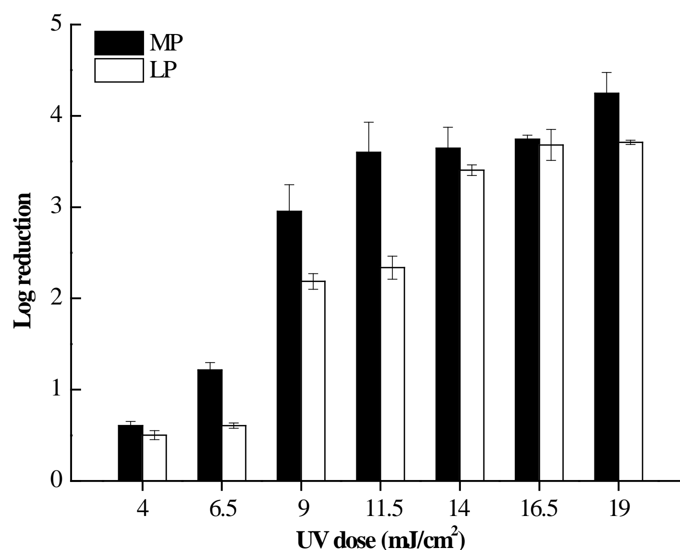

Fig. 2-2 UV inactivation of *E. faecalis* by MP and LP UV disinfection in sterile distilled water. Error bars represent standard deviations of three experiments

Effect of salinity on UV inactivation

Comparison of Log removal of *E. faecalis* by two types of UV lamps under different salinity conditions is shown in Fig. 2-3. It was observed that at 1% or higher, salinity had an enhanced impact on MP UV disinfection at high UV doses more than 14 mJ/cm^2 while a weakened impact on LP UV disinfection from 4 to 19 mJ/cm^2. After LP UV exposure, UV inactivation was significantly suppressed in artificial seawater and natural seawater than in sterile distilled water with both a lower log reduction and lower inactivation rate (k1) as shown in Fig. 2-3B and Table 2-2. Interestingly, after MP UV exposure, the lowest disinfection rates was obtained in sterile distilled water and apparently, the level of log reduction and inactivation rate at high UV doses more than 14 mJ/cm^2 were found to be higher when increasing the

solution salt concentration as illustrated in Fig. 2-3A and Table 2-2. Differences in impact of salinity on UV disinfection of *E. faecalis* were observed between LP and MP UV lamps. Given that DNA damage is caused mainly by the light around the wavelength of 254 nm, one plausible interpretation for higher Log reduction with increased salt concentration under MP UV irradiation is the damage of intercellular components other than DNA due to salt stress.

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 UV inactivation rate decreased when increasing the solution salt concentration, whereas Shang et al. (2009) observed higher salinity resulted in higher level of inactivation of fecal coliform bacteria by UV both in the presence and in absence of TiO_2. It is known that organic matter and inorganic ions exposed to UV light can not only absorb UV light (Wright and Cairns, 1998) but also form radicals that interact with bacteria (Buschmann et al, 2005). Hence the effect of salinity on UV inactivation is likely dependent on the sum of both UV light attenuation and radical formation.

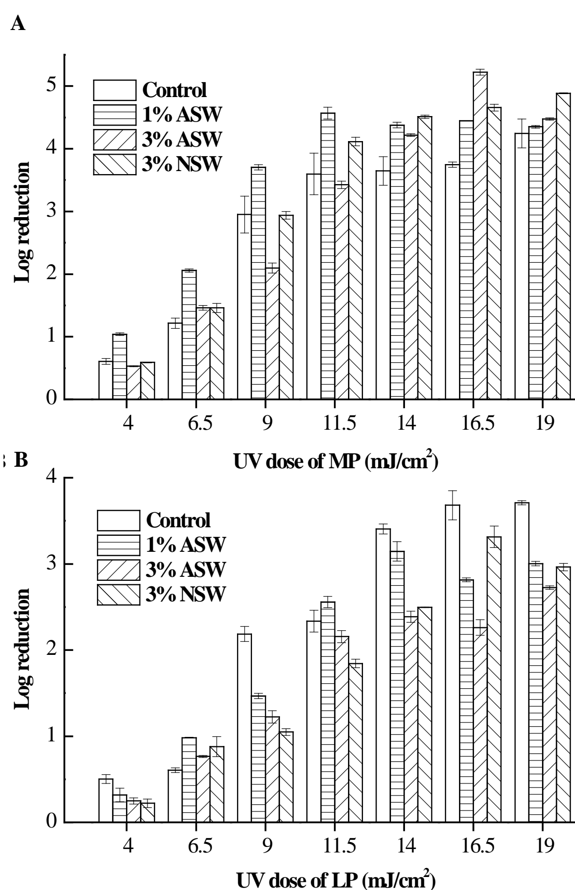

Fig. 2-3 The effect of salinity on UV inactivation of *E. faecalis* by a MP and b LP UV disinfection. Error bars represent standard deviations of three experiments. ASW: artificial seawater, NSW: natural seawater

Table 2-2 Kinetic parameters of the double first order kinetic model applied to salinity experiments

Bacterium	UV lamp	Suspension solution	Salinity (%)	k_1 (s^{-1})	k_2 (s^{-1})	σ	r^2
E. faecalis	LP	DI water	-	0.0229	0.00500	0.9996	0.9421
		ASW	1	0.0225	0.00131	0.9990	0.9154
			3	0.0170	0.00174	0.9986	0.9265
		NSW	3	0.0182	0.00332	0.9997	0.9088
	MP	DI water	-	0.509	0.00889	0.9999	0.9621
		ASW	1	0.6205	0.0205	0.99995	0.9579
			3	0.5500	0.0299	0.99998	0.9298
		NSW	3	0.5157	0.0367	0.99997	0.9468

Note: DI water: distilled water.

Effect of turbidity on UV inactivation

Response of *E. faecalis* to MP or LP UV inactivation at three levels of turbidity (1, 10 and 30 NTU) is given in Fig. 2-4. Following MP and LP UV disinfection, lower UV inactivation levels were obtained when turbidity was higher than 1 NTU as compared to those of the controls at 4 mJ/cm^2 for MP and in the range of 4-11.5 mJ/cm^2 for LP. In addition, the value of σ was noted to decrease with increasing levels of water turbidity (Table 2-3), which was in good agreement with studies of Hu et al. (2007). It was also noted that as UV fluence increased to 6.5 mJ/cm^2 for MP and 14 mJ/cm^2 for LP, the influence on *E. faecalis* inactivation efficiency at different levels of turbidity became less noticeable, indicating that at high turbidity, UV disinfection performance can be improved by increasing UV doses especially when MP UV disinfection was employed.

Turbidity affects UV disinfection process in two ways: they may decrease the UV transmittance of the water and affect dose delivery or may shield microorganisms from UV light, thus altering the characteristics of the dose response curve (Laurel et al., 2004). Gullian et al. (2012) studied the effect of turbidity on the UV effectiveness of removing heterotrophic bacteria (HB) from two commercial recirculating aquaculture systems (RAS) and found that the effectiveness of UV disinfection decreased with increasing turbidity level, and the UVC disinfection in RAS2 was less efficient than in RAS1. Dehghani et al. (2013) investigated the effect of turbidity on inactivation efficiency of larva and adult Rhabitidae in municipal water, and reported that increase of

turbidity up to 25 NTU decreased inactivation efficiency of larvae and adult nematodes from 100% to 66% and 100% to 64% respectively after exposure to a LP UV dose of 14.4 mJ/cm^2. Given that the reflection and absorption of UV light has been considered in obtaining weighted UV doses used in the present study, it is likely that small kaolin clay particles (2.649 μm) in diameter may shield smaller microorganisms such as *E. faecalis* and thus compromise UV inactivation.

Effect of temperature on UV inactivation

Fig. 2-5 and Table 2-4 illustrate the results of *E. faecalis* inactivation following MP and LP UV disinfection under various temperature conditions. There was a decreasing trend of inactivation rates and levels as the temperature increased, despite log reduction increased a little bit but not remarkable after MP UV at low temperature when the UV dose was greater than 14 mJ/cm^2. Likewise, a lower LP UV inactivation level was observed in the freezing treated waterborne microorganisms in general (Gao and Williams, 2013; Williams et al., 2011). Higher inactivation rates and levels were observed at very high temperature (45 or 50 °C), and lower inactivation rates and levels at very low temperature in wastewater (10 or 5 °C) (Abu-ghararah, 1994; Mounaouer and Abdennaceur, 2012). The lower inactivation rates and levels at 4 °C than those at 25 °C might be explained by the resistance to UV in order to survive the extreme temperature.

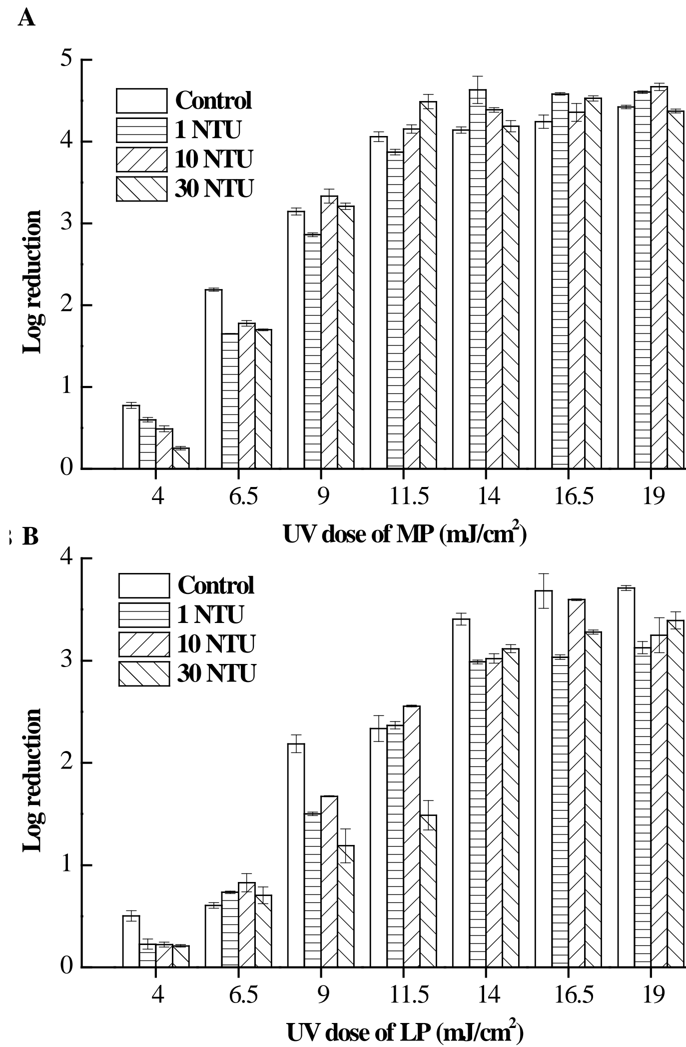

Fig. 2-4 The effect of turbidity on UV inactivation of *E. faecalis* by a MP and b LP UV disinfection. Error bars represent standard deviations of three experiments

Table 2-3 Kinetic parameters of the double first order kinetic model applied to turbidity experiments

Bacterium	UV lamp	Turbidity (NTU)	σ	r^2
E. faecalis	LP	1	0.999702	0.952023
		10	0.9997	0.9499
		30	0.98483	0.92644
	MP	1	0.99994	0.96459
		10	0.99994	0.94442
		30	0.99993	0.90764

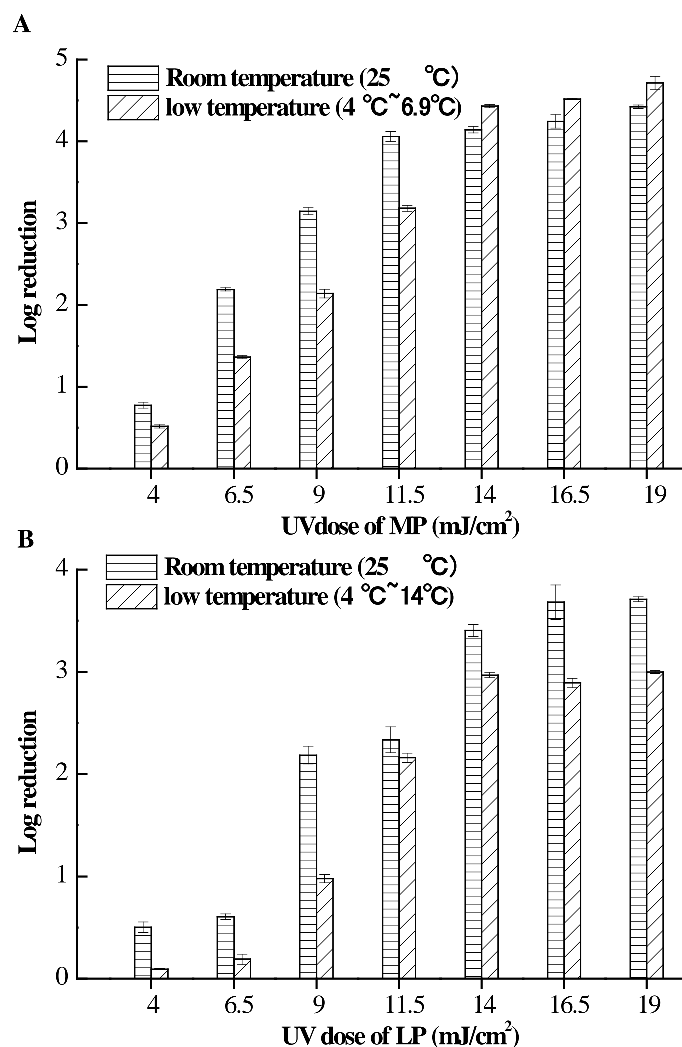

Fig. 2-5 The effect of temperature on UV inactivation of *E. faecalis* by a LP and b MP UV disinfection. Error bars represent standard deviations of three experiments. Low temperature ranges from 4-6.5 °C for MP UV and 4-14 °C for LP UV.

Table 2-4 Kinetic parameters of the double first order kinetic model applied to temperature experiments

Bacterium	UV lamp	Temperature (℃)	k_1 (s^{-1})	k_2 (s^{-1})	σ	r^2
	MP	4~6.9	0.47493	0.01609	0.99997	0.95072
E. faecalis		25	0.509	0.00889	0.9999	0.9621
	LP	4~14.0	0.0151	0.0151	0.9994	0.8896*
		25	0.0229	0.00500	0.9996	0.9421

(*) Data fitting that presented a low goodness of fit (R^2<0.9).

2.3.2 Photoreactivation and Dark repair of *E. faecalis* after UV disinfection

Fig. 2-6 shows the percentage log repair of *E. faecalis* in light or dark following LP and MP UV disinfection in sterile distilled water (control experiments). It can be seen that when a germicidal UV dose of 16.5 mJ/cm^2 was applied, the maximal percentage of photoreactivation achieved within 6 h was 14.5% and 45.5%, after MP and LP irradiation, respectively. It was reported previously that MP UV exposure resulted in a greater reduction in photolyase activity than did LP UV exposure (Hu and Quek, 2008), and thus may contribute to a lower degree of photoreactivation. In terms of dark repair, it is evident that dark repair levels are much lower than those for photoreactivation, no matter which type of UV lamp was used. A maximum of 22.7% log dark repair was achieved after 6 h following LP UV disinfection, whereas a decrease in the bacterial concentration over incubation time was detected in darkness after MP exposure, indicating that MP UV radiation which has a broad wavelength spectrum may have induced some delayed mutagenic effects in the cells which continued to kill the cells after disinfection, as is the case for *E. coli* NCIMB 9481 (Quek and Hu, 2008b).

Effect of salinity on photoreactivation and dark repair

Fig. 2-7A shows repair levels following 6 h exposure to fluorescent light and dark conditions after LP and MP UV disinfection at various salinity levels.

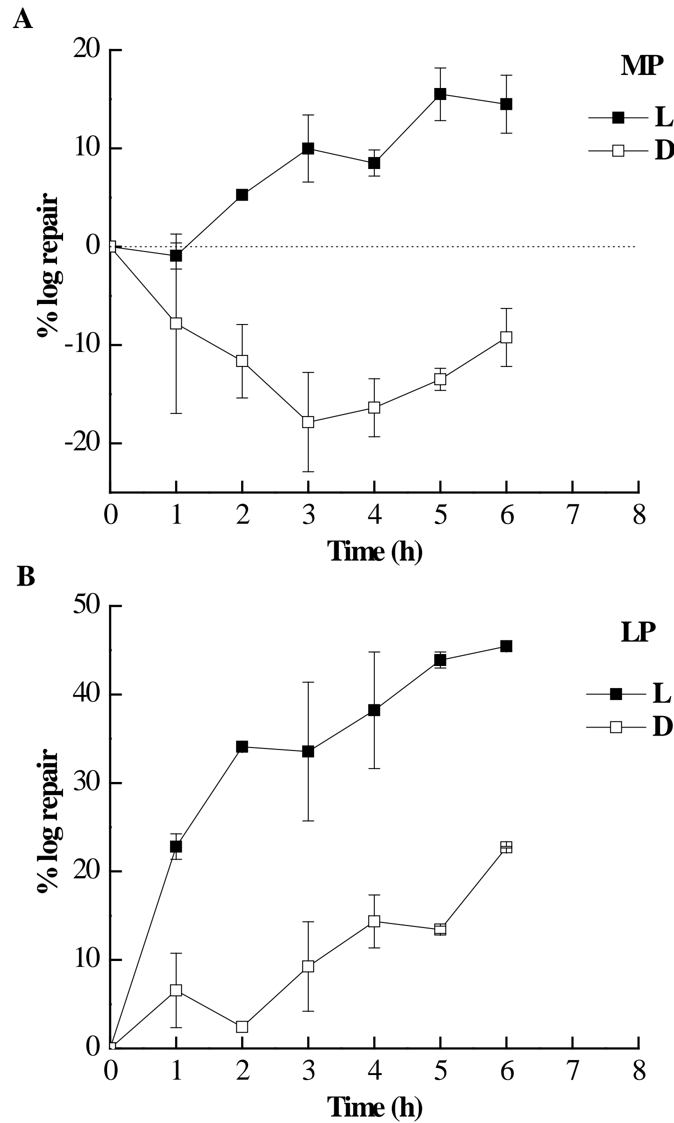

Fig. 2-6 Percentage log repair of *E. faecalis* after exposure to fluorescent light or incubation in the dark following a MP and b LP UV disinfection in sterile distilled water. Error bars represent standard deviations of three experiments. L: light, D: dark.

After the MP UV exposure, the final recovery values were increased at salinity levels above 1% under both light and dark conditions, suggesting that both photoreactivation and dark repair could be promoted, which may enhance the risk of discharging MP UV-treated *E. faecalis* in the brackish and marine water environment. One possible explanation is that more removal of CPDs, which is one of the two major classes of UV-induced damage (~75%) (Thoma

1999) were observed in the presence or absence of light after MP UV irradiation (Fig. 2-7B). Given that both *E. faecalis* and *Dunaliella salina* are salt tolerant (Byappanahalli et al., 2012; Ramos et al., 2011), high salinity may cause the expression of CPD photolyase in *E. faecalis* as in *D. salina* by leading to DNA double-strand breaks (by NaCl) and DNA oxidative damage (by H_2O_2) (Cheng et al., 2007). It is possible that more repaired CPDs by photolyase in light after MP and LP UV irradiation under salt stress applies to the increase in bacteria concentrations. For LP exposure, after 6 h of exposure to fluorescent light, there was no significant difference in the final log recovery percentages between 1% artificial seawater and control groups, whereas enhanced bacterial regrowth was observed in either 3% artificial seawater or 3% natural seawater (Fig. 2-7A). Dark repair was suppressed when the salinity level was 1% or higher, which may probably because *E. faecalis* could not repair UV-induced pyrimidine dimers in the genomic DNA in the dark (Fig. 2-7B) and therefore failed to perform survival recovery.

In contrast, conflicting results were reported in previous studies showing the repressive effects of salinity on photoreactivation. Chan and Killick (1995) compared photoreactivation in both synthetic sea water and under isotonic conditions at 15°C after a 95% reduction in initial viable cell count by LP UV irradiation and found that photoreactivation was slower and a lower maximum recovery was obtained for those cells in a saline environment. The ability of *E.coli* to photoreactivate declines sharply above a 30% in synthetic sea water (0.9%) and levels off at 70% of the maximum salinity (2.1%). Additionally, Baron and Bourbigot (1996) also observed that when the salinity of effluent

reached an average of 2.4% after 3 h incubation, the photo repair rates of *E. coli* were very small (0.0003 for UV doses above 44 mJ/cm^2) and no repair was observed for *enterococci*, implying that photoreactivation would not pose high risk in marine water environment. However, Oguma et al. (2013) mimicked the salt condition that UV-treated wastewater was discharged to coastal areas and studied the effects of different NaCl concentrations on photoreactivation.

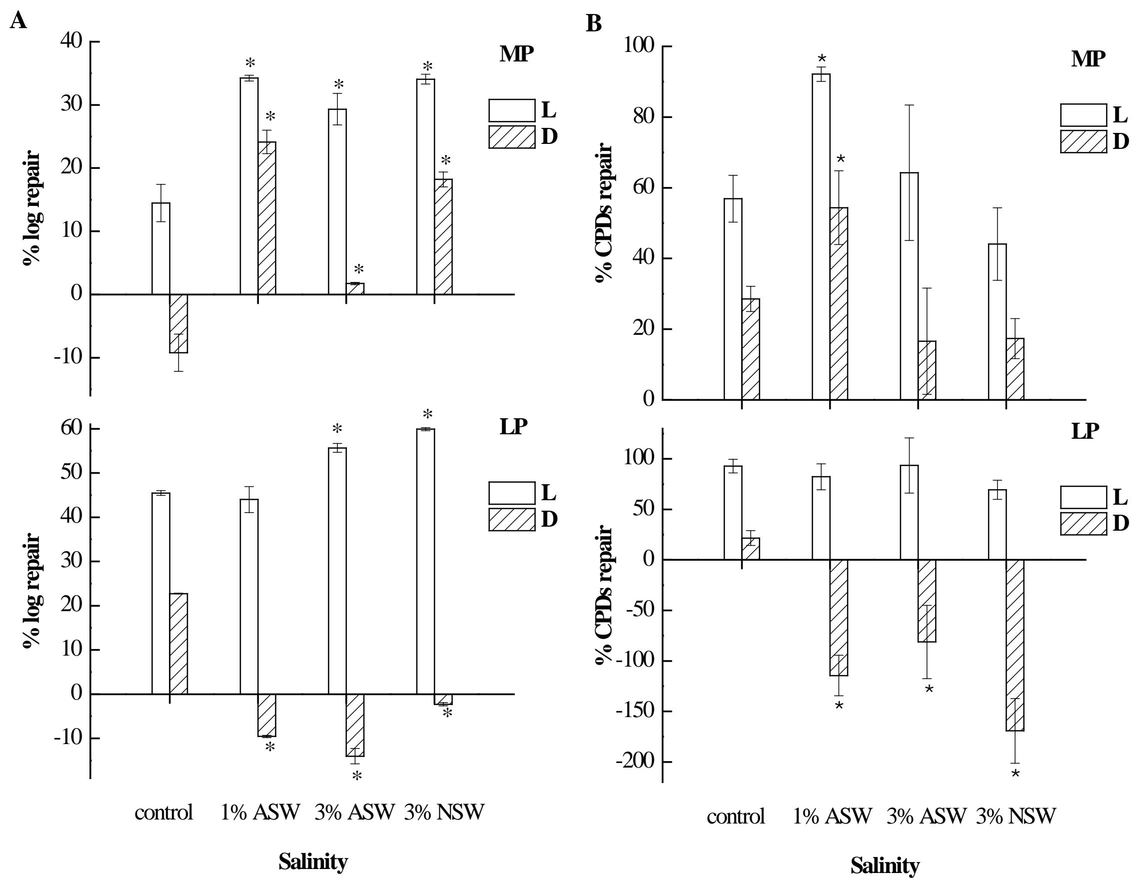

Fig. 2-7 (A) Percentage log repair and (B) CPDs repair of *E. faecalis* after 6 h exposure to fluorescent light or incubation in the dark following MP and LP UV disinfection in sterile distilled water or at various salinity levels and constant light intensity of 12 kLux. Error bars represent standard deviations of three experiments. L: light, D: dark. ASW: artificial seawater, NSW: natural seawater. Asterisks denote values that are significantly different (*$p < 0.05$) from the control value.

The results showed that photoreactivation of *E. coli* was significantly suppressed in NaCl solution at 2.4% or higher but not affected in NaCl solution at 1.9% or lower, which demonstrated that photoreactivation was not always suppressed in seawater when the salinity was rather low. However, such inhibition effects of *E. coli* on photoreactivation were not found in *E. faecalis* in this study, which needs further evaluation. This study also reveals that effects of salinity on dark repair seem to be related with different UV sources. It can be inferred that in brackish areas, higher LP UV doses need to be considered to prevent photoreactivation and dark repair, whereas in seawater, the promoting effects of salinity on regrowth process of *E. faecalis* need to be taken into account at designing the disinfection system.

Effect of turbidiy on photoreactivation and dark repair

From Fig. 2-8, it can be seen that after MP exposure, the effects of turbidity on photoreactivation were negligible when turbidity was lower than 30 NTU. It is difficult to explain the increased levels of photoreactivation when turbidity reached 30 NTU. In theory, high turbidity may decrease light intensity reaching targeted organisms, and thus inhibit photoreactivation, which may be one plausible interpretation for repressive effects on the final photorepair achieved after LP exposure when turbidity was 1 NTU or higher. The correlation between turbidity and photoreactivation after MP or LP exposure was not statistically significant in line with previous studies (Lindenauer and Darby, 1994). As shown in Fig. 2-8A, dark repair was detectable when

turbidity was higher than 10 NTU following MP exposure. Further studies should be conducted to uncover the underlying mechanism. On the contrary, after LP UV exposure, dark repair levels decreased with an increase of turbidity above 10 NTU (Fig. 2-8A), which agrees with the trends observed in the repair of CPDs in the dark (Fig. 2-8B), and also confirms that the less efficient removal of CPDs is directly related to the decrease in bacteria concentrations in high turbidity.

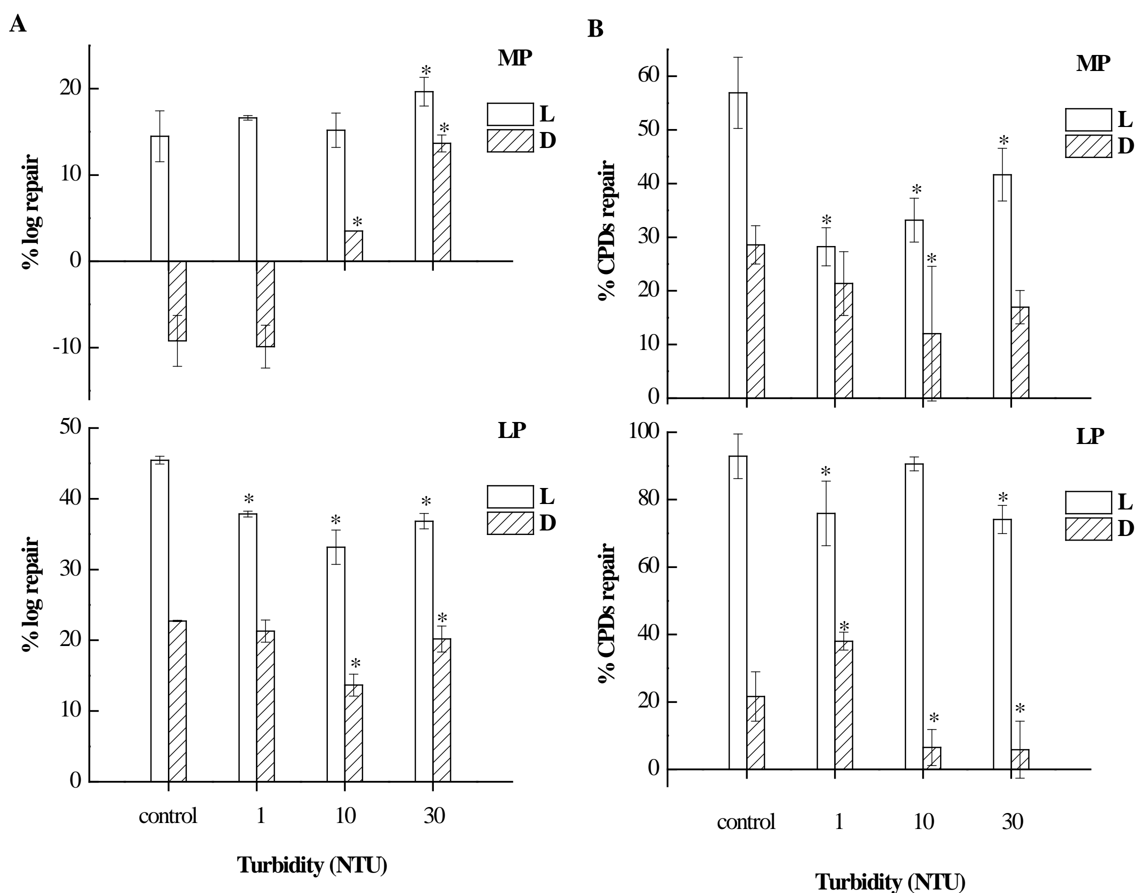

Fig. 2-8 (A) Percentage log repair and (B) CPDs repair of *E. faecalis* after 6 h exposure to fluorescent light or incubation in the dark following LP and MP UV disinfection in sterile distilled water or at various turbidity levels and constant light intensity of 12 kLux. Error bars represent standard deviations of three experiments. L: light, D: dark. Asterisks denote values that are significantly different (*p < 0.05) from the control value.

Based on the results, it seemed that effect of turbidity on photoreactivation and dark repair varies widely between these two UV sources, and LP lamps are superior to MP lamps against photoreactivation and dark repair when turbidity is higher than 1 NTU.

Effect of temperature on photoreactivation and dark repair

Fig. 2-9 (A) shows the percentage log recovery after 6 h of incubation when irradiated suspensions were incubated at different temperatures. After MP UV disinfection, the percentage of photoreactivation of 14.5 % and 0.885 %, respectively, was detected at 25 and 4 °C, indicating the suppressed photoreactivation after MP exposure in cold areas or during winter. Results regarding effects of temperature on photoreactivation could be well explained by the fact that significantly more CPDs were repaired at 25 °C than at 4 °C (Fig. 2-9B). No regrowth was observed in the dark at either 25 or 4 °C after MP UV exposure. Temperature was found to have a more significant impact on dark repair than on photoreactivation after LP exposure. Photoreactivation was slower when the temperature was reduced. Interestingly, there was no significant difference in the extent of photoreactivation when the temperature changed from 25 °C to 4 °C after LP UV treatment compared to MP UV treatment, whereas the repair rate was still higher at 25 °C than that at 4 °C within the first two hours following LP UV disinfection, which could be because at 25 °C, most of photo repair occurred within the first two hours, followed by levelling off of the curves after that. In accordance with results of

MP UV inactivation, higher temperature could reach higher level of dark repair of *E. faecalis* after LP UV exposure.

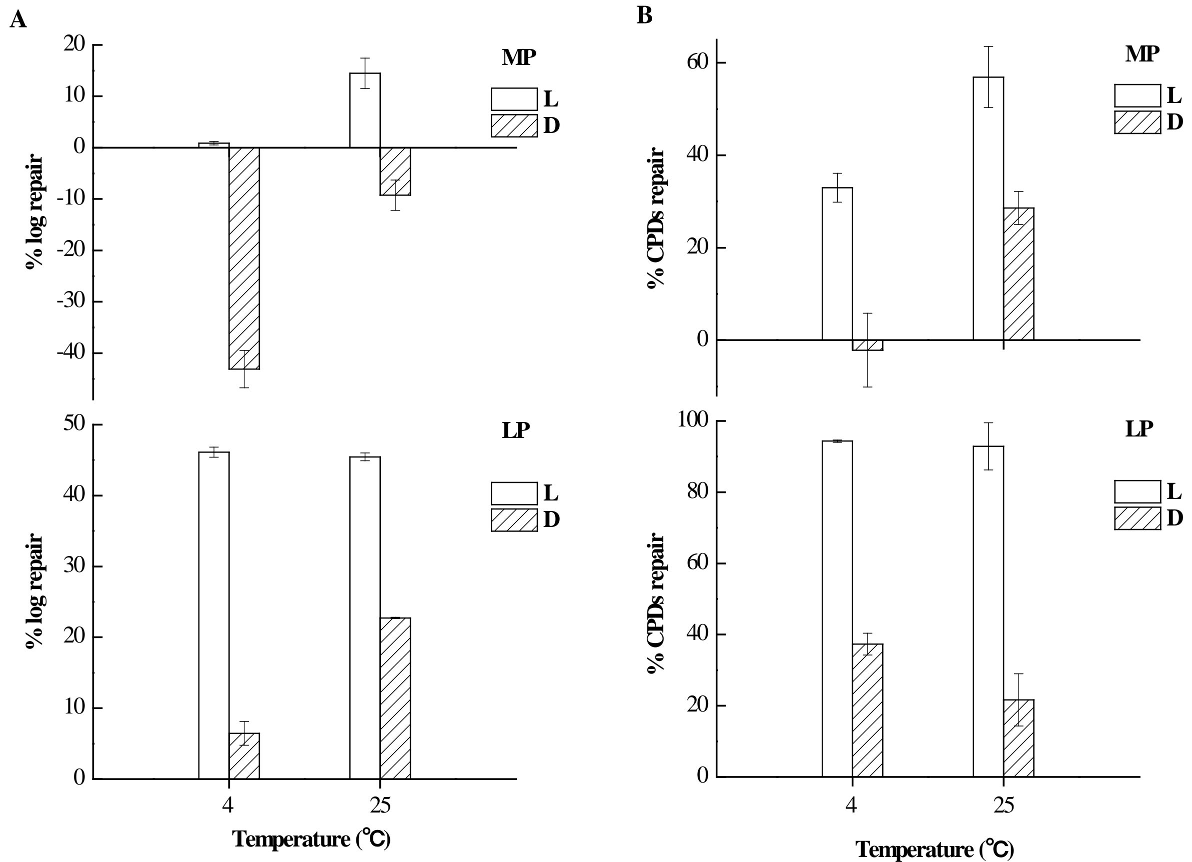

Fig. 2-9 (A) Percentage log repair and (B) CPDs repair of *E. faecalis* after 6 h exposure to fluorescent light or incubation in the dark following LP and MP UV disinfection in sterile distilled water at various temperatures and constant light intensity of 12 kLux. Error bars represent standard deviations of three experiments. L: light, D: dark. Asterisks denote values that are significantly different (*$p < 0.05$) from the control value.

Given that an enzymatic and biological process, photoreactivation can be influenced by temperature, the effects of which are not always consistent among different indicator microorganisms. Kelner (1949) reported that the photoreactivation rate of the actinomycete, *S. griseus* ATC 3326 after LP UV

exposure increased with rise in temperature up to about 50°C. Salcedo et al. (2007) quantified the effect of the temperature on photoreactivation kinetic of three bacterial indicators (total coliforms, faecal coli forms and faecal streptococci) by a logistic model and also found that the extent of photoreactivation after UV-C irradiation was favored by elevated temperatures (5-30 °C). However, Chan and Killick (1995) found that temperature had no significant effect on the reactivation of a wild strain of *E.coli* in a saline environment after LP UV exposure although a slight rate increase is evident above 20 °C. Quek and Hu (2008a) reported that for both *E. coli* strains (ATCC 11229 and ATCC 15597), photoreactivation levels following LP and MP UV disinfection were higher under near-optimum growth temperatures (23 or 37 °C) than those with too high (50 °C) or too low (4 °C) temperatures, whereas the photoreactivation rates are independent of temperature. In the present study, in respect of *E. faecalis*, low temperature was found to have a negative impact on photoreactivation after MP and LP exposure.

In line with previous reports that showed greater dark repair levels at higher temperature (Salcedo et al., 2007) after UV-C irradiation, in the current study it is evident that incubation at 25 °C was more favorable to the recovery from LP UV stress than at 4 °C in the dark. Dark repair experiences much more complex pathways and does not directly reverse DNA damage but replaces the damaged DNA with new and undamaged nucleotides, as is different from photoreactivation (Britt, 1995). CPDs can be removed by nucleotide excision repair (NER), a multistep, multienzyme process (Sinha and Häder, 2002). NER has been demonstrated to increase with temperature between 5 °C and

28 °C in yeast (Giese et al., 1957), which is one of the pathways of dark repair. Based on our results for *E. faecalis*, the dark repair of CPDs are temperature-dependent after either MP or LP exposure as in yeast.

2.4 Conclusions

In conclusion, based on final inactivation levels and the kinetics of inactivation obtained in this study, in terms of UV disinfection performance, MP UV lamps were effective in *E. faecalis* disinfection in a saline environment (1% or higher), whereas increased salinity levels hindered inactivation LP UV disinfection. Both the presence of turbidity (above 1 NTU) and low temperature adversely affects the inactivation efficiency after both MP and LP exposure especially at low UV doses. Higher MP UV doses of greater than 14 mJ/cm^2 appear to minimize the negative effects of turbidity and temperature on *E. faecalis*. In terms of repair capacity, salinity had a beneficial effect on photoreactivation after both LP and MP exposure, whereas the effect of salinity on the dark repair levels are dependent upon UV sources, that is, dark repair can be promoted after MP exposure while suppressed after LP exposure with salt concentration above 1%. Both photoreactivation and dark repair can be promoted with high turbidity up to 10 NTU after MP exposure, whereas an inverse relationship between turbidity and repair levels were observed after LP exposure. As expected, low temperature was found to adversely affect reactivation of *E. faecalis* following MP and LP UV disinfection. All these three factors demonstrated an important role in the inactivation and repair capability when UV light is used to treat ballast water.

Considering that UV-treated wastewater is exposed or discharged to marine water environment in many countries, results of this study provide significant implications for the management of public health.

CHAPTER 3 Effect of salinity on low- or medium-pressure UV disinfection of *Vibrio cholerae*

3.1 Background

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). *V. 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.

Studies on UV inactivation of *V. cholerae* focused mostly on LP UV lamps (Das and Das, 1983; Das et al., 1981; Hoyer, 1998; Wilson et al., 1992), whereas little was known about the efficiency of MP UV disinfection and the comparison of the inactivation characteristics of LP and MP UV lamps, although installations employing MP UV disinfection have also increased in recent years.

To our knowledge, the effects of different salt concentrations on UV radiation is not well documented yet, although salinity is one important factor influencing UV performance, as is the case for *E. 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 an indicator microorganism after MP and LP UV disinfection. Both the cellular study and ELISA-based assay were used to investigate the inactivation efficiency. The relationship between the amount of CPDs and cell numbers of *V. cholerae* after UV irradiation was evaluated in the present study.

3.2 Materials and Methods

3.2.1 Microorganisms

V. cholerae NCTC 7253 was purchased from the United Kingdom National Collection of Type Cultures (NCTC). 1 colony of the *V. cholerae* culture from agar plate was inoculated into 30 mL of nutrient broth and shook overnight at 37 °C to prepare 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 5,000 rpm 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 approx. 10^6 CFU/mL for the UV irradiation study.

3.2.2 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 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 were determined as previously described by Bolton and Linden (2003) and Zimmer and Slawson (2002). 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.

3.2.3 Salinity experiments

V. cholerae were resuspended in two types of water (artificial seawater (ASW) and natural seawater (NSW)). ASW was prepared as described by Lleo` 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 hyposmotic 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 2-1.

3.2.4 Cultivation assay

From appropriate dilutions of the microcosms, the total number of *V. cholerae* was examined by spread plate on nutrient agar consisting of nutrient broth 13 g, agar 15 g per litre. Colonies were counted after 24 h incubation at 37 °C and recorded as CFU/mL.

3.2.5 Enzyme-linked immunosorbent assay (ELISA)

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 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 before and after UV irradiation at 5 mJ/cm^2.

3.2.6 Data analysis

The Log reduction of the test microorganisms and the kinetics of UV disinfection were calculated using Equation 1 and Equation 3 applied to *E. faecalis* in Page 34-35, Chapter 2.

All experiments were conducted three times to ensure reproducibility of the experimental data. Data are presented in mean ± standard deviation.

3.3 Results and Discussion

3.3.1 UV inactivation of *V. cholerae*

Fig. 3-1 shows the inactivation of *V. cholerae* following MP and LP UV disinfection in sterile distilled water (the controls). It can be seen that for both types of UV radiation, at high UV doses (long exposure to UV light), the inactivation rate decreased and tailed off (tailing effect), which is possibly due to shielding or clumping of the bacteria. However, such inactivation behavior was not 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. It seemed that different strains of *V. cholerae* may have different UV sensitivity, as demonstrated for *E. coli* (Malley et al., 2004; Sommer et al., 1998, 2000). It was also found that when 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 the more intense radiation and broader wavelength spectrum emitted by MP UV lamps that may cause damage to intercellular biomolecules other than DNA (Kalisvaart 2004). At higher doses (≥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*. Additionally, k_1 was 1.673 and 0.0826 for MP and LP lamps respectively (Table 3-1), which showed that inactivation of MP UV disinfection was faster than LP UV disinfection.

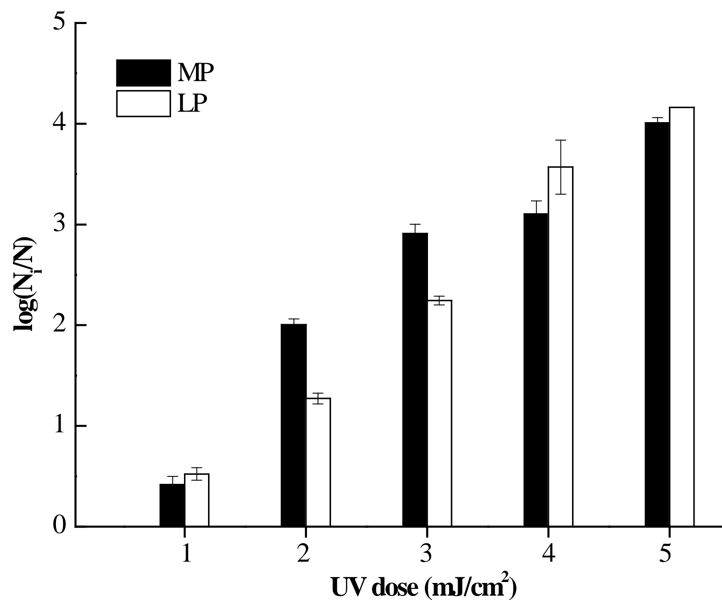

Fig. 3-1 UV inactivation of *V. cholerae* by MP and LP UV disinfection. Error bars represent standard deviations of three experiments

Previous studies have shown the sensitivity of *E. coli* ranged from 10 to 15 mJ/cm^2 (MP) (Quek et al., 2006) and 5 to 11 mJ/cm^2 (LP) (Butler et al., 1987;

Hollaender, 1942) for a 4 Log10 inactivation, while for *E. faecalis* or *V. cholerae*, a higher UV dose more than 19 mJ/cm^2 or 5 mJ/cm^2 was needed to result in the same level of reduction, revealing that in respect of the type of microorganism exposed to UV lamps, the sensitivity to UV light follows the sequence: *V. cholerae* > *E.coli*> *E. faecalis*, which was in good agreement with previous studies (Coohill and Sagripanti, 2008).

3.3.2 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 Fig. 3-2. 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 or natural seawater than in sterile distilled water with a lower log reduction and inactivation rate (k_1) shown in Fig. 3-2 and Table 3-1.

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 UV inactivation rate decreased when increasing the solution 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. It is known that organic matter and inorganic ions exposed to UV light can not only absorb UV light (Wright and Cairns, 1998) but also form radicals that interact with bacteria (Buschmann et al, 2005). The overall

effect of salinity on UV inactivation is likely dependent on whether the UV light attenuation is greater than the advantages due to radical formation. Results indicated that salinity had a detrimental effect on inactivation of *V. cholerae* at high UV doses. Therefore, ballast water salinity should be decreased before it passes through UV disinfection unit in actual operation.

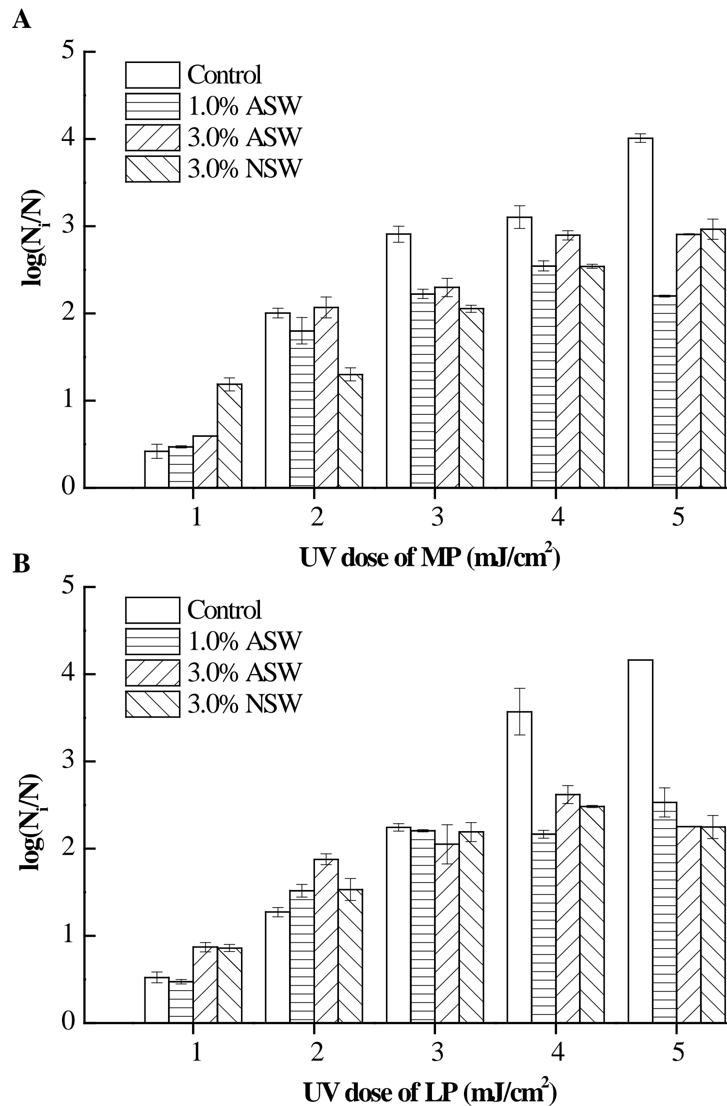

Fig. 3-2 The effect of salinity on UV inactivation of *V. cholerae* by a MP and b LP UV disinfection. Error bars represent standard deviations of three experiments. ASW: artificial seawater, NSW: natural seawater

Table 3-1 Kinetic parameters of the double first order kinetic model applied to salinity experiments

Bacterium	UV lamp	Suspension solution	Salinity (%)	k_1 (s^{-1})	k_2 (s^{-1})	σ	r^2
V. cholerae	LP	DI water	-	0.0826	0.0826	0.9000	0.9834
		ASW	1	0.0707	0.00342	0.9950	0.9743
			3	0.0813	4.50E-16	0.9958	0.9710
		NSW	3	0.0793	0.00100	0.9954	0.9899
	MP	DI water	-	1.673	0.687	0.9816	0.9741
		ASW	1	1.208	0.100	0.9925	0.9083
			3	1.607	0.253	0.9934	0.9950
		NSW	3	1.332	0.573	0.9000	0.9883

Note: DI water: distilled water.

3.3.3 DNA damage using ELISA assay

As shown in Fig. 3-3, less CPDs were formed with the increasing salinity after both MP and LP exposure, which was in accordance with the decreasing net log reduction in cell numbers at salinities of 1% or 3%. Hence, the cell number reduction in *V. cholerae* may be due largely to DNA damage, as is the case for *A. variabilis* (Sakai et al., 2007). Further support is provided by the study of Cairns and 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.

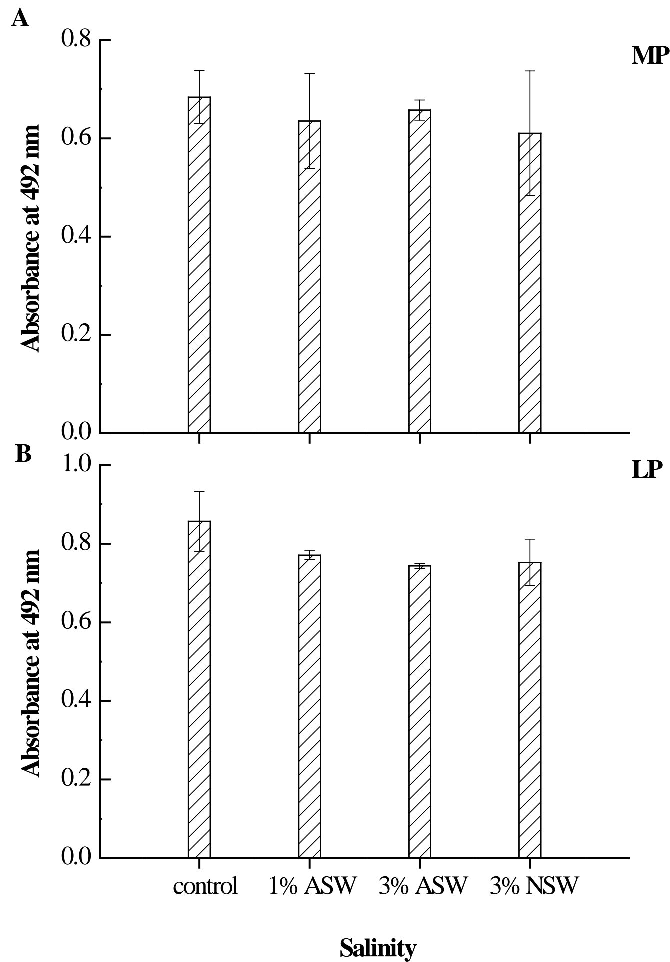

Fig. 3-3 Effect of salinity on formation of CPDs after MP and LP exposure at 5 mJ/cm^2. Absorbance at 492 nm was used as a measure of induced CPDs. Error bars represent standard deviations of three experiments. ASW: artificial seawater, NSW: natural seawater

3.4 Conclusions

In summary, *V. cholerae* was inactivated by either MP or LP UV irradiation. MP lamp leads to a higher disinfection efficiency than LP; At high UV doses, the inactivation rate decreased and tailed off. In general, high salinity can suppress inactivation effects of MP or LP UV irradiation especially at high UV doses. DNA damage is likely to contribute to cell number reduction. This study illustrates that salinity affects the inactivation efficiency of MP and LP

disinfection for ballast water treatment. 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.

CHAPTER 4 CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

Overall, the study is mainly concerned with the effects of salinity, turbidity and temperature on inactivation performance (for *E. faecalis* and *V. cholerae*) and repair potential (for *E. faecalis*) including photoreactivation and dark repair after MP and LP UV disinfection. The inactivation and repair characteristics across a range of salinities (0, 1%, 3%), turbidity (0, 1, 10, 30 NTU) and temperature (4 °C and 25 °C) of LP and MP UV lamps were compared. Additionally, with the aim of understanding the inactivation mechanisms during UV irradiation and subsequent repair, the amount of CPDs, one of the major types of DNA damage, was determined by ELISA assay, and the correlation between CPDs and inactivated bacteria was investigated. Several conclusions can be drawn from this study as follows:

1) *V. cholerae* is more sensitive to MP and LP UV than *E. faecalis*, and tailing effect is observed for the two microorganisms. The UV disinfection curves can be modelled by a double first-order equation.

2) MP UV exposure resulted in higher inactivation efficiency against these two microorganisms than LP UV exposure.

3) Effect of salinity: For *E. faecalis*, MP UV lamps were effective in a saline environment (1% or higher), whereas increased salinity level hindered LP

UV disinfection. For *V. cholerae*, high salinity (1% or higher) can suppress inactivation effects of MP and LP UV irradiation especially at high UV doses (≥3 mJ/cm^2 for MP and ≥4 mJ/cm^2 for LP). Salinity presented a beneficial effect on photoreactivation after both MP and LP exposure, whereas the effect of salinity on the dark repair is however dependent upon UV sources, that is, dark repair can be promoted after MP exposure while suppressed after LP exposure with salt concentration (1% or higher).

4) Effect of turbidity: For *E. faecalis*, the presence of turbidity (1 NTU or higher) adversely affects the inactivation efficiency after both MP and LP exposure. Higher MP UV doses of greater than 14 mJ/cm^2 appear to minimize the negative effects of turbidity on *E. faecalis*. Both photoreactivation and dark repair can be promoted with high turbidity above 10 NTU after MP exposure, whereas an inverse relationship between turbidity and repair levels were observed after LP exposure.

5) Effect of temperature: For *E. faecalis*, the presence of low temperature (4-6.5 °C for MP UV and 4-14 °C for LP UV) adversely affects the inactivation efficiency after both MP and LP exposure. Higher MP UV doses of greater than 14 mJ/cm^2 appear to minimize the negative effects of temperature on *E. faecalis*. Low temperature was found to adversely affect reactivation of *E. faecalis* following MP and LP UV disinfection.

6) The accumulation and removal of CPDs is likely to contribute to the changes of cell numbers for *V. cholerae*, whereas it seems that no clear positive correlation was found between the CFU viability assay and the ELISA assay for *E. faecalis*.

4.2 Recommendations

1) Other than these three environmental factors (salinity, turbidity and temperature), other water characteristics could be investigated for their influence on the efficiency of UV disinfection and/or regrowth following UV disinfection, such as particle size, number of particles and particle size distribution, TOC, SS, given that natural seawater contains a variety of factors other than these three environmental factors in the present study, which may potentially influence inactivation/reactivation in the marine water environment.

2) Considering that practical UV doses applied are usually around 40 mJ/cm^2, it would, therefore, be useful to have photoreactivation data of the indicator at such high UV doses.

3) Other types of DNA damage induced by UV radiation such as 6-4PPs and Dewar isomer of 6-4PPs needs to be quantified aiming for better estimating the impacts of environmental conditions on DNA damage and photorepair.

4) For *V. cholerae*, further research is necessary to evaluate effects of typical environmental conditions such as salinity, photoreactivating light intensity and temperature on photoreactivation and dark repair following MP or LP exposure.

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