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Effects of water quality on inactivation and repair of *Microcystis viridis* and *Tetraselmis suecica* following medium-pressure UV irradiation

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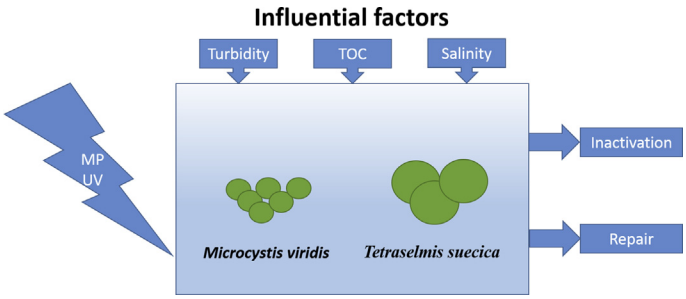
HIGHLIGHTS

- The inactivation and repair of *Microcystis viridis* and *Tetraselmis suecica* were evaluated.
- *T. suecica* was more sensitive to UV inactivation than *M. viridis*.
- Turbidity, TOC and salinity adversely affected MP UV disinfection efficiency generally.
- The levels of reactivation were dependent upon these three factors especially TOC.

GRAPHICAL ABSTRACT



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ABSTRACT

The transfer of invasive organisms by ballast-water discharge has become a growing concern. UV treatment has become an attractive ballast water treatment technology due to its effectiveness, no harmful disinfection byproducts and easiness to handle. Two robust algae strains *Microcystis viridis* and *Tetraselmis suecica* were selected as indicator organisms to determine efficiency of medium-pressure (MP) UV-treatment on ballast water. Inactivation and potential repair of these two algae strains following MP UV irradiation were assessed under various turbidity, total organic carbon (TOC) and salinity conditions. The investigated range of UV doses was from 25 to 500 mJ/cm^2. For *M. viridis*, results indicated that disinfection efficiency was negatively correlated with all of these three factors at low doses (25–200 mJ/cm^2). Photoreactivation and dark repair were promoted at high TOC levels (6–15 mg/L) with about 6–25% higher repair levels compared with those in distilled water, whereas no significant impacts were identified for turbidity and salinity on both of the photoreactivation and dark repair. For *T. suecica*, increased turbidity and TOC levels both hindered the performance of UV irradiation at high doses (200–500 mJ/cm^2). Suppressive effects on photoreactivation and dark repair were consistently observed with changes of all of the three factors. In conclusion, generally these three factors resulted in repressive effects on UV disinfection efficiency, and TOC played a more significant role in the levels of reactivation than the other two. The responses of *T. suecica* to these three factors were more sensitive than *M. viridis*.

1. Introduction

Ballast water is water carried by ships to ensure stability, trim and structural integrity. But by this way, it has become a primary

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vector for transference of various species with bacteria and virus-like particles (VLPs) dominating ballast water biota around the world (Kim et al., 2015), which has negative impacts on the environment through factors such as competition for food, altered substrate/ambient temperature and light availability (Sutherland et al., 2001). For example, Cholera infections could result from discharge of ballast water (McCarthy and Khambaty, 1994). Nearly all known harmful algal bloom species have been documented in viable form from ship's ballast water (Hallegraeff, 2015). 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.

Ultraviolet (UV) radiation, a tried, tested method in water/waste water management has been adopted, accounting for almost 25% of the current installations (Lloyd's Register, 2010). It is very effective to kill most of the organisms carried by the ballast water: invertebrates and their eggs (Raikow et al., 2007), viruses (Guo et al., 2010; Hijnen et al., 2006), bacteria (Hijnen et al., 2006; Rubio et al., 2013), protozoan (oo)cysts (Hijnen et al., 2006), phytoplankton (Tao et al., 2013) and other microbes. The two most commonly used UV lamps are medium-pressure (MP) and low-pressure (LP) mercury UV lamps, the former emitting a broad spectrum of wavelengths in the UV radiation region ranging from 200 to 400 nm (Masschelein, 2002), whereas the latter emits monochromatic UV radiation at 254 nm. Malley (1999) found that a single MP UV lamp has the same disinfection capacity as 25 LP UV lamps with the same size because of its higher intensity, which makes the MP UV lamps become a cost-effective alternative to LP UV lamps. Since the 1990s, MP UV lamps have become more and more popular and the number of water treatment plants applying MP UV lamps continues to increase.

However, repair capability of microorganisms via photoreactivation or dark repair is one of the limitations of UV disinfection technology (Schwartz et al., 2003). DNA repair is a potential drawback of UV disinfection, which is prevalent among many organisms such as bacteria (Goosen and Moolenaar, 2008; Hu and Quek, 2008), cyanobacteria (Levine and Thiel, 1987), plants (Britt, 1996) and can deteriorate the disinfection performance of UV radiation. In addition, several factors have been reported to affect UV inactivation and repair after inactivation, such as turbidity (Passantino et al., 2004), salinity (Oguma et al., 2013; Rubio et al., 2013) and organic matters (Cantwell et al., 2008; Ou et al., 2011). However, the effects of these factors on the UV inactivation and repair of microalgae have little been studied, and there could be difference between algae and bacteria in terms of water quality effects due to their different cellular sizes and compositions. 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 turbidity, TOC and salinity on the inactivation and repair of microalgae for comparison with the previous works. Hence, the limitations of UV technology such as poor penetration and high requirements for pretreatment can be better addressed when treating ballast water.

Microcystis viridis (5 μm in diameter) and *Tetraselmis suecica* (9.5 μm in diameter) were selected as indicators of cyanobacteria and chlorophyta respectively to highlight potential different responses between species. At present there is no discharge standard for the majority of bacteria and eukaryotes <10 μm in ballast water. However, microalgae is very resistant to UV radiation (Holzinger and Lutz, 2006) and if it is not treated well, algal bloom can break out, which will cause undesirable odour to the water and generate algal toxin (Babaran et al., 1998). Hence, the aims of the present work were to point out the efficiency of MP UV toward inactivation of *M. viridis* and *T. suecica,* and the effects of turbidity, TOC and salinity on inactivation efficiency and reactivation phenomena for better control of the growth of these two microalgae.

2. Materials and methods

2.1. Microorganisms

M. viridis was obtained from Freshwater and Invasion Biology Lab (FIBL) and *T. suecica* was provided by Tropical Marine Science Institute (TMSI) at National University of Singapore. The cultivation medium for *M. viridis* and *T. suecica* were MLA (Bolch and Blackburn, 1996) and F2 medium (Guillard and Ryther, 1962), respectively. These two microalgae were cultured in cell culture flask on the cultivation shelf at 25 ± 1 °C under a photoperiod of 12 h of light and 12 h of dark (12 L:12 D) at 3000–4000 lux.

Log-phase microalgae culture was used to study the UV disinfection and repair performance in this study. The cells were collected by centrifugation (4500 g, 5 min), washed twice with 9 ml of distilled water, and subsequently suspended in distilled water or natural or synthetic water under different levels of turbidity, TOC and salinity, achieving a concentration of approximately 10^6 cells/mL.

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 a MP (1 kW) UV lamp. 10 mL of the diluted microalgal suspension was dispensed into a 6 cm diameter sterile plastic Petri dish and exposed to MP UV radiation. The investigated UV doses ranged from 25 to 500 mJ/cm^2 and were determined as previously described by Zimmer and Slawson (2002). Microalgal suspensions were stirred throughout the irradiation process. Triplicate 100 μL samples were taken before and after irradiation for microalgae viability quantification via real-time polymerase chain reaction (RT-PCR), while the rest of the sample was covered and used for photoreactivation and dark repair studies.

(i) Turbidity effect study. Kaolin clay (mean particle size: 2.649 μm) with tendency to swell and 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 10–15 NTU (Waite et al., 2003), whereas the variability in the turbidity of seawater between locations and over time has been reported in previous studies (2–30 NTU, Desormeaux et al., 2009; <10 NTU, Lauri et al., 2010). Therefore, UV exposure was performed in three levels of turbid 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.).

(ii) TOC effect study. Humic substances are one of the principal organic constituents in natural water with concentrations in the range of several mg/L to several tens of mg/L (Wang et al., 2012). The concentration of humic substance in natural water is in the range of 0.03–30 mg C/L (Shinozuka, 1996). In the present study, the concentration of humic acid was from 3 to 15 mg/L and within the limit of 30 mg C/L. Humic acid (Sigma-Aldrich, Switzerland) stock solution was prepared by stirring the humic acid solution overnight, and followed by filtration with 0.45 μm membrane. After that, the solution was diluted to get 3, 6, and 15 mg/L TOC. TOC-V_{CSH} Total Organic Carbon (TOC) analyzer (Shimadzu, Japan) was employed to measure TOC of samples.