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Evaluation of residual toxicity of hypochlorite-treated water using bioluminescent microbes and microalgae: Implications for ballast water management

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ABSTRACT

Total residual oxidants (TRO) in treated ballast water can produce various disinfection by-products (DBPs) depending on local conditions, such as salinity and organic matter content in water. Because TRO and DBPs are known to be harmful to aquatic organisms and humans, ecotoxicity tests have been proposed for screening the residual toxicity before discharging treated ballast water. In the present study, we aimed to address the decay rates and toxicity changes of TRO under various conditions in salinity, initial TRO concentrations, and residence time of TRO. In addition, the toxicological sensitivities of bioluminescent bacteria *Vibrio fischeri* and a commonly-used microalgae *Skeletonema costatum* relative to the residual toxicity of TRO and six selected DBPs were determined. Decay rate of TRO concentration increased as a function of salinity and was affected by the initial concentrations of TRO. Unexpectedly, significant bioluminescence inhibition was observed for hypochlorite-treated water at $< 0.1\ mg\ L^{-1}$ TRO (expressed as Cl_2), which is a lower concentration than the maximum allowable discharge concentration (MADC) to marine waters established by the International Maritime Organization (IMO). The ecotoxicological thresholds of no observed effective concentration and median effect concentration for all tested DBPs were about 3–10 times lower for *V. fischeri* than for *S. costatum*. The results indicate that bioluminescent microbes possess an ecologically-relevant sensitivity to both TRO and DBPs in ballast water. In general, bioassay using *V. fischeri* was potentially more effective than microalgae for screening the total toxicity of TRO and DBPs in treated ballast water, especially given that ballast water usually contains a highly variable and complex mixture of toxicants.

1. Introduction

The discharge of ballast water has been a concern since it was known to be a major route by which invasive aquatic species occur and spread into new habitats (Ruiz et al., 1997). Alien invasive marine species often has threatened native ecosystems, adversely affecting local aquatic-based economic activities (such as fisheries) and human fatalities. To minimize such problems, the International Maritime Organization (IMO), in 2004, adopted to propose the international convention for the control and management of ship ballast water and sediment, which requires that discharged ballast water should meet specific water quality standards with respect to the concentration of living organisms (IMO (International Maritime Organization), 2004). The standards also make it mandatory for ships to install a ballast water management system (BWMS) on board.

Electrolysis, ultraviolet irradiation, ozonation, and thermal treatment are typically used for treating ballast waters (Delacroix et al., 2013; Duan et al., 2016; Tsolaki and Diamadopoulos, 2010; Werschkun et al., 2014). Although electrolysis which most technically efficient and cost-effective treatment approach is widely-used, it creates both free chlorine (Cl_2) and chlorine compounds in ballast water (Duan et al., 2016). These active substances (ASs) are defined as concentration of total residual oxidants (TRO), quantified as free chlorine (IMO, 2007). The highly reactive TRO are adversely impact physiological and

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metabolic processes of phytoplankton by damaging cell membranes, leading to leakage of intracellular materials, reduction in photosynthetic efficiency, and denaturation of proteins and nucleic acids (Virto et al., 2005). This is why the IMO requires that TRO be neutralized before being discharged into aquatic environments. In addition, approval for the use of ASs, including chlorine, must be granted by the Marine Environment Protection Committee (MEPC) of the IMO in accordance with mandated procedures established for BWMS (G9 protocol) (IMO, 2007, 2008).

The formation of disinfectant by-products (DBPs) which impact aquatic organisms and marine ecosystems have been a problem for most BWMS using ASs to treat ballast water. The most-frequently occurring DBPs in chlorinated ballast water include bromoform (BF), monobromoacetic acid (MBAA), bromodichloromethane, and bromoacetonitrile (Fabbricino and Korshin, 2005). The formation of DBPs has been observed variously depending on environmental parameters such as salinity, residence time, bromide content, and initial properties of the ballast water (Delacroix et al., 2013; Gregg et al., 2009; Richardson et al., 2007; Zhang et al., 2013). Previous studies have examined how DBPs are formed during the treatment freshwater used for drinking (Delacroix et al., 2013; Werschkun et al., 2014), but there is still limited information on the formation, fate, and effects of DBPs in treated seawater (Čulin and Mustać, 2015; Fisher et al., 2014). DBPs could potentially cause cytotoxicity, carcinogenicity, and mutagenicity in organisms (Richardson et al., 2007; Werschkun et al., 2014). However, these toxicities cannot be neutralized and some DBPs can persist for long periods of time in marine environments, and could bioaccumulate in organisms (Gregg et al., 2009; Lee et al., 2015). Thus, the IMO requires that ecotoxicological tests and chemical analyses of potential DBPs in seawater be tested prior to discharge. In fact, the IMO mandates that at least two different types of water tests be conducted (e.g., seawater, brackish water, and/or freshwater) before the Marine Environment Protection Committee provides approval for a BWMS to operate.

For many approved BWMS, it is difficult to assess treated ballast water within a practical time frame. This is because most DBP analyses and ecotoxicity tests take days to weeks to perform, even by trained staff in well-managed laboratories. Thus, there is a need for compliance monitoring and ecotoxicity tests for ballast water that are both simpler to use and can be more rapidly performed. Currently, under the G9 protocol, testing of microalgae, invertebrate, and vertebrate (e.g., fish) taxa must be examined in whole-effluent toxicity (WET) tests for ballast water after being treated. Of these three taxa, microalgae are the most frequently utilized test organisms because they are highly sensitive to treated ballast water (IMO, 2007).

Even though bacteria are a very important component of aquatic ecosystems and they have been already established as standard test organisms by the International Organization for Standardization (ISO, 1134-2008), toxic effects of ASs and/or DBPs on bacteria have not been applied (ISO, 2007). It would be a practical, rapid, and sensitive assessment tool to use a microbial assay with a bioluminescent bacterium for residual toxicity evaluation of treated ballast water. The specific aims of this study were to: (1) measure decay rates of TRO (total residual oxidants) under various salinity conditions and initial TRO concentrations; (2) evaluate changes in ecotoxicity after injecting hypochlorite (as TRO) relative to water salinity and residence time of the TRO; and (3) compare toxicological sensitivities of the bioluminescent bacteria (*Vibrio fischeri*) and the commonly-used microalgae (*Skeletonema costatum*) by determination of residual toxicity of TRO and six selected DBPs.

2. Materials and methods

2.1. Experimental settings

We created a stock solution by adding sodium hypochlorite (NaOCl) (Sigma-Aldrich, St. Louis, MO) to millipore water filtered through a 0.22 μm filter. This stock solution was injected into artificial seawater (for use in our TRO decay tests) in an amount sufficient to produce initial residual oxidant concentrations of 0.31, 0.63, 1.3, 2.5, 5.0, and 10 mg L^{-1} (expressed in TRO as Cl_2) for use in our TRO decay tests (Dataset I) (Table 1). A TRO nominal concentration gradient from 0.15 to 5 mg L^{-1} was established for Dataset II, whereas a gradient from 0.08 to 10 mg L^{-1} was established for Dataset III. TRO decay was determined periodically: Dataset I provided a long-term (120 h) evaluation of TRO (sampled at 0, 24, 48, 72, 96, and 120 h), whereas Dataset II provided a short-term (24 h) evaluation of TRO (sampled at 0, 1, 2, 3,

Table 1
Experimental design of the three Datasets (I, II, and III) examined in this study.

	Dataset I	Dataset II	Dataset III	
Specific purpose	Determination of TRO decay rates at varying conditions of salinity and initial concentrations	Evaluation of toxicity changes after injecting TRO at (1) three salinity conditions and (2) over time	Comparison of toxicological sensitivity between assays of *V. fischeri* and *S. costatum* on TRO and 6 DBPs	
Test species		*V. fischeri*	*V. fischeri*	*S. costatum*
Experimental conditions				
Exposure concentrations of TRO (mg L^{-1})	0.31–10 (6 levels)	(1) 5 (2) 0.15–5 (6 levels)	0.08–10 (9 levels)	0.08–10 (9 levels)
Exposure concentrations of DBPs (mg L^{-1})	–	–	0.78–100	[a]7.81–1000 (8 levels) [b]3.91–500 (8 levels)
Salinity treatments (psu)	2.7, 21.3, and 33.2 ± 0.1	(1) 2.7, 21.3, and 33.2 (± 0.1) (2) 33.2 ± 0.1	33.2 ± 0.1	33.2 ± 0.1
Temperature (°C)	25 ± 1	25 ± 1	15	20
Test duration (hours)	120	120 and 24	0.5	72
Initial cell concentrations (cells mL^{-1})	–	–	–	1–2 × 10^4
Replicates	3	3	3	3
Measurement	TRO concentration	Luminescence inhibition (EC_{50})	Luminescence inhibition (NOEC, LOEC, & EC_{50})	Growth inhibition (NOEC, LOEC, & EC_{50})
Data presented in	Fig. 1	Fig. 2	Figs. 3–4 and Table 2	

[a] Concentrations for bromate and chloroform.
[b] Concentrations for bromoform, monochloroacetic acid, dichloroacetic acid, and monobromoacetic acid.