Study on the killing of oceanic harmful micro-organisms in ship’s ballast water using oxygen active particles

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Abstract. Global Environment Facility has identified that the spread of marine invasive alien species is one of the four major risk factors threatening the safety of global marine environments. Ballast water discharge is the main cause of biological invasion. With physical methods of strong electric field ionization discharge at atmospheric pressure, O₂ and sea water (gaseous) were ionized, and then dissociated to a number of oxygen active particles (ROS) such as ·OH, O₂⁺, H₂O⁺, etc. ROS was injected into 0.6 t h⁻¹ ballast water treatment system to form high concentration ROS solution in order to kill the harmful micro-organisms in ballast water. According to the land-based test standard of International Maritime Organization (IMO) Guidelines for Approval of Ballast Water Management Systems (G8), this paper concludes that single-cell algae of 3.0×10⁴ cell ml⁻¹ and bacteria of 2.0×10⁴ cfu ml⁻¹ were killed by ROS solution of 2.0 ppm. Death rate could reach almost 100%. The results meet the requirements of Regulation D-2 of International Convention for the Control and Management of Ships’ Ballast Water and Sediments completely.

1. Introduction
Invasion of native biotas by non-indigenous species is a threat to the integrity of biotic communities, the economy and even human health. The introduction of invasive marine species into new environments by ship's ballast water has been identified as one of the four greatest threats to the world’s oceans [1]. With the development of economic globalization, marine shipping industry has occupied an important part of world trade, and its cargo has carried more than 90 percent of all goods in world trade [2]. Ships’ ballast water as a major bio-invasion pathway is increasing environmental threats [3]. Ballast water containing marine plankton is carried by ship. When the ship reaches other harbor, the ballast water with the plankton including bacterial virus, algae, sea fish, small invertebrates, spores, etc is discharged into local water [4]. According to report, approximately 80000 ships all over the world carry more than 10 billion tons of ballast water every year [5]. Migratory species in the ballast is about 10000 every day in the global scope. So far, the number is still increasing. Every year, biological invasion of ballast water of the global scope causes the economic losses about billions of dollars, and the number is increasing year after year.

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In order to reduce the effects of discharge of ballast water on ecological environment, International Maritime Organization (IMO) issued “Ships ballast water and sediment control and management of International Convention” in February 2004. In the Convention, from 2009 onwards, all new ships should be installed the ballast water treatment equipments. By 2016, all ships must be installed the ballast water treatment equipments. At present, there are several methods for treating ballast water. The most common techniques are as follows: ballast water exchange, ultraviolet, electrolytic and ozone [6-9]. However, these methods can’t kill the microorganism in ballast water completely. The treated ballast water can’t reach D2 emissions standards formulated by IMO.

Advanced oxidation technology (AOT) based on the classical chemical oxidation method [10], which can produce a large number of •OH and induce a series of •OH chain reactions, resulting in removing the organisms and other pollutants and eventually become H₂O and CO₂ [11, 12]. The technique provides a green pollution-free new approach for the treatment of ballast water.

2. Materials and methods

2.1 Discharge process of strong electric field at atmospheric pressure

In our research, the plasma discharge of strong electric-field was consist of large numbers of micro-streamers. This process involved a well-distributed, diffuse and stable discharge phenomenon akin to glow discharge. In micro-streamer channels, lots of active particles produced giving rise to a series of plasma chemistry reactions.

![Figure 1. Schematic diagram for the formation of micro-streamer in strong-field ionization discharge.](image)

Under the conditions of the 0.47 mm discharge gap, a power supplying with the frequency in the range of 5-20 kHz and the peak voltage in the range of 8-20 kV, the strong electric field could be obtained in the discharge gap. When electric field strength (E) was higher than 100 kV·cm⁻¹, the average electron energy was higher than 10 eV, electron density was higher than 10¹⁴ cm⁻³ and the ionization duty cycle was higher than 2%. As shown in figure 1, the average electron energy of the electrons in the electric field was greater than 10 eV. Particularly in the discharge channel, the electron temperature was more than 12 eV. Electron energy was greater than the ionization energy of N₂ and H₂O. Figure 2 was a structure diagram of plasma source.
2.2 Formation of oxygen active particles

In the atmospheric electric field discharge, the active particles ROS contained •OH, O₂⁺, O₂⁻, O₃, H₂O₂, H₂O₂, HO₂⁻, etc., and •OH was the primary active particle. •OH was generated by three reaction pathways, as shown in figure 3 [9, 13]. Firstly, H₂O₂ can be decomposed inside the plasma to give HO₂⁻ radical. HO₂⁻ was an important initiator of generating of •OH, which serves as the main reaction. The reaction rate constant of producing •OH was 2.2 × 10⁶ L (mol s)⁻¹ which was three times higher than the HO⁻ average reaction rate constant of 70 L (mol s)⁻¹. Under the action of HO₂⁻, active particles such as O₃, O₂⁻, etc., were efficient to generate •OH.

2.3 Experimental systems and parameters

In our research, we developed 0.6 t h⁻¹ ballast water treatment equipment and the core technology was plasma source (figure 4). This equipment was used to deal with harmful micro-organisms in ballast...
water. Original sea water was obtained from Guanglu Island, Dalian. The salinity was 28.1 with practical salinity unit (PSU) and the temperature was 18.7 °C. As the detection index for concentration of ROS, TRO was defined as all the oxidants which could kill harmful micro-organisms. TRO was mainly composed of ·OH and other active oxygen particles such as HO₂⁻, O₂²⁻, HO₃⁺, O₂⁺, H₂O, etc, which were generated during the ROS chain reactions in ballast water. Experiments lasted for 5 days, including control tank and treated tank tests.

![Figure 4. Schematic diagram for 0.6 t h⁻¹ ballast water treatment equipment.](image)

According to G8, phytoplankton as the research objects should be selected in less than 50 microns in size and five species in three phyla. Therefore, phytoplankton were selected as follows: *Tetraselmis*, *porphyridiun*, *Cylindrotheca*, *Alexandrium* and *Karenia mikimotoi*, whose concentration were 0.70×10⁴ cell ml⁻¹, 0.72×10⁴ cell ml⁻¹, 0.68×10⁴ cell·ml⁻¹, 0.70×10⁴ cell·ml⁻¹ and 0.67×10⁴ cell·ml⁻¹, respectively. Total concentration of algae was 3.47×10⁴ cell·ml⁻¹. Heterotrophic bacteria should be present in a density of at least 10⁴ living bacteria per millilitre, so three kinds of bacteria (*Heterotrophic bacteria*, *Escherichia coli*, *Enterococcus*) were selected as the experimental bacteria, concentration were 2.03×10⁴ cfu ml⁻¹, 0.96×10⁴ cfu ml⁻¹ and 0.72×10⁴ cfu/ml⁻¹, respectively. Concentrations of ROS solution were ranged from 0-2.0 ppm.

3. Results and Discussion

The experimental results for the ballast water treatment were shown in table 1. The result presented that single-cell algae of 3.0×10⁴ cell·ml⁻¹ and bacteria of 2.0×10⁴ cfu·ml⁻¹ were killed by ROS solution of 2.0 ppm. After detecting 150 mL treated water sample, the results showed that the concentration of algae and bacteria were 7 cell·ml⁻¹ and 3 cfu ml⁻¹, respectively. Death rate reached almost 100 %. It reached the requirement of Regulation D-2 of International Convention for the Control and Management of Ships’ Ballast Water and Sediments completely. The ROS concentration in the treated tank declined over time during storage for 5 days. The TRO in the treated tank was advantageous to inhibit the re-growth of algae and bacteria. Until the fifth day, the micro-organisms did not reanimate, indicating that harmful micro-organisms in ballast water were rapidly killed by ROS solution.

Different algae and bacteria had different morphology, so the treatment effects of ROS were also different. As shown in figure 5, with the increase of ROS solution concentration, death rate of *Alexandrium*, *porphyridiun* and *Cylindrotheca* increased gradually, which indicated the survival rate of algae declined gradually. But the same kind of algae with different original concentration had different lethal concentration of ROS solution. For the concentration of 0.7×10⁴ cell ml⁻¹ of *Alexandrium*, when ROS solution concentration increased to 1.2 ppm, its lethal effect reached to inflection point and a large number of deaths appeared. Equally, for the concentration of 0.72×10⁴ cell ml⁻¹ of *porphyridiun*, its death inflection point also appeared in 1.2 ppm of ROS solution. However, for *Cylindrotheca*, whose concentration was 0.68×10⁴ cell ml⁻¹ and less than concentrations of *Alexandrium* and *porphyridiun*, the death of inflection point extended to 1.25 ppm. In comparison to
three kinds of algae with the same concentration, *Cylindrotheca* died with higher concentration of ROS solution due to the morphology structure. *Cylindrotheca* belonged to the *Bacillariophyta*, and its surface had chitin carapace that could resist ROS, therefore, greater dose of ROS solution was needed.

**Table 1.** Five day ballast water treatment experiment.

| Storage time | 0 h | 48 h | 120 h |
|--------------|-----|------|-------|
| item         | control | treated | control | treated | control | treated |
| **TRO (mg L⁻¹)** | 0 | 2.0 | 0 | 0.21 | 0 | 0 |
| *Tetraselmis* (cell ml⁻¹) | 0.70×10⁴ | 1 | 0.40×10⁴ | 0 | 0.21×10⁴ | 0 |
| *Porphyridium* (cell ml⁻¹) | 0.72×10⁴ | 0 | 0.38×10⁴ | 0 | 0.20×10⁴ | 0 |
| *Cylindrotheca* (cell ml⁻¹) | 0.68×10⁴ | 5 | 0.42×10⁴ | 0 | 0.25×10⁴ | 0 |
| *Alexandrium* (cell ml⁻¹) | 0.70×10⁴ | 1 | 0.37×10⁴ | 0 | 0.19×10⁴ | 0 |
| *Mikimoto* (cell ml⁻¹) | 0.67×10⁴ | 0 | 0.41×10⁴ | 0 | 0.21×10⁴ | 0 |
| Total algae (cell ml⁻¹) | 3.47×10⁴ | 7 | 1.98×10⁴ | 0 | 1.06×10⁴ | 0 |
| **Heterotrophic bacteria (cfu ml⁻¹)** | 2.03×10⁴ | 3 | 1.96×10⁴ | 1 | 1.92×10⁴ | 1 |
| *Escherichia coli* (cfu ml⁻¹) | 0.96×10⁴ | 1 | 0.91×10⁴ | 0 | 0.87×10⁴ | 0 |
| *Enterococcus* (cfu ml⁻¹) | 0.72×10⁴ | 0 | 0.7×10⁴ | 0 | 0.66×10⁴ | 0 |

In figure 6, two kinds of bacteria *Escherichia coli* and *Enterococcus* had no significant difference in the lethal dose due to the simple structure of bacteria and ROS was effective for them. *Escherichia coli* and *Enterococcus* began to show a large number of deaths in 0.8 ppm of ROS solution. The death rate reached approximately 100 % when the concentration increased to 1.5 ppm.

**Figure 5.** Relationship between the concentration of algae and the lethal concentration of ROS.

**Figure 6.** Relationship between the concentration of bacteria and the lethal concentration of ROS.
4. Conclusions
With physical methods of strong electric field ionization discharge at atmospheric pressure, $\text{O}_2$ and sea water (gaseous) are ionized, and then dissociate to a number of oxygen active particles (ROS) such as $\cdot\text{OH}$, $\text{O}_2^+$, $\text{H}_2\text{O}^+$, etc. In order to kill the harmful micro-organisms in ballast water, ROS is injected into $0.6 \text{t h}^{-1}$ ballast water treatment system to form a high concentration ROS solution. In transport process of the ship ballast water, harmful micro-organisms are killed rapidly and efficiently.

Algae of $3.47\times10^4 \text{cell ml}^{-1}$ and bacteria of $2.03\times10^4 \text{cfu ml}^{-1}$ are killed by the 2.0 ppm of ROS solution. The experimental results show that the concentrations of micro-organisms are all less than 10 unit ml$^{-1}$. Death rate reaches approximately 100%. The micro-organisms do not reanimate within 120 h. The treated water reaches the requirements of regulation D-2 of International Convention for the Controlling and Management of Ships’ Ballast Water and Sediments completely.

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