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The effects of organic compounds on inactivation efficacy of *Artemia salina* by neutral electrolyzed water

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ABSTRACT

The introduction of aquatic non-indigenous organisms and pathogens via ships ballast water is considered as a great treat to global marine ecosystems, costal economy and public health. This issue can be efficiently addressed by electrolytic treatment technology. In this work, the effects of organic compounds on inactivation efficacy of neutral electrolyzed water were studied by bench scale test and land-based test with *Artemia salina* as an indicator organism. In bench scale test, both glucose and chitosan significantly accelerated TRO decay of neutral electrolyzed water, while the other kinds of dissolved organic compounds (sodium citrate and sucrose) and particle organic compounds (corn starch) had no significant effect on TRO decay. Both glucose and chitosan weakened the inactivation efficacy of neutral electrolyzed water, especially at 24 h. The results of land-based test also indicated that the inactivation efficacy of ballast water management system not only depended on TRO initial concentration, but also on the property of organic compounds. The presence of glucose and chitosan indicated the need of high level of initial TRO concentration to reach good inactivation efficacy. The results of this study may be useful to decide the initial TRO concentration in ballast water treatment with electrolysis technology.

1. Introduction

Ballast water (BW) is commonly used to maintain the balance and structural integrity of ships during the voyage (Qi and Eames, 2015). The introduction of aquatic non-indigenous organisms and pathogens via ships ballast water is considered to be one of the greatest threats to global marine ecosystems, coastal economy and public health (Carney et al. 2011; First et al., 2013; Hua and Hwang, 2012; Niimi and Reid, 2013). In order to regulate the discharge of ballast water and reduce the risk of introducing invasive species by ballast water, the International Maritime Organization (IMO) adopted *The International Convention for the Control and Management of Ships Ballast Water and Sediments* in 2004. Ships are required to treat ballast water to meet regulation D-2 in accordance with the timetable listed in the convention.

Therefore, a number of ballast water treatment technologies are being developed and tested for their abilities to prevent the introduction of non-indigenous species. These technologies include filtration (Tang et al., 2006), hydrocyclone physical separation techniques (Veldhuis et al., 2006), various oxidizing and non-oxidizing biocides (Carbona et al., 2010; Maranda et al., 2013; Wright et al., 2009; Slooten et al., 2014), ultraviolet treatment, thermal treatment, sonication (Gavand et al., 2007; Holm et al., 2008), ozonation (Perrins et al., 2006) and so on. Among them, electrolytic treatment technology has distinguished itself as the most promising technology, due to the advantages of onsite generation of oxidants, good inactivation efficacy, low energy consumption and low foot print (Jung et al., 2013; Lacasa et al.,2013; Nanayakkara et al., 2011; Zhang et al., 2013; Wu et al., 2011).

According to pH, electrolyzed water is categorized into acidic electrolyzed water (electrolyzed oxidizing water) and neutral electrolyzed water (NEW). The electrolyzed water used in the treatment of ballast water is mainly referred to as neutral electrolyzed water, which is produced by the electrolysis system without a membrane separating the anode and the cathode. The main active constituents of neutral electrolyzed water are sodium hypochlorite, chlorine, bromide, hypobromous acid, hydrogen peroxide, oxygen free radical, hydroxyl radical and other disinfection byproducts (DBPS). These oxidants produced by electrolysis can oxidize the tissue, enzyme and DNA/RNA of organisms and lead to their death. The level of oxidants in water is measured as total residual oxidant (TRO).

Neutral electrolyzed water has a broad inactivation efficacy on organisms such as *Bacillus subtilis* spore (Jung et al., 2013), *Escherichia coli* (Lacasa et al., 2013; Nanayakkara et al., 2011;

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Nanayakkara et al., 2012; Perrins et al., 2006), *Enterococcus faecalis* (Nanayakkara et al., 2011), *Dunaliella salina* (Wu et al., 2011), *Microcystis aeruginosa* (Liang et al., 2005), and *Artemia salina* (Lacasa et al., 2013; Tsolaki et al., 2010). The efficacy of neutral electrolyzed water in the treatment of ballast water depends on TRO concentration and holding time. An increase in water salinity has a beneficial effect on inactivation efficacy (Lacasa et al., 2013). Conversely, high dissolved organic carbon has a negative effect on inactivation efficacy. Bryan C. Nielsen study showed 3.0 mg/L TRO was the minimum dosage to sufficiently inactivate the organisms in ballast water. In order to sufficiently inactivate organisms in the presence of high organic carbon, the TRO concentration should be not less than 13 mg/L (Nielsen, 2006). Meanwhile, chlorine is highly selective towards organic compounds. For the reaction of chlorine with organic compounds, second order rate constants can vary over more than 10 orders of magnitude (Deborde and Gunten, 2008). Therefore using total organic carbon as a probe to decide the initial TRO concentration has the potential to overestimate or underestimate the dosage of TRO. However, few of the previous studies provided much information about the effect of organic compounds on the inactivation efficacy of neutral electrolyzed water.

The objective of this study is therefore to investigate the effects of organic compounds on the inactivation efficacy of neutral electrolyzed water with *A. salina* as an indicator. The results would be useful to decide the initial TRO concentration in ballast water treatment.

2. Materials and methods

2.1. Preparation of Artemia salina larvae

Natural seawater from Jiaozhou Bay was used throughout the experiment process. *A. salina* in the form of dehydrated cysts was provided by Tianjin Fengnian Aquaculture Co., Ltd. *A. salina* was hatched with the procedure described by E. Tsolaki (Tsolaki et al., 2010). *A. salina* cysts were placed in seawater at 3 g/L and illuminated by natural light. After 24 h incubation, instar I-nauplii were separated from the unhatched cysts and hatching debris, and then transferred into a beakers containing 0.45 μm filtered natural sea water. The active larvae were chosen for further experiments.

2.2. Bench scale test

2.2.1. Preparation of neutral electrolyzed water

Neutral electrolyzed water (NEW) was prepared by electrolyzing marine water. Electrolysis was performed with an advanced electrochemical system (PARSTAT 2273, Priceton Applied Research, USA) under galvanostatic conditions. A 5500 mL single cell was used. A commercial dimensional stable anode (DSA) (Supplied by Xian Taijin Industrial Electrochemical Technology Co., Ltd.) was used as an anode electrode. The anode electrode was made of Ti and covered by Ru-Ir-Ta foil. The geometric area of the anode was 4 cm^2. The cathode electrode was a Pt rod. The electric current was 0.8 A.

2.2.2. The influence of organic compounds on the decay of TRO

To determine the influence of organic compounds on TRO decay, appropriate amounts of glucose, sucrose, sodium citrate, chitosan and corn starch were added into brown bottles respectively. NEW was freshly prepared using the method in 2.2.1. Immediately after TRO analysis, NEW was divided into bottles, with 1000 mL NEW in each bottle. The concentrations of organic compound in the bottles were in the range of 1–10 mg/L. All the tests were performed in triplicate. The group with no organic compounds was used as blank. At 2 h, 24 h and 120 h, 50 mL NEW was taken from bottles and used to analyze TRO concentrations.

TRO was measured according to the standard method of 4500 Cl-B method I (Eaton et al., 2005).

2.2.3. The influence of organic compounds on the inactivation of Artemia salina

To determine the influence of organic compounds on the inactivation of *A. salina* by NEW, 125 mL brown bottles were used. Appropriate amounts of glucose and chitosan were added to bottles respectively. 100 mL fresh NEW parallel with the experiment in 2.2.2 was added to each 125 mL bottle. The concentrations of organic compound were in the range of 1–7 mg/L. Then 15 *A. salina* nauplii larvae were added to each bottle. The bottles were stored in darkness at 20 °C for 120 h. All the tests were performed in triplicate. The group only with sea water was used as control. At 2 h, 24 h and 120 h, the samples were observed by the zooplankton counter under 3D digital microscope (HIROX, KH-8700, USA). The live-dead judgment was made according to the movement of organisms. The completely motionless larvae were counted as dead.

The mortality of *A. salina* was calculated with the following equation:

Mortality $(\%)=(N_0-N_t)/N_0\times 100$,

where, N_0 was the initial live number of *A. salina* and N_t was the live number at time t.

2.3. Land-based test

2.3.1. Test facility

The land-based testing facility with flow rate of 250 m^3/h was established in Tuandao harbor, Qingdao in accordance with the IMO Guidelines (G8 Guidelines for Approval of Ballast Water Management Systems). There were four simulated tanks in total, each with a volume of 250 m^3. Two tanks (culture tanks) were used for preparing challenge water. The other two tanks were used

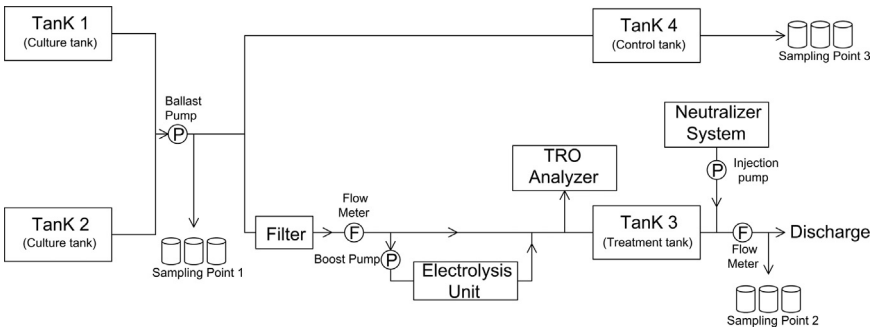

Fig. 1. Flow chart of ballast water treatment and sampling points of the land based test.