Ballast water treatment and bacteria: Analysis of bacterial activity and diversity after treatment of simulated ballast water by electrochlorination and UV exposure☆

Nanna B. Petersen [a], Torben Madsen [b], Mikkel A. Glaring [a], Fred C. Dobbs [c], Niels O.G. Jørgensen [a,*]

[a] Section for Microbial Ecology and Biotechnology, Department of Plant and Environmental Sciences, University of Copenhagen, 1871 Frederiksberg, Denmark
[b] DHI, Agern Alle 5, 2970 Hørsholm, Denmark
[c] Ocean, Earth & Atmospheric Sciences, Old Dominion University, 406 Oceanography & Physical Sciences Building, Norfolk, VA 23529, USA

HIGHLIGHTS

- Bacterial regrowth was found in ballast water treated by UV and electro-chlorination.
- Potential pathogens (*E. coli*, *Vibrio* spp., enterococci) survived both treatments.
- γ-*Proteobacteria* replaced α-*Proteobacteria* as dominant bacteria after regrowth.
- Treatment-introduced cell damage caused only minor release of nutrients for bacteria.
- Activity rather than abundance of bacteria indicates effects of treatment.

GRAPHICAL ABSTRACT
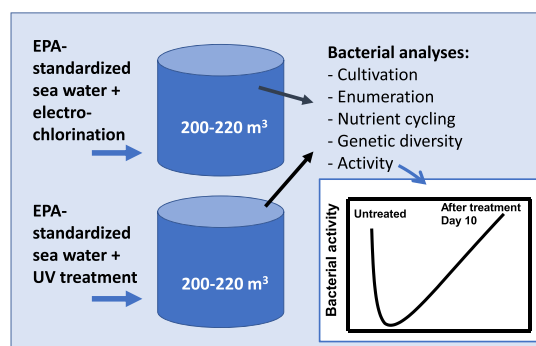
ARTICLE INFO

ABSTRACT

Effects of ballast water (BW) treatment by ultra-violet (UV) light and electrochlorination (EC) on survival, activity and diversity of marine bacterioplankton and release of organic matter from cell damage were examined at discharge in a large-scale BW test facility (250 m³ tanks) at Hundested harbour, Denmark. The tests were performed in accordance with the requirements for type approval testing by International Maritime Organization (IMO) and US Coast Guard. After treatment, the water was held in the tanks for one day (EC) before discharge, or 6 days (UV, including also a final UV re-treatment) before discharge. In the discharged and treated water, numbers of viable bacteria and bacterial growth rate had decreased significantly relative to the untreated water, but the total number of bacteria only was reduced in the EC-treated water. After additional storage for up to 10 days in small-scale laboratory incubations, significant regrowth of bacteria was observed after either treatment. Sequencing of 16S rRNA gene amplicons demonstrated that α-*Proteobacteria* initially were dominant, but γ-*Proteobacteria* dominated after regrowth. Bacteria used to document BW treatment efficiency (*E. coli*, *Vibrio* spp., enterococci) survived both treatments; neither treatment reduced the risk of pathogen dispersal. Concentrations of amino acids in the water were used as indicators of treatment-induced cell damage and demonstrated higher concentrations at discharge, but only after the EC treatments. Our results indicate that activity of bacteria, rather than their abundances, should be used when examining effects by ballast water treatment on microorganisms and that none of the examined treatment technologies could eliminate pathogenic bacteria.

© 2018 Elsevier B.V. All rights reserved.

1. Introduction

Large volumes of seawater and fresh water are transported between national and international ports by ships as ballast water (BW). The average content of BW in oceangoing vessels has been estimated to be about 37% of their cargo capacity (David, 2015). Assuming an international seaborne cargo of 9.35 billion tons (estimate for 2013), this means about 3.1 billion tons of water are carried annually in ballast tanks (David, 2015). Since most natural waters contain about 10^9 bacteria·L^{-1} (Kirchman, 2008), ships globally could potentially carry about 3×10^{21} bacteria in their ballast tanks. In addition to bacteria, naturally occurring microalgae constitute another but smaller group of microorganisms in ballast water. Typically, phytoplankton makes up between 0.1 and 10% of the bacterial abundance in natural waters, but large seasonal and local variations may occur (Kirchman, 2008). Zooplankton is also commonly found in ships´ ballast tanks but their abundance and composition depend on geographical location and season (Hernandez et al., 2017).

To comply with requirements by the International Maritime Organization (IMO) and the United States Coast Guard, ballast water should be treated at uptake and/or discharge to reduce the number of organisms in discharged water to meet the ballast water discharge standard (IMO, 2016; US Coast Guard, 2012). The standard includes requirements for three types of bacteria that serve as indicators for the effect of treatment on bacterial abundance. Discharge standards for bacteria are <250, 100 and 1 culturable cell per 100 mL water for *Escherichia coli*, intestinal enterococci and toxigenic *Vibrio cholerae*, respectively. For viable cells ≥10 μm and <50 μm, such as phytoplankton, fewer than 10 cells must be present per mL.

Techniques applied to reduce the content of microorganisms in BW include addition of chemicals, production of reactive molecules, e.g. by electrochlorination (EC) of sea water, ultra-violet (UV) radiation, ozone, deoxygenation and heating (Goncalves and Gagnon, 2012). Treatment with these approaches has been found efficient in an immediate reduction of both phytoplankton and bacteria, often to densities close the detection limit (First and Drake, 2014; Hess-Erga et al., 2010). However, if the treated water is stored in ballast tanks after treatment, regrowth of both bacteria and phytoplankton may occur. In some studies, regrowth by phytoplankton was observed a few days after UV radiation, while chlorination was more efficient in killing algal cells (First and Drake, 2014; Martinez et al., 2013). In contrast, other studies showed regrowth of phytoplankton a few days after treatment with UV light, electrochlorination and chemical chlorination (Stehouwer et al., 2015). Phytoplankton cells can persist in ballast tanks and have been shown to survive there for at least 23 days in darkness (Kang et al., 2010). In contrast, many zooplankton organisms appear to die a few days after intake of water in ballast tanks (Gollasch et al., 2000).

As for bacteria in ballast water, most studies have focused on survival of pathogens after treatment, and knowledge on the regrowth capacity by natural bacterial communities is limited (Grob and Pollet, 2016). In 2010, Hess-Erga et al. showed regrowth of heterotrophic bacteria (functionally defined as bacteria capable of growing on agar plates containing selected organic nutrients) after simulated ballast water treatment. For bacterial genera that include potentially pathogenic species, such as *Vibrio* and *Escherichia*, regrowth after treatment-mimicking techniques used for ballast water has been examined. Thus, Tryland et al. (2010) found that disinfection by ozone, UV light and ClO$_2$ initially reduced populations of heterotrophic bacteria and *Vibrio* spp., but regrowth of both occurred after 5 days. Similarly, growth of *V. cholerae* and *V. parahaemolyticus* was stimulated two days after treatment of sea water by UV light, ozone and chlorine, but the presence of natural bacterioplankton reduced the growth of *Vibrio* spp. (Wennberg et al., 2013). Rubio et al. (2013) observed a similar regrowth of *E. coli*, after an initial reduction, following treatment with solar and UV light and advanced oxidation processes.

In addition to chemical and physical methods for reduction of bacteria in ballast water, bacterial abundance in ballast tanks is probably also controlled by natural biological processes, especially grazing by aquatic protists (Dobbs and Rogerson, 2005). Indeed, protists were found to be important in keeping bacterial densities low in ballast tanks (Seiden and Rivkin, 2014). Zooplankton as well as particles (aggregates) may impact abundance, survival and growth of bacteria after treatment, the former by grazing and the latter by functioning as refuges (Tang et al., 2011).

Nutrients released in the form of organic matter from senescent and dead organisms, e.g., dead and dark-injured algae (Carney et al., 2011; Lasternas and Agusti, 2014) may stimulate regrowth of bacteria and this phenomenon is also expected to happen in treated ballast water. Supporting this idea, Hess-Erga et al. (2010) found approximately a 50% increase in dissolved organic carbon (DOC) after treatment of sea water by UV light or ozone. Similarly, dissolved organic matter from cyanobacteria stimulated growth of *V. cholerae* and *V. vulnificus* as well as the total bacterial community (Eiler et al., 2007).

In an attempt to address how onboard-ship treatment of ballast water may affect bacterial communities, we analyzed activity and diversity of bacteria at discharge of water treated by commercial equipment for ocean-going vessels. The treatments were UV exposure and electrochlorination (electrolysis of water to produce a chlorinated solution) and were conducted at a land-based test facility in connection with biological efficacy tests for type approval of ballast water management systems (BWMS). In agreement with US Coast Guard and IMO regulations, a holding time of one day was applied after the electrochlorination before water was discharged, while a holding time of 6 days was applied after the UV treatment to reduce the risk of regrowth microorganisms in the discharged water due to their DNA repair systems (Weber, 2005). Onboard a ship, UV treatment may be practiced at intake of water into the ballast tanks, and when the ballast tanks are emptied.

Before treatment of the simulated ballast water (challenge water), concentrations of particulate and dissolved organic carbon (POC and DOC), total suspended solids (TSS), as well as of the living organisms in different size classes, were adjusted to meet IMO and US Coast Guard requirements (IMO, 2016; US Coast Guard, 2012). The US Coast Guard requirements imply that type approval tests of BWMS at a land-based facility shall be conducted according to the principles in the ETV protocol (US Environmental Protection Agency, 2010). After treatment and storage in the 250 m^3 tanks, bacterial dynamics in the discharged water were followed in the laboratory in small-scale incubations. Survival and regrowth of bacteria were examined by cultivation and microscopy, and activity of the bacterial populations was determined by measuring growth rate estimated by incorporation of [^{3}H] thymidine into DNA. Free dissolved amino acids and combined amino acids, e.g. in peptides and proteins, are important intracellular components in living cells (references in Supplementary material) and therefore, changes in amino acid concentrations in the discharged water, relative to the natural harbour water, may indicate cell damage caused by the treatments. Therefore, pools of free and combined amino acids were measured before treatment and in the discharged water, and bacterial utilization of the amino acids was determined from peptidase enzyme activity and assimilation of free amino acids. Finally, the bacterial communities were characterized by 16S rRNA amplicon sequencing before and after treatment.

To our knowledge, this is the first study on effects of ballast water treatment technologies that combines abundance, activity, nutrient uptake and diversity of bacteria at discharge and that examines the potential for regrowth of bacteria surviving the treatments. Based on results from published studies on effects of BW treatments, we expected that a few bacterial species might survive the treatments and initiate regrowth after discharge, and that organic matter from damaged cells was important in this regrowth. Further, we assumed that hypochlorite