Science of the Total Environment 409 (2010) 247–255

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Efficacy and environmental acceptability of two ballast water treatment chemicals and an alkylamine based-biocide

Stéphanie La Carbona [a], Satu Viitasalo-Frösen [b,1], Daniel Masson [c], Jukka Sassi [d], Samuel Pineau [e,2], Maiju Lehtiniemi [b], David Corroler [a,*]

[a] Equipe de Recherche en Physico-Chimie et Biotechnologies EA 3914, Université de Caen Basse-Normandie, Bd Maréchal Juin, 14032 Caen cedex, France
[b] Marine Research Centre, Finnish Environment Institute (SYKE), PO Box 140, 00251 Helsinki, Finland
[c] IFREMER, Laboratoire L.E.R.P.C., Avenue du Mus-de-Loup, Ronce les bains, 17390 La Tremblade, France
[d] VTT Technical Research Centre of Finland, Maritime Operations and Environment, P.O. Box 1000, FI-02044 VTT, Finland
[e] Centre for Marine and Biological Corrosion, 55 rue de Beuzeville, BP9, 50120 Equeurdreville, France

ARTICLE INFO

Article history:
Received 30 June 2010
Received in revised form 22 September 2010
Accepted 3 October 2010
Available online 5 November 2010

Keywords:
Ballast water treatment
Biocides
Mexel® 432/336
Peraclean® Ocean
Seakleen®
Environmental acceptability

ABSTRACT

Ship's ballast waters transport large numbers of organisms which may become invasive in coastal regions. One option to address this problem is the use of biocides as ballast water treatment (BWT). Efficacy and environmental acceptability of three commercial active substances (the BWT biocides Peraclean® Ocean and Seakleen®, and alkylamine-based biocide Mexel® 432/336) were tested against three bacteria species, two vegetative microalgae and one zooplanktonic larva, in 10 and 30 Practical Salinity Unit (PSU) waters. In both salinities, PeraClean® Ocean was the most effective biocide against bacteria causing >90% mortality at 20 mg/l, compared with 50 mg/l for Mexel® 432/336 and >500 mg/l for Seakleen®. Regarding zooplankton, Seakleen® was the most effective chemical causing 90% mortality in 24 h at concentrations <6 mg/l ($LC90_{24\ h}$) in both salinities, compared with 23 and 26 mg/l for Mexel® 432/336 and 370 and 480 mg/l for PeraClean® Ocean in 10 and 30 PSU, respectively. Similar pattern of efficacy was obtained for microalgae in 30 PSU: effective concentrations inducing 50% growth inhibition in 4 days were ≤1.6 mg/l for Seakleen®, ≤10.1 mg/l for Mexel® 432/336 and ≤30.9 mg/l for PeraClean® Ocean. Our work highlighted that treated waters displayed residual toxicity after 24 h still inducing mortality depending on the organism and biocide. However Mexel® 432/336 is the only biocide which had no impact on oyster larvae development at effective concentration. Altogether our data showed that Mexel® 432/336 was the only biocide displaying a broad spectrum efficacy in concentrations <50 mg/l and not toxic for oyster larvae development at this concentration. However residual toxicity of treated waters for any organism should be taken into account in BWT systems utilising biocides.

1. Introduction

Invasive aquatic species are one of the greatest threats to the world's oceans, and may cause severe environmental, economic and public health impacts (Bax et al., 2003; Pimentel et al., 2001). Ship's ballast waters are one of the most important ways for different forms of life to be transported and consequently to establish reproducing populations into new marine environments (Drake and Lodge, 2004; Galil, 2000; Lavoie et al., 1999). It has been estimated that 3 to 10 billion tons of ballast water is transferred each year all over the world with different species from main trophic groups (David et al., 2007), such as zooplankton (Bailey et al., 2003; Duggan et al., 2006), phytoplankton (Burkholder et al., 2007; McCarthy and Crowder, 2000), fish (Ricciardi and MacIsaac, 2000) and several millions of viral and bacterial particles (McCarthy and Khambaty, 1994; Ruiz et al., 2000). Moreover, biofilms on ballast tank coatings and sediments at the bottom of ballast tanks can contain numerous organisms including pathogens and, consequently, represent another risk of biological invasions (Drake et al., 2005, 2007). Finally, bacteria can associate with planktonic species and survive in seawater for long periods (Signoretto et al., 2004, 2005). As examples of harmful impacts, introduction of the zebra mussel from Europe (*Dreissena polymorpha*) into the Great Lakes (Canada and USA) generated billion dollars of expenditure for fouling control and cleaning of infested installations; *Vibrio cholerae* caused an outbreak of cholera on Latin America coastal in 1991, evidently due to ballast waters originating from Asia;

* Corresponding author. Tel.: +33 2 31 56 71 22; fax: +33 2 31 56 73 03.
E-mail address: david.corroler@unicaen.fr (D. Corroler).
[1] Present address: City of Helsinki Environment Centre, P.O. Box 500, FIN-00099, City of Helsinki, Finland.
[2] Present address: ACCOAST SA, Espace Atlantis, 7 Parc d'Activité Doaren Molac, 56 610 ARRADON, France.

0048-9697/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2010.10.006

invasion of dinoflagellates from south-east Asia (*Gymnodinium* and *Alexandrium*) in Australian waters, caused paralytic shellfish poisoning and thus affected the local shellfish farming and the coastal economy in general. To tackle this growing threat, a diplomatic conference adopted the International Convention for the Control and Management of Ship's Ballast Water and Sediments in 2004 to prevent the potentially devastating effects of the spread of harmful aquatic organisms carried by ship's ballast waters (IMO, 2004). Several treatment methods have been developed in order to limit introduction of new invasions in the future such as exchange of ballast waters (95% volumetric exchange) in the open sea, i.e. by a minimum of 200 m depth and 50–200 mi from the coast. However, a recent study showed that ballast water exchange was not sufficiently effective in removing all organisms from the ballast tanks in regional seas (McCollin et al., 2008), although this method could not be totally excluded (Costello et al., 2007; Tomaru et al., 2010). In this context, finding alternative effective BWT methods is crucial. Significant amount of research efforts by scientific and engineering research establishments around the world are underway and several technologies applying mechanical and physical treatments are now being considered: filtration and hydrocyclonic separation (Cangelosi et al., 2007), deoxygenation (McCollin et al., 2007), UV (D.F. Raikow et al., 2007), ultrasound or a combination of these methods, e.g. filtration or hydrocyclon as a primary treatment and ultraviolet light as a secondary one (Sutherland et al., 2001). Other methodologies involve also chemical treatments (biocides), which are an attractive alternative due to their potential for destroying a wide range of organisms and for relatively easy applicability to existing and future vessels. However, most of the biocides were developed for drinking water or sewage treatments instead of salt water applications. Therefore efficacy of these biocides in ballast waters has to be addressed. Two distinct classes of biocides are proposed as potential BWT chemicals in terms of efficacy, environmental acceptability, safety, cost-effectiveness, and corrosion concerns: inorganic oxidants (H_2O_2, Cl_2, peracetic acid [PAA], and Br) and organic oxidants (quinones, i.e. juglone and menadione). Studies have already been performed with chlorine dioxide (Gregg and Hallegraeff, 2007), glutaraldehyde (D.E. Raikow et al., 2007; Sano et al., 2003), hydrogen peroxide (Kuzirian et al., 2001), quinones (Chelossi and Faimali, 2006; Gregg and Hallegraeff, 2007; Raikow et al., 2006; Wright et al., 2007, 2009) and peracetic acid (de Lafontaine et al., 2008, 2009; Gregg and Hallegraeff, 2007). Some of these active substances such as vitamin K3 (menadione), chlorine dioxide or peracetic acid are already commercialised as SeaKleen® (Garnett, Inc., Atlanta, USA), Vibrex® (Grayson, Australia) and PeraClean® Ocean (Degussa AG, Germany), respectively. To date, twelve systems applying active substances have received final approval from International Maritime Organization (IMO), including the SEDNA® Ballast Water Management System using PeraClean® Ocean. In addition, 24 other systems using active substances have received IMO Basic Approval (Gregg et al., 2009; IMO, 2010).

In this study we assessed efficiency of three biocides on bacteria, phytoplankton and zooplankton populations and environmental acceptability of the treated waters. Two of the tested chemicals are the BWT biocides PeraClean® Ocean and Seakleen®. The third one is Mexel® 432/336, a formulation of alkylamines, which has never been tested in ballast water treatments before. It derives from Mexel® 432/0 which is already successfully used in cooling water treatment of industrial installations. Consequently, it meets the criteria of safe handling and displays properties such as liquefying effects on sludge, action on biofilms and anticorrosion effects, which are of high interest in the ballast tank context. Moreover, Mexel® 432/336 is in accordance with the Directive 98/8/EC of the European Parliament and the Council of February 16, 1998 concerning biocidal products. The aim of this work was to compare the efficiency of the three biocides on different trophic levels and to evaluate their toxicity.

2. Materials and methods

2.1. Biocides

PeraClean® Ocean was a newly opened bottle containing 15% peracetic acid (Evonik Degussa GmbH). Seakleen® was supplied as a liquid solution at 500 g/l (Vitamar LLC, USA). Mexel® 432/336 was provided as a white solution at a concentration of 500 g/l (Mexel®, France). All stock and working solutions were prepared in filtered (0.2 μm, Sartorius) seawater immediately before use. Biocide concentrations in the water prior to the test and after 24 h were determined as follows: for PeraClean® Ocean, acid peracetic concentration was estimated by colorimetric method with strips (Merckoquant®, Merck Chemicals); concentration of menadione (i.e. Seakleen®) was measured by spectrophotometry at 337 nm; and Mexel® 432/336 concentration was estimated by a specific kit measurement supplied by the manufacturer. For each biocide and concentration, two consecutive incubations were carried out, i.e. the "effect" test to study mortality or growth inhibition in freshly prepared test medium and the "after-effect" test to study whether the used test medium had lost toxicity during a retention time of 24 h (suggested by the manufacturers).

2.2. Biocide "effect" test on bacteria

Biocide assays were performed on 3 bacteria species isolated from a sampling campaign on seven ships in three different ports of France. Following culture on specific medium, isolated colonies were biochemically (API gallery, Biomerieux) and molecularly (16S rRNA sequencing, Eurofins MWG Operon) characterized. The three selected species were: *Staphylococcus kloosii*, a gram positive bacterium which can be responsible for nosocomial infections; *Escherichia coli* O157: H7, a pathogenic gram negative bacterium which is a fecal contamination indicator; and *Aeromonas* sp., another gram negative germ which is commonly found in marine environment and can be pathogenic for animals and humans. For "effect" determination, separate containers of 250 ml of filtered seawater from the English Channel adjusted to 10 or 30 PSU, were inoculated with 10^5 cells/ml from an overnight culture. This level of contamination was consistent with literature data (Mimura et al., 2005; Ramaiah et al., 2005). Initial cell concentration was checked (T0) and defined as colony-forming unit (CFU)/ml by inoculating appropriate dilutions in mass in nutritive agar (Biokar Diagnostics) and counting developed colonies after 48 h of incubation at 30 °C. Tens of 250 ml were then transferred in tubes before addition of one of the biocides at various concentrations. Control incubations were run without biocides. PeraClean® Ocean was applied at concentrations ranging from 0.5 to 20 mg/l, Seakleen® from 100 to 500 mg/l and Mexel® 432/336 from 2 to 50 mg/l. Representative concentrations are shown. Tubes were incubated in darkness at 17 °C for 24 h and the cell concentration after 24 h of incubation was determined (CFU/ml). The mortality percent was then calculated relative to the cell concentration at T0 and concentrations leading to at least 90% mortality were defined as effective. Three independent assays were performed for each tested concentration and each bacterium.

2.3. Biocide "effect" test on Artemia sp

Bioassays with zooplankton were carried out with the brine shrimp *Artemia* sp., extensively used as a test species in various toxicity tests (e.g. Nunes et al., 2006) and widely tolerant to various salinities. The test medium was 0.2 μm filtered natural seawater from northern Baltic Sea adjusted to 10 or 30 PSU with hw-Meersalz aquarium salt. *Artemia* nauplii were obtained by hatching from resting eggs under optimal conditions (25–28 °C, salinity 10 or 30 PSU, depending on the test trial). One to four days old naupliar stages