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Electrochemical disinfection of simulated ballast water using *Artemia salina* as indicator

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ABSTRACT

This work examined the potential of electrochemical disinfection to treat simulated ballast water with *Artemia salina* (*A. salina*) as an indicator organism. The effect of contact time (residence time in the electrolytic cell) and current density were investigated. Furthermore, the formation of disinfection by-products (trihalomethanes) was also examined. Under conditions of single pass through the electrolytic cell, a current density of 135 mA/cm^2 and a residence time of around 1 min were required for 100% mortality of *A. salina*. Dissolved organic carbon due to cell lysis increased by 1–2 mg/L, while the formation of chlorination by-products, expressed as trihalomethanes was very small (less than 10 μg/L at 135 mA/cm^2).

1. Introduction

Ballast water is water carried by ships to ensure stability, trim and structural integrity. Ballast water contains plenty of microorganisms, phytoplankton, zooplankton, etc. Most species carried in ships' ballast water do not survive the voyage. Most of those that do, do not survive when discharged into the new environment. However, under certain circumstances some species do survive to form viable populations, and may become serious pests. Invasive aquatic species is considered one of the greatest threats to the world's oceans posing a threat to the local marine ecological system [1,2]. Carlton et al. [3,4] has estimated that more than 3000 species are transported by ships each day, and some 40 recent of invasions have been mediated by ballast water. The appearance of invasive species is often accompanied by economic damage.

Shipping moves over 80% of the world's commodities and transfers large volumes of ballast water annually. The International Maritime Organization (IMO) estimates that approximately 10 billion tonnes of ballast water are transported and exchanged annually. The IMO has developed guidelines in order to regulate discharges of ballast water. The IMO also has the authority to evaluate and approve appropriate ballast water treatment technologies, regarding technical, cost, environmental, as well as health effects to the ambient and human environments. Ballast water exchange is currently the best-available measure to reduce the risk of transfer of harmful aquatic organisms. There are two main types of ballast water exchange: sequential or reballasting and flow through or continuous flushing. The first one involves completely emptying of ballast tanks (individually or in sequence) and refilling them with open ocean water; the second concerns partially emptying and refilling of the tanks [5]. However, there are serious limitations with this method including ship-safety limitations. Even when it can be fully implemented, this technique is less than 100% effective in removing organisms from ballast water.

During the last two decades several technologies have been tested for the treatment of ballast water [6]. These methods include:

A. Physical separation techniques, such as filtration [7–9] or hydrocyclone separation [10].
B. Application of UV radiation [11–14].
C. Conventional disinfection methods including ozonation [15–20] and chlorination [21–23].
D. Application of biocides as part of chemical treatment techniques [24–30].
E. Other systems, such as sonication [31–34].

Electrolytic treatment technologies are also another alternative method. Electrochemical oxidation has been applied in recent years over a wide range of industrial wastewaters, such as textile industry effluents [35], leachate [36], as well as in disinfection of drinking, swimming pool and sea water [37]. Li et al. [38] reported electrochemical treatment applications in electrochlorination, generation of lethal oxidants, destruction caused by the electric field or inactivation by energy rich intermediate products.

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Electrolytic disinfection of water is usually based on the electrochemical production of hypochlorite and/or hypochlorous acid from the chloride content of water. Sometimes, the addition of an electrolyte (most commonly NaCl) is required. There are two categories of electrochemical disinfection devices resulting in direct or indirect electrolysis. The first one uses direct electrolysis which is capable to interfere directly with the influent stream, while the latter uses a concentrated brine solution to generate an anolyte – a mixture of oxidising species [39]. A wide range of electrode materials is used for the anode and the cathode, such as graphite rods, ruthenium mixed oxides, $Ti/TiO_2–RuO_2–IrO_2$, Ti/Pt, Ti/PbO_2 and $TiMnO_2$, Ti/Pt, Ti/PbO_2, $Ti/PdO–Co_3O_4$, $TiRhO_xTiO_2$ [37], Ti/Ta/Pt/Ir [40]. Recently, boron-doped diamond electrodes produced from thin film conductive diamond deposed on highly doped polycrystalline or monocrystalline silicon substrates have been successfully used in several applications [36,41–44].

A possible disadvantage of electrochemical disinfection is the production of chlorination by-products. Many disinfection substances could react with the organic matter naturally present in water and/or other organic substances to yield disinfection by-products. Trihalomethanes and haloacetic acids are the most dominant species of chlorination by-products. Concentration of free chlorine and bromine, pH, temperature and the level of organic matter are parameters that control the production of disinfection by-products. It should be mentioned that residual chlorine may also pose an environmental risk due to its toxicity to aquatic organisms. As such, a final dechlorination step is recommended. This could be accomplished by the use of a reduction agent, such as sodium thiosulfate, sodium dioxide or sodium metabisulfite or even by the use of absorption on active carbon.

Electrochemical disinfection is a potential treatment of ballast water. Disinfection of seawater is effective since it has a chloride content of 19 g/L. The seawater electrolytic process produces free chlorine from natural seawater taken on board. It is a cost effective technique as far as there is no need of storing and transporting chemical substances. Another advantage of the electrolytic system is that electrolytic disinfection may take place on board during the deballasting (discharge) procedure with a flow through electrode design. In this way, corrosion of ballast tanks may be avoided.

Matousek et al. [45] produced sodium hypochloride through an electrolytic system at concentrations higher than 3 ppm. The results showed that bacteria were removed at 99.99% levels, phytoplankton and mesozooplankton more than 99%. Nakayama et al. [46] conducted laboratory-scale experiments using a titanium nitride covered anode for the electrochemical inactivation of marine bacteria (*Vibrio alginolyticus*). In all experiments, 98.7% of *V. alginolyticus* cells were inactivated in 30 min. Dang et al. [47] applied electrolysis to ballast water in a small-scale pilot plant with *Artemia* sp. as the indicator microorganism. *Artemia* sp. was removed from ballast tanks at levels higher than 95%. It should be emphasized that in the majority of the studies, as well as in the IMO guidelines, bacteria are used as indicators. However, higher organisms may not be as susceptible to treatment methods as bacteria are, and therefore there is a need to further evaluate these methods.

This work examined the potential of electrochemical disinfection to treat simulated ballast water. A lab scale experiment was set up in order to evaluate the electrochemical unit and the experiments made up using simulated seawater. *Artemia Salina*, a small crustacean, was chosen as an indicator organism. *A. salina* was preferred over bacteria, because the latter are easily amenable to chlorine disinfection. *A. salina* is a good indicator for testing ballast water treatment, because it has the benefit of easy handling and hatching. It does not require special equipment for measuring alive and dead *A. salina*. The effect of contact time (residence time in the electrolytic cell) and current density on *A. salina* mortality rates was investigated. Furthermore, the formation of disinfection by-products (trihalomethanes) was also examined.

2. Materials and methods

2.1. Test organism

Throughout the experimentation process artificial seawater (ASW) was used. This was prepared by diluting unrefined sea salt to tap water (concentration 30 g/L) to final salinity of 30‰. The simulated sea water had the same conductivity as the actual sea water.

A. salina in the form of dehydrated cysts was provided by the Hellenic Centre of Marine Research. *A. salina* cysts were kept refrigerated (4–5 °C) in the absence of light. Prior to use, they were hatched into nauplii larvae for 24 h. To initiate the growth procedure a volume of 25 mL of the cysts was placed in 1 L of artificial seawater. A water basin bath was used to keep the temperature at 28 °C [48]. Continuous aeration was provided through constant air flow. The hatching of *A. salina* was complete after a period of 24 h. Then, the *A. salina* suspension was diluted to 200 L of artificial seawater (feed water). Continuous aeration was also provided.

2.2. Experimental unit

The experimental unit consisted of a 200 L capacity feed tank, peristaltic pump, flow meter, and the electrolytic cell with its power supply and thermostat. The thermostat was used for safety reasons. Its role was to cut off electricity when the temperature of the water in the cell exceeded 35 °C. The electrolytic cell (supplied by Water Safe S.A. [49]) consisted of a SS 316 L cathode tube (7.5 cm diameter and 23 cm long). The anode was located in the center of the cathode tube and was made of Ti and Ta alloy measuring 2.5 cm diameter and 17 cm long. The anode tube was covered by Pt–Ir alloy foil approximately 0.25 mm in thickness. The area of the anode was calculated 133.45 cm^2. According to cell specifications, the cell could achieve current densities ranging from 30 to 352 mA/cm^2. The power supply could maintain constant current at the desired level with only minor adjustments of applied voltage.

2.3. Experimental procedure

Prior to the experimental evaluation of the electrolytic treatment, two sets of experiments were conducted in order to investigate the influence of (i) residual chlorine and (ii) total residual chlorine plus sodium thiosulfate on *A. salina* population The objectives of the preliminary experiments were twofold: first, to examine the efficiency of hypochlorite itself as inactivation agent; and second, to ensure that all residual chlorine (hypochlorite) in the samples taken for analysis had been destroyed and therefore the actual mortality of *Artemia* was only due to the electrochemical treatment inside the electrolytic cell. As such, lower doses of hypochlorite were preferable, because under actual on-board treatment large storage facilities for hypochlorite would be required. The experiments were conducted in conical flasks of 500 mL volume. *A. salina* was hatched as described above and the artificial seawater with the *A. salina* nauplii was added to the conical flask followed by the addition of chemicals. Samples were taken at 5, 10, 15, 30 and 45 min and analyzed for alive *A. salina* nauplii.

In case of the addition of sodium thiosulfate, a volume of 2 mL of 1N sodium thiosulfate solution ($Na_2S_2O_3·5H_2O$) was added to the conical flask. This amount is sufficient to reduce up to 200 mg/L of residual chlorine.

i. Influence of total residual chlorine.

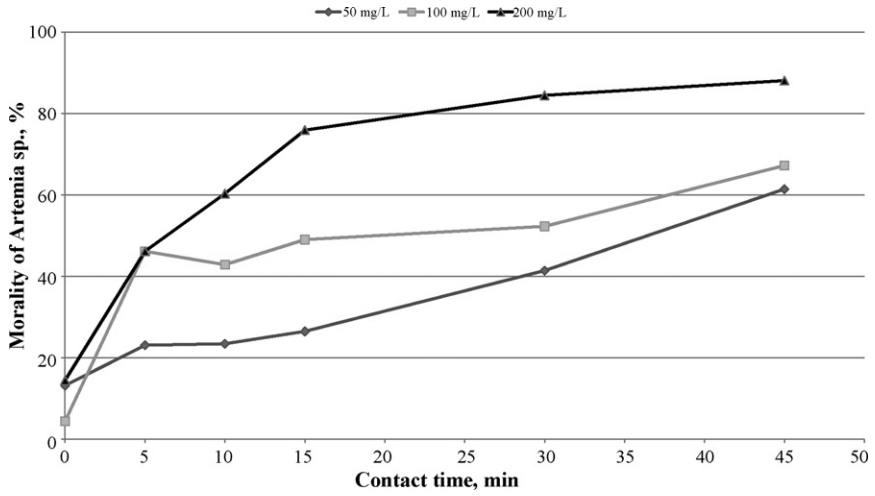

Fig. 1. Influence of 50, 100 and 200 mg/L residual chlorine on the mortality rate of *Artemia*.

Appropriate amounts of 2 g/L sodium hypochlorite stock solution were added to the conical flask in order to achieve levels of 50, 100 and 200 mg/L free residual chlorine.

ii. Influence of residual chlorine and sodium thiosulfate on *A. salina* population.

In this series of preliminary experiments both sodium thiosulfate solution and sodium hypochlorite stock solutions were added in the conical flasks in order to study their possible effect on mortality of *A. salina*.

Following the preliminary experiments, the operation of the electrolytic unit on mortality of *A. salina* was evaluated. Four different flow rates were used, namely 50, 100, 200 and 300 L/h, resulting in residence times of 65.5, 32.8, 16.4 and 10.9 s, respectively. The voltage input tested for each residence time ranged from 0 to 20 V. These values corresponded to current values from 0 to 47 A. The mechanical stress control was performed with the run at 0 V.

The system was operated continuously and samples of disinfected effluent were collected from the sampling point. Two samples of 500 mL each were collected in beakers: the first beaker contained 2 mL sodium thiosulfate stock solution (1N) in order to instantly destroy free chlorine produced during electrolysis; the other beaker did not contain any sodium thiosulfate. The former sample was used for the measurement of alive *A. salina* organisms, dissolved organic carbon (DOC) and trihalomethanes (chlorination by-products). The latter sample was used for the measurement of total residual chlorine. The sampling procedure for each flow rate (residence time) and current was repeated in triplicate.

2.4. Analytical methods

Sampling and measurement of *A. salina* (before and after electrochemical treatment) was done in triplicate. The volume of each sample used for the measurement of *A. salina* was 5 mL. Each sample was transferred into Petri dishes (90 mm in diameter), where the measurement of *A. salina* was conducted with a colony counter apparatus. The living-dead judgment was made according to organism movement. The coefficient of variation (CV, standard deviation over mean) of the *Artemia* sp. measurements was less than 0.17. *A. salina* numbers were expressed as number of organisms per liter.

Residual chlorine was measured according to standard method 4500 Cl-B method I [50]. Acetic acid and potassium iodide used in titrometric determination of residual chlorine were purchased from Carlo Erba and Fluka, respectively. Salinity was measured with a Crison microCM 2002 conductivity meter. Dissolved organic carbon (DOC) was measured with a Shimadzu 500 A TOC analyzer.

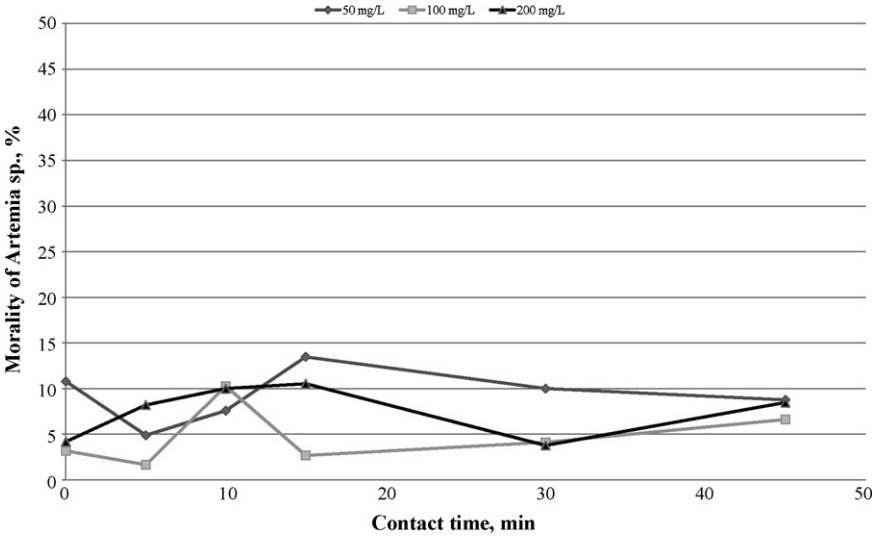

Fig. 2. Influence of 50, 100 and 200 mg/L residual chlorine and sodium thiosulfate (0.0004 M) on the mortality rate of *Artemia*.

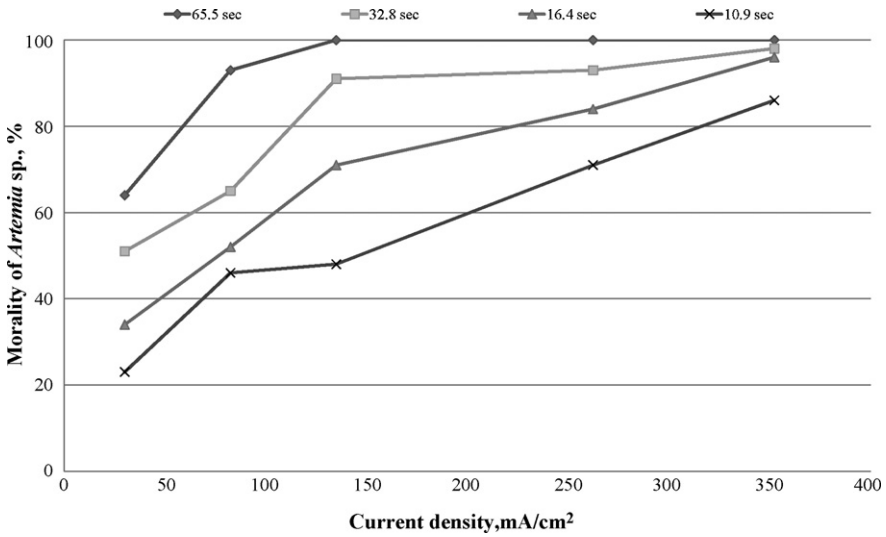

Fig. 3. Mortality rate of *Artemia* as a function of residence time and current density.

Prior to analysis the samples were filtered through a membrane filter with a pore size of 0.45 μm. Disinfection by-products were measured using head space solid phase micro-extraction (SPME) as the pre-concentration stage and gas chromatography (Carlo Erba) using electron capture detector (ECD). The analytical method is described in detail in Antoniou et al. [51]. This method is capable of measuring 14 chlorinated volatile organic compounds (VOCs) in water and wastewater samples with the Limit of Quantification for trichloromethane (chloroform), bromodichloromethane, dibromomethane and bromoform being 4.6, 2.5 1.5 and 1 ng/L, respectively.

3. Results and discussion

As mentioned in the experimental procedure, two preliminary sets of experiments were conducted in order to investigate the effect of residual chlorine and the combination of residual chlorine and sodium thiosulfate on *A. salina* population.

The effect of sodium hypochlorite (free residual chlorine) on *A. salina* mortality is depicted in Fig. 1. Three different concentrations of residual chlorine, namely 50, 100, 200 mg/L, were applied. The chlorine contact time ranged from 0 to 45 min. In general, *A. salina* mortality increased with increasing chlorine dose and contact time. High *A. salina* mortality rates (over 75%) were only observed at high chlorine concentration (200 mg/L) and contact times longer than 15 min. The last set of the preliminary experiments examined the effect of sodium thiosulfate addition to chlorinated *A. salina* brine. As shown in Fig. 2, the mortality of *A. salina* did not exceed 14% for all chlorine doses and contact times, indicating that having achieved dechlorination of the water sample significantly reduced *A. salina* mortality.

The operation of the electrolytic unit on the mortality of *A. salina* was investigated in the subsequent set of experiments. Four different flow rates were applied which corresponded to four residence times. Fig. 3 shows *Artemia* sp. mortality rate as a function of current density and residence (contact) time. The higher the residence time and the higher the current density, the higher the mortality rate of *A. salina*. Under conditions of residence time of 65.5 s and current density of 135 mA/cm^2 complete mortality of *A. salina* was achieved. However, for all other current density values *A. salina* mortality was high, but not 100%. The results of Fig. 3 clearly indicate that the electrolytic treatment was very efficient

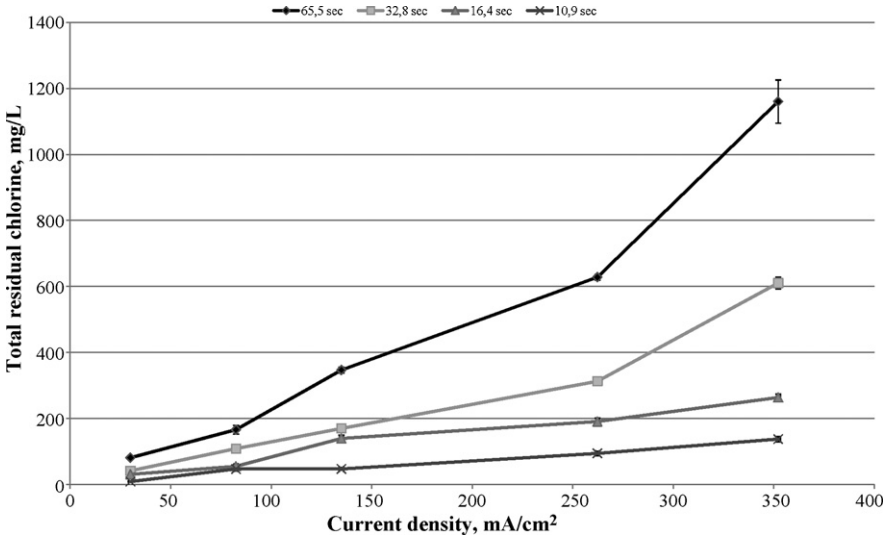

Fig. 4. Production of total residual chlorine as a function of residence time and applied current density (mean values ± SD; $n = 9$).

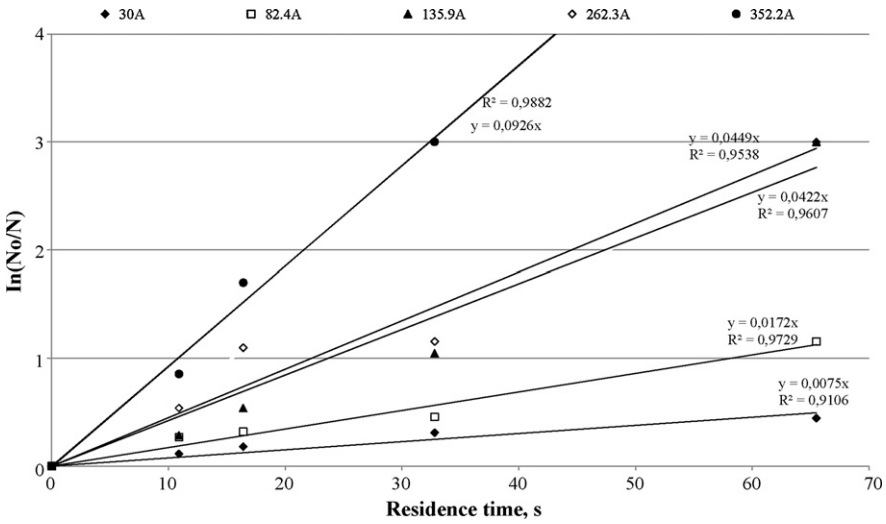

Fig. 5. Modeling *Artemia* survival rate with first-order kinetics (Eq. (2)).

even at contact times as low as 1 min. This indicates that it is possible to disinfect ballast water efficiently under ballast water discharge (deballasting) conditions. These results are in agreement with the work by Dang et al. [47], who studied *Artemia* sp. mortality by electrochemical treatment at pilot level. In their work *Artemia* sp. mortality around 95% was reported under conditions of 4 ppm initial chlorine concentration and 15 A current. In addition, electrochemical inactivation of *A. salina* was as high or even more efficient as compared to other disinfection methods [6].

Concerning the production of chlorine during electrolytic treatment, Fig. 4 presents residual chlorine concentration as a function of residence time and current density. The higher the current density and residence time, the higher the residual chlorine concentration. By comparing the levels of residual chlorine generated under electrolytic conditions, it appears that very high concentrations are achieved in very short residence times. For instance, at 135 mA/cm^2 the level of chlorine was 475 mg/L at a residence time of 65.5 s. The electrolytic treatment was more effective than sodium hypochlorite, since for the same level of residual chlorine *A. salina* mortality was higher under electrolytic treatment as compared to the addition of sodium hypochlorite (Figs. 1, 3 and 4). This indicates that the inactivation mechanism was a combination of hypochlorite disinfection, as evidenced by the high chlorine levels being generated (Fig. 4), as well as direct oxidation at the anode surface.

The disinfection process is usually modeled by the Chick–Watson equation which considers the first-order kinetics for the microorganism survival rate. In electrochemical treatment, first-order kinetics along with second order and pseudo-first-order kinetics have also been used [36,52]. If we also assume first-order order kinetics for *A. salina*, then the *A. salina* survival rate may be expressed as:

$$\frac{dN}{dt} = -k'N \tag{1}$$

where N: *A. salina* population at time t; t: time, seconds; k': first-order kinetic coefficient.

If we further assume that the electrolytic cell has the characteristics of a Plug Flow Reactor (PFR), then an *A. salina* balance will

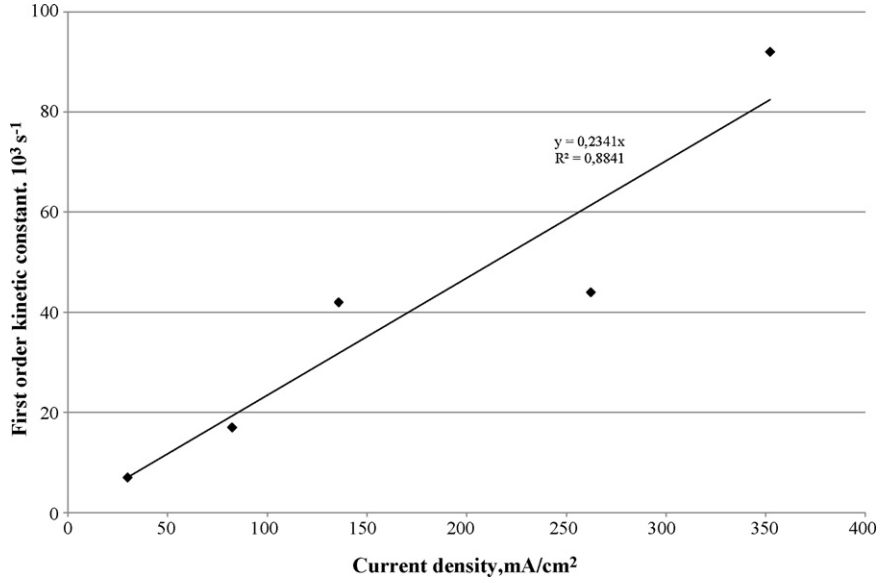

Fig. 6. Relationship between first-order kinetic coefficient and current density.

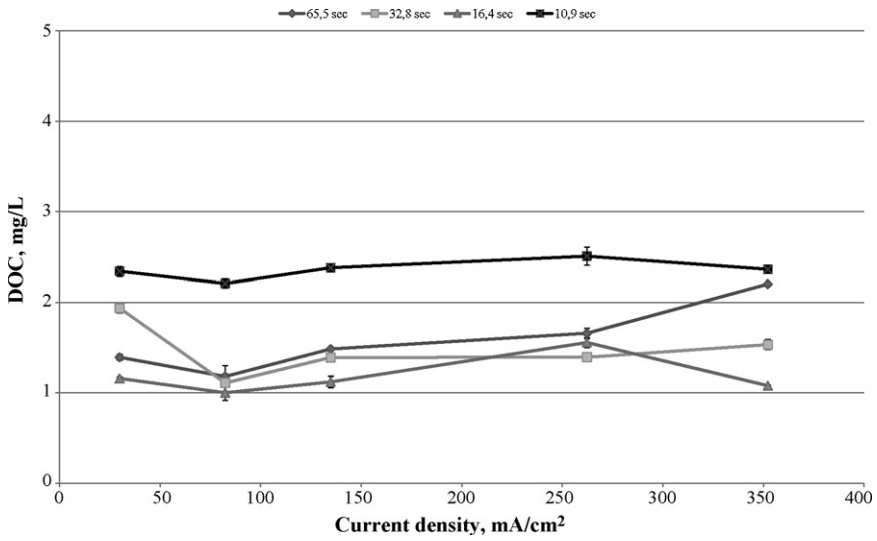

Fig. 7. DOC values as a function of residence time and applied current density (mean values ± SD; $n = 3$).

yield:

$$\ln \frac{N_o}{N_\tau} = k'\tau \quad (2)$$

where N_o: initial *A. salina* population; N_τ: *A. salina* population at time τ; τ: space-time (residence time).

It is estimated as:

$$\tau = \frac{V}{Q} \quad (3)$$

where V: electrochemical cell volume, L; Q: ballast water flowrate, L/s.

The results of the modeling technique are presented in Fig. 5. The linear trend in the data indicates that first-order kinetics may adequately describe the experimental results. The values of the correlation coefficients are above 0.90 indicating a good fit of the first-order model. The slope of the straight line is the value of the first-order kinetic coefficient. These values ranged between 0.0075 and 0.0926 s^{-1}. Gusmao et al. [53] investigated the disinfection of water containing *Staphylococcus aureus* with a 0.08 M sodium sulfate as electrolyte. A treatment time of 58 min was needed to reach total sterile suspension. The first-order kinetic constant was 0.0033 s^{-1}. This value is up to one third of those values estimated in this study, because the electrolyte used in the former work did not promote the production of chlorine. Furthermore, Fig. 5 shows that there is a distinct difference in process efficiency for various values of current density: the higher the current density, the larger the value of the kinetic coefficient. The values of the kinetic coefficients are plotted against current density in Fig. 6. As can be seen, there is a linear relationship between the kinetic coefficient and current density. Given that the inactivation of *Artemia*. Therefore, the overall kinetic model describing *A. salina* survival rates can be written as

$$\ln \frac{N_o}{N_t} = kI\tau \quad (4)$$

where I: current density; k: overall first-order kinetic coefficient, 0.234×10^{-3} mA^{-1} s^{-1} cm^2.

Under electrolytic conditions death of *A. salina* resulted in cell lysis and increase in total organic carbon of the brine. Fig. 7 shows the dissolved organic carbon (DOC) concentration as a function of current density and residence time. The increase in DOC concentration ranged from 1 to 2.5 mg/L. The DOC value of the synthetic brine after *A. salina* inoculation and prior to electrochemical treatment was around 1 mg/L. Following the increase in DOC, the production

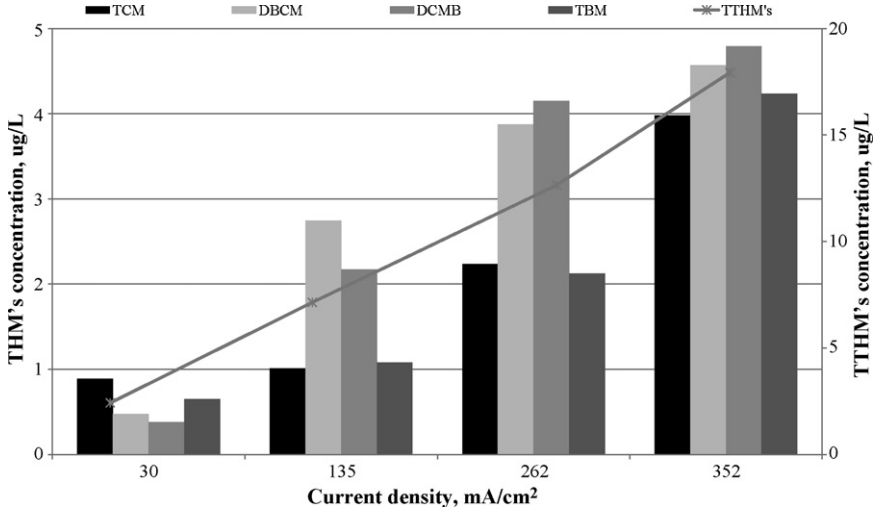

Fig. 8. Concentration of TCM (chloroform); DBCM (dibromomethane); DBCM (dichloromethane); TBM (bromoform) and TTHM's (total trihalomethanes) at a residence time of 32.8 s.

of disinfection by-products was examined. Trihalomethanes were the most important group detected. Fig. 8 presents the level of individual compounds, as well as total trihalomethane concentration as a function of current density for residence time of 32.8 s. The total trihalomethane concentration was around 6.5 μg/L. Although there are no guidelines related to trihalomethane levels in ballast water, the values reported in this work are significantly lower than those set for drinking water (100 μg/L in the European Union) [54]. The low level of trihalomethanes produced during the electrolytic treatment was due to the low reaction (residence) time.

Power consumption ranged from 0.07 kWh/m^3 (for low current density and low residence time) to 19.2 kWh/m^3 (for high current density and high residence time). Under optimal treatment conditions (current density 135 mA/cm^2; residence time 65.5 s; mortality rate 100%) power consumption was 3.6 kWh/m^3.

4. Conclusions

In the current study we examined the potential of electrochemical disinfection to treat simulated ballast water with *A. salina* as an indicator organism. The following conclusions arose from the investigation.

- Sodium hypochlorite was quite an effective disinfectant. Mortality rates over 75% were achieved at high chlorine concentration (200 mg/L) and contact times longer than 1.5 min.
- Electrochemical treatment was very efficient in achieving high mortality rates. A current density of 135 mA/cm^2 and a residence time of around 1 min could achieve 100% mortality of *A. salina*. Under these conditions, the concentration of residual chlorine was around 400 mg/L.
- Dissolved organic carbon concentration increased by 1–2 mg/L, while the concentration of trihalomethanes generated was less than 10 μg/L for current density 135 mA/cm^2.
- Power consumption was between 0.07 and 19.2 kWh/m^3. Under optimal treatment conditions (current density 135 mA/cm^2; residence time 65.5 s; mortality rate 100%) power consumption was 3.6 kWh/m^3.

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