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Seawater disinfection by chlorine dioxide and sodium hypochlorite. A comparison of biofilm formation

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Abstract Continuous seawater disinfection by chlorine dioxide (ClO_2) was studied at residual concentrations of 0.2 and 0.4 mg ClO_2 L^{-1} and compared with sodium hypochlorite (NaClO) disinfection at 1 mg L^{-1} of free chlorine. The results revealed that both disinfectants decrease the biological activity and cell counts in seawater. When NaClO was used, both the cell counts and the adenosine triphosphate (ATP) level were diminished (1.8 log and 76 %, respectively); however, when ClO_2 was used, the ATP level decreased to the same level as with NaClO (78–84 %), but the cell counts were reduced only weakly (~0.1 log). The biofilm concentration in seawater without disinfectants reached 700 pg ATP cm^{-2} after 40 days, whereas in the treated lines, the biofilms remained below 1 pg ATP cm^{-2} irrespective of the disinfectant and dose used. ClO_2 generated much less trihalomethanes than NaClO (<1 vs. 154 μg L^{-1}). Bromoform (77–96 %) was the predominant chemical species found in disinfected seawater.

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1 Introduction

Seawater disinfection is a common strategy against biofouling, a general operational problem associated with the biofilms formed by microorganism growth in water systems. Biofouling is common in plants that handle seawater, such as desalination plants, cooling towers and power plants, but bacterial growth in distribution systems is also a matter of concern (Choi et al. 2002; Chowdhury 2012; Flemming 2002; Matin et al. 2011; Taylor 2006). Disinfection is also used in ballast water, aquaculture and thalassotherapy (Organization 2005; Parinet et al. 2012; Summerfelt 2003). The most common strategy for seawater disinfection is the addition of chlorine-based disinfectants, like chlorine, hypochlorite, chlorine dioxide and chloramines, by continuous, intermittent or shock dosages; but UV and advanced oxidation processes, such as ozonation, are also used (Aieta and Berg 1986; Marconnet et al. 2011; Mayan Kutty et al. 1995; Metz et al. 2011; Van Geluwe et al. 2011). Since the 1900s, free chlorine, in the form of either chlorine gas or hypochlorite, has been the most commonly used disinfectant due to its high efficiency and low cost compared with others (Kim et al. 2009; Micale et al. 2009; Volk et al. 2002). Nonetheless, disinfection by chlorine is often associated with the formation of trihalomethanes (THMs) and other compounds, collectively referred to as disinfection by-

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products (DBPs) (Agus et al. 2009; Deborde and von Gunten 2008; Liang and Singer 2003; WHO 2008). DBP formation is influenced by the bromide concentration, which it is higher in seawater (65–71 mg L^{-1}) than in freshwater (<0.2 mg L^{-1}) (Agus et al. 2009; Flury and Papritz 1993). Most of these DBPs—and specially the brominated ones—are carcinogenic, mutagenic and persistent (Petrucci and Rosellini 2005; Richardson et al. 2003). Consequently, they can have adverse effects on marine and human life. Unfortunately, reverse osmosis membranes cannot efficiently retain them and prevent their delivery to the environment (Agus et al. 2009; Dalvi et al. 2000; Fogelqvist and Krysell 1991; Lattemann and Höpner 2008). More specific studies on DBP occurrence, abundance, speciation and kinetics, and their consequences and effects can be found elsewhere (Allonier et al. 1999; Krasner et al. 2006; Liang and Singer 2003; Richardson et al. 2007; Sorlini and Collivignarelli 2005). However, they are outside the scope of this work and hence will not be further discussed.

Although chlorine is currently the most commonly used disinfectant, there is a clear need for a more health- and environment-friendly product with the same efficiency. In this regards, chlorine dioxide (ClO_2) has become a feasible alternative, given its high and selective oxidation potential (Agus et al. 2009; Rav-Acha 1998). Furthermore, because ClO_2 does not react with bromides, the formation of DBPs is lower compared to chlorine. The disadvantages of ClO_2 are its low stability and the production of chlorite (~70 % of the applied dose) and chlorate, which may cause health problems in sensitive people (Aieta and Berg 1986; Kim et al. 2009; Mayan Kutty and Al-Jarrah 1991; Petrucci and Rosellini 2005; Rav-Acha 1998).

The aim of the present study was to compare the ability of ClO_2 and hypochlorite (NaClO) to reduce biofilms in seawater and to determine their disinfection efficiency in terms of bacterial cell biomass and biological activity. Moreover, given the adverse effects of their associated DBPs, the occurrence of THMs in disinfected waters was also examined.

2 Materials and Methods

2.1 Experimental Setup

The experiments were carried out at the "Zona d'Aquaris Experimentals" (ZAE) of the Institut de Ciències del Mar (CSIC) in Barcelona, Spain. Raw Mediterranean seawater was permanently pumped into the ZAE from 300 m offshore at 10 m depth. The main physico-chemical characteristics of the seawater used (Table 1) are representative of Mediterranean coastal seawater.

The experimental setup (Fig. 1) consisted of an open-circulation system, where seawater fed four experimental containers (18 cm×13 cm×6.5 cm) arranged in parallel at a flow of 185 mL min^{-1}. At the entrance of each vessel, the disinfectant was continuously supplied to guarantee the final concentrations of the chemicals in the whole volume with a residence time of ~6 min. Line 1 constituted the control treatment with no disinfectant added. In lines 2A and 2B, ClO_2 was continuously added at a final concentration of 0.2 and 0.4 mg L^{-1} of ClO_2, respectively, according to typical ClO_2 doses (EPA 1999; Gagnon et al. 2004; Petrucci and Rosellini 2005). Finally, in line 3, sodium hypochlorite (NaClO) was supplied at a final concentration of 1 mg L^{-1} of free Cl_2 in order to compare the results with typical chlorine residual disinfectant.

The whole system was maintained in the dark at 20 °C.

ClO_2 was obtained by diluting sodium chlorite ($NaClO_2$) and hydrochloric acid (HCl) (Eq. 1) using a Oxiperm®Pro OCD-162 5-D/G (Grundfos Alldos, Spain) that produces a ~2 g L^{-1} of ClO_2 per batch. NaClO was diluted using a 5 % (*w*/*v*) commercial solution (Ref. 212297.1211, Panreac, Spain).

$$5\ NaClO_2 + 4\ HCl \rightarrow 4\ ClO_2 + 5\ NaCl + 2\ H_2O \quad (1)$$

The concentration of each chemical throughout the experiment was determined as indicated in Section 2.2.

Table 1 Characteristics of (raw) seawater

Parameter	Units	Value (± Std. Dev.)
pH	–	8.0
Salinity	g L^{-1}	38
Temperature	°C	17±1
Turbidity	NTU	0.63±0.05
DOC	mg C L^{-1}	0.93±0.01
A_{254}	m^{-1}	1.04±0.07
$SUVA_{254}$	L mg C^{-1} m^{-1}	1.12±0.08

DOC dissolved organic carbon, A_{254} absorbance at λ=254 nm, $SUVA_{254}$ specific UV absorbance at λ=254 nm

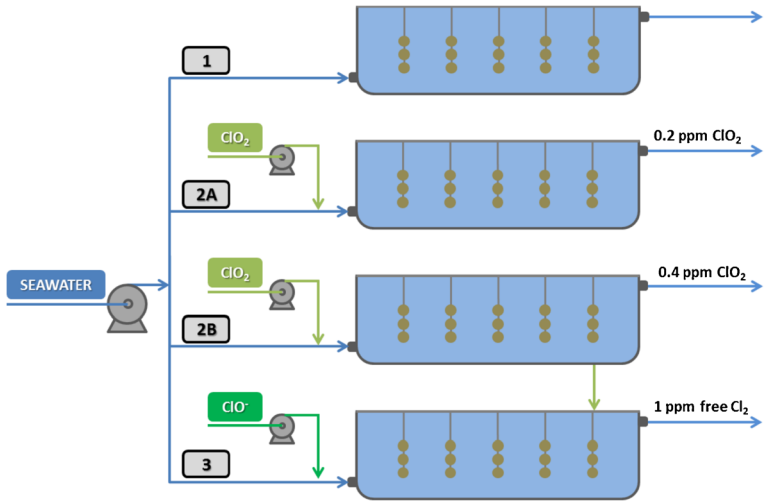

Fig. 1 Scheme of the treatment lines. *Line 1*, control (seawater); *line 2A*, addition of ClO2 at a final concentration of 0.2 mg L^{-1}; *line 2B*, addition of ClO2 at a final concentration of 0.4 mg L^{-1}; *line 3*, addition of NaClO to a final concentration of 1 mg L^{-1} of free Cl2. *Yellow circles* represent the non-porous glass beads suspended in the containers to track biofilm formation

To evaluate the efficacy of the disinfectant treatments, samples were taken at regular intervals for determining the bacterial biomass and activity in the outlet streams of the containers and analysed according to Section 2.3. Furthermore, to track biofilm formation, several sets of three non-porous glass beads (bead surface area= 1.9 cm^2) were anchored using a stainless steel wire and submerged in each of the experimental containers (Fig. 1). Measurements of the microbial activity on the beads' surface were conducted throughout the experiment, and microscopic observations of the beads' surface were conducted on the last day (Section 2.4).

2.2 Free Cl_2 and ClO_2 Determination

The concentrations of free Cl_2 (the proxy for NaClO) and ClO_2 were analysed according to the *n*,*n*-diethyl-*p*-phenylenediamine (DPD) method (Standard Methods 4500-Cl G and 4500-ClO_2 D, respectively (Standard Methods 1999)). Thus, seawater samples were measured immediately after collection using a pocket colorimeter (Cat. No. 58700-01, Hach-Lange) and the powder pillow performance method (Ref. 14070-99 for free Cl_2 and 27709-00 for ClO_2, Hach-Lange).

2.3 Bacterial Biomass and Activity in Seawater

2.3.1 Adenosine Triphosphate

Adenosine triphosphate (ATP) is a biomolecule present in all living microorganisms, and its concentration in seawater can be used as an indirect measure of the biological activity (Hammes et al. 2010). Therefore, it can be a useful tool for assessing disinfection efficiency. In this study, we estimated the ATP concentrations in the water in subsamples obtained before and after filtration through 0.22-μm membrane filters (Nuclepore, Whatman). The unfiltered subsample corresponded to the total ATP (ATP_{total}), including both particulate and dissolved ATP. Filtration retained most bacterial cells, so the filtered subsample corresponded to the dissolved, extracellular or cell-free ATP (ATP_{free}). The cellular ATP (i.e. that associated with living bacterial cells) was calculated indirectly by subtracting the acellular ATP from the total ATP levels. The ATP concentration was measured using the BacTiter-Glo™ Microbial Cell Viability Assay (Promega Biotech Iberica, Spain) and a GloMax® 20/20 luminometer (Promega Biotech Iberica, Spain). In an Eppendorf tube, 100 μL of seawater sample and 100 μL of ATP reagent were mixed and agitated with an orbital mixer, at room temperature for 3 min. Then, 30 s later, the luminescence signal was recorded as relative light units (RLU). RLU values were converted to nanogram ATP per litre using an ATP standard dilution series (A3377, Sigma-Aldrich). All the ATP analyses were performed in triplicate.

2.3.2 Total Cell Counts by Flow Cytometry

The total cell counts (TCC) were measured by flow cytometry, a rapid and sensitive technique routinely used in aquatic microbial ecology to estimate bacterial abundance (Gasol and Del Giorgio 2000; Hammes et al. 2008; Hoefel et al. 2003). The samples (1.8 mL) were

first fixed with 1 % paraformaldehyde (Sigma-Aldrich) and 0.05 % glutaraldehyde (Sigma-Aldrich) for 10 min in the dark. The samples were then stained with 2 μL of SYBR®Green I (1:10 dilution in dimethyl sulfoxide, Molecular Probes), mixed, incubated in the dark for 15 min and immediately frozen at −80 °C. The samples were thawed before being analysed using a FACSCalibur flow cytometer (BD Biosciences) equipped with a laser, emitting at a wavelength of 488 nm at an event rate of 38 μL min^{-1} for 2 min. The data acquisition was performed using CellQuest (BD Biosciences). Fluorescent latex beads (0.92 μm, Polysciences) at 10^6 beads mL^{-1} were added as an internal standard.

2.4 Biofilm Formation

2.4.1 Biofilm Analysis by ATP Measurement

The amount of biofilm formed on the glass beads surface was assessed indirectly over the time-course based on the ATP concentration. On the day of sampling, a bead from each treatment line was carefully removed and placed in an Eppendorf tube containing 200 μL of phosphate buffer (pH=7.0) and mixed with 200 μL of ATP reagent (G8231, Promega Biotech). After mixing for 3 min, 200 μL of supernatant was collected and processed as described above (Section 2.3.1).

2.4.2 Biofilm Observation by Scanning Electron Microscopy

On day 40, one bead from each treatment line was collected and air-dried. Each bead was mounted on a scanning electron microscopy (SEM) stub with adhesive double-sided carbon conductive discs and sputter-coated with gold–palladium. The beads were then viewed using a Hitachi S-3500N scanning electron microscope operating at 5 kV.

2.5 Analysis of Trihalomethanes

Samples for the measurement of THMs (BDCM, bromodichloromethane; TBM, bromoform; TCM, chloroform; DBCM, dibromochloromethane) produced during the treatments were collected at three different dates during the experiment (t=8, 15 and 22 days) from each line. With the aim of measuring the maximum DBP concentrations, the THM samples were not quenched but were kept in the dark at room temperature for 72 h. Afterwards, the samples were stored at 4 °C until the analysis. The THM measurements were performed by headspace sample introduction and gas chromatography/mass spectrometry (HS/GC/MS, with a Thermo Fisher Scientific TRACE GC Ultra gas chromatograph coupled to a DSQII mass spectrometer with a TriPlus automatic injector headspace module) with a THM detection limit of at least 1 μg L^{-1}. More details regarding THM method can be found elsewhere (EPA 2003; Pérez Pavón et al. 2008).

3 Results and Discussion

3.1 Seawater Disinfection

In the flow cytometry analysis, the dot plots representing Side Scatter (SSC) vs. green fluorescent signal (FL1) corresponding to the SYBR® Green I-stained DNA allowed us to distinguish the microbial cells from the background noise. In addition, based on the FL1 intensity, two specific clusters could be distinguished: the so-called low- (L-DNA) and high- (H-DNA) nucleic acid content bacteria (Gasol and Del Giorgio 2000; Hammes et al. 2008; Lebaron et al. 2001). Figure 2 shows the flow cytometry dot plots obtained for the treatments lines.

The bacterial abundance in seawater was high in the control (line 1) and in the ClO_2 treatments (lines 2A and 2B), whereas hypochlorite caused a clear decrease in the total cell numbers (line 3). The total bacterial abundance and biological activity (ATP concentration) are plotted together in Fig. 3 to show the effect of the disinfection on the two parameters.

When NaClO was used as a disinfectant, almost all (98 %) the bacterial cells were disrupted, releasing the intracellular material, including cellular ATP. Therefore, both the total cell number and the cellular ATP level diminished (1.8 log units and 76 %, respectively) compared with untreated seawater. However, the effect on the bacteria was much different when ClO_2 was used. Although the cellular ATP levels decreased due to cellular inactivity (78–84 %), only small reductions in the total cells counts (~0.1 log) were observed, suggesting of a different mechanism of bacterial cell inactivation. The results indicate that when ClO_2 is used, the biological activity of the cells decreases without losing cell integrity. In other words, ClO_2 does not seem to cause

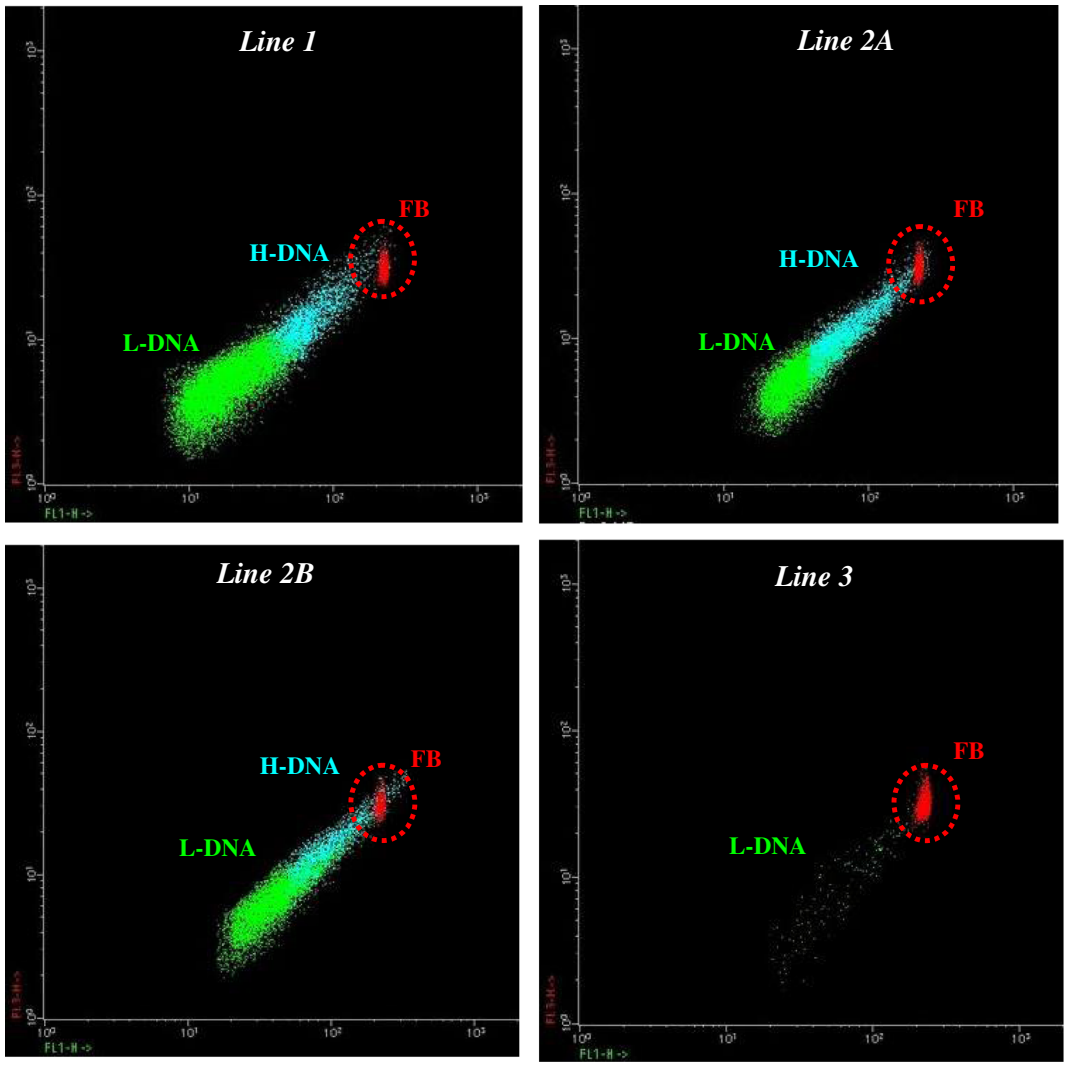

Fig. 2 Flow cytometry dot plots (red fluorescent signal in the *y*-axis vs. green fluorescent signal in the *x*-axis) of outlet water samples from seawater (*line 1*), 0.2 mg L^{-1} ClO2 (*line 2A*), 0.4 mg L^{-1} ClO2 (*line 2B*) and 1 mg L^{-1} free Cl2 (*line 3*). *FB*, calibration fluorescent beads

substantial physical damage to bacterial cells, although other subtle mechanisms of cell inactivation may be at play (Aieta and Berg 1986; Lelieveld et al. 2005). The present study also shows that measurements of both the total cell numbers and the cell viability (e.g., ATP) parameters are required to determine the effects of disinfecting agents on bacteria and drawing the correct mechanistic conclusions (Siebel et al. 2008).

Altogether, our results suggest that despite both disinfectants have different inactivation mechanisms, both are useful to inactivate the growth in seawater environment. Even though, other studies suggest that less ClO_2 is needed to achieve the same level of disinfection, especially in already contaminated spaces that involve a significant concentration of microorganisms, because it is more selective than chlorine (Aieta and Berg 1986; Gagnon et al. 2004; Petrucci and Rosellini 2005).

3.2 Biofilm Formation and Visualisation

3.2.1 Biofilm Formation Capacity

The degree of biological colonisation of the glass bead surface in the different treatment lines was determined by the total ATP concentration (Fig. 4).

In the absence of disinfectants (line 1—Seawater control), the biological activity increased exponentially in a two-step mode until day 10, consistent with

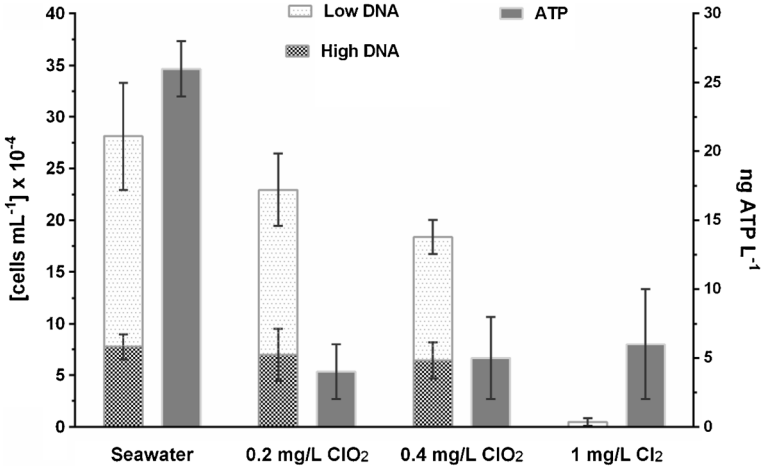

Fig. 3 Total cell numbers (low- and high-DNA bacteria), determined by flow cytometry, and cellular ATP levels in the four treatments: seawater (line 1), 0.2 mg L^{-1} ClO2 (line 2A), 0.4 mg L^{-1} ClO2 (line 2B) and 1 mg L^{-1} free Cl2 (line 3). (*Vertical bars* correspond to std. dev., n=6.)

previous studies (Abdul Azis et al. 2001; Gagnon et al. 2004). Afterwards, the activity remained constant, at approximately 700 pg ATP cm^{-2}, until the end of the experiment (day 41).

When a disinfectant was used (lines 2A, 2B and C), the biological activity on the bead surface remained very low in comparison with the control (line 1). Indeed, when disinfectants were added, ATP levels were almost 3 orders of magnitude lower than in the control. Thus, with 0.2 mg L^{-1} of ClO_2 addition (line 2A), ATP levels did not reach 1 pg ATP cm^{-2} in the stationary phase, and was even lower, at approximately 0.1 pg ATP cm^{-2}, when the seawater was treated with either 0.4 mg L^{-1} of ClO_2 or 1 mg L^{-1} of free Cl_2. Therefore, disinfection by continuous doses of either ClO_2 or free chlorine effectively inhibited biofilm formation in seawater. Interestingly, the effect was largely similar in the three treatments, with no significant differences between the 0.4 mg L^{-1} ClO_2 and the 1 mg L^{-1} Cl_2 doses.

To compare our results with those reported in the literature, we calculated a parameter analogous to the so-called biofilm formation rate (BFR). The BFR (van der Kooij et al. 1995a, b) is evaluated in a biofilm monitor consisting of a vertically glass column containing glass cylinders. Water flows through this column at a fixed flowrate. Then cylinders are collected at regular intervals, sonicated in sterile water and the suspended biomass concentration is assessed with ATP analysis. The BFR value is calculated from the linear increase of the biomass concentration on the surface of cylinders as

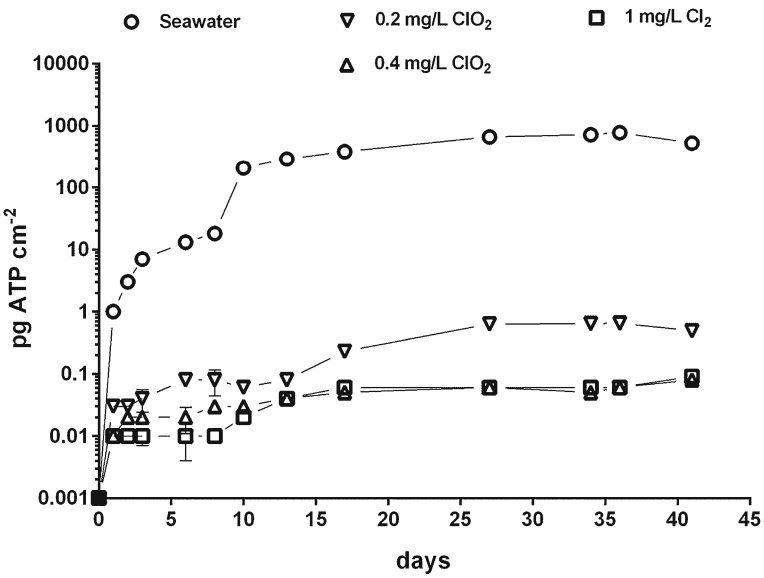

Fig. 4 Biological activity (total ATP) on the glass bead surface over time in the four treatments: seawater (line 1), 0.2 mg L^{-1} ClO2 (line 2A), 0.4 mg L^{-1} ClO2 (line 2B) and 1 mg L^{-1} free Cl2 (line 3). (*Vertical bars* correspond to std. dev., n=3.)

Table 2 Biofilm formation rate (BFR) obtained from different water treatments

Water source	BFR [pg ATP cm^{-2} day^{-1}]	Reference
Mediterranean coastal seawater	62[a]–100[a]	Simon et al. (2013); this study
Seawater+ClO_2 at final conc.: 0.2–0.4 mg L^{-1}	<1[a]	
Seawater+free Cl_2 at final conc.: 1 mg L^{-1}		
Treated groundwater	11	Kruithof et al. (1998); van der Kooij et al. (1995a)
Treated river water	38	
Treated surface water	15	
Treated groundwater+100 μg of acetate-C L^{-1}	382	
Drinking water	4	van der Kooij et al. (1995b)
Drinking water+10 μg of acetate-C L^{-1}	362	
Ultrafiltered surface water	<1	Kruithof et al. (1998)
Treated waters in Netherlands	<30	van der Kooij et al. (2003)

[a]Note that our BFR parameter do not correspond exactly to the BFR described in the bibliography

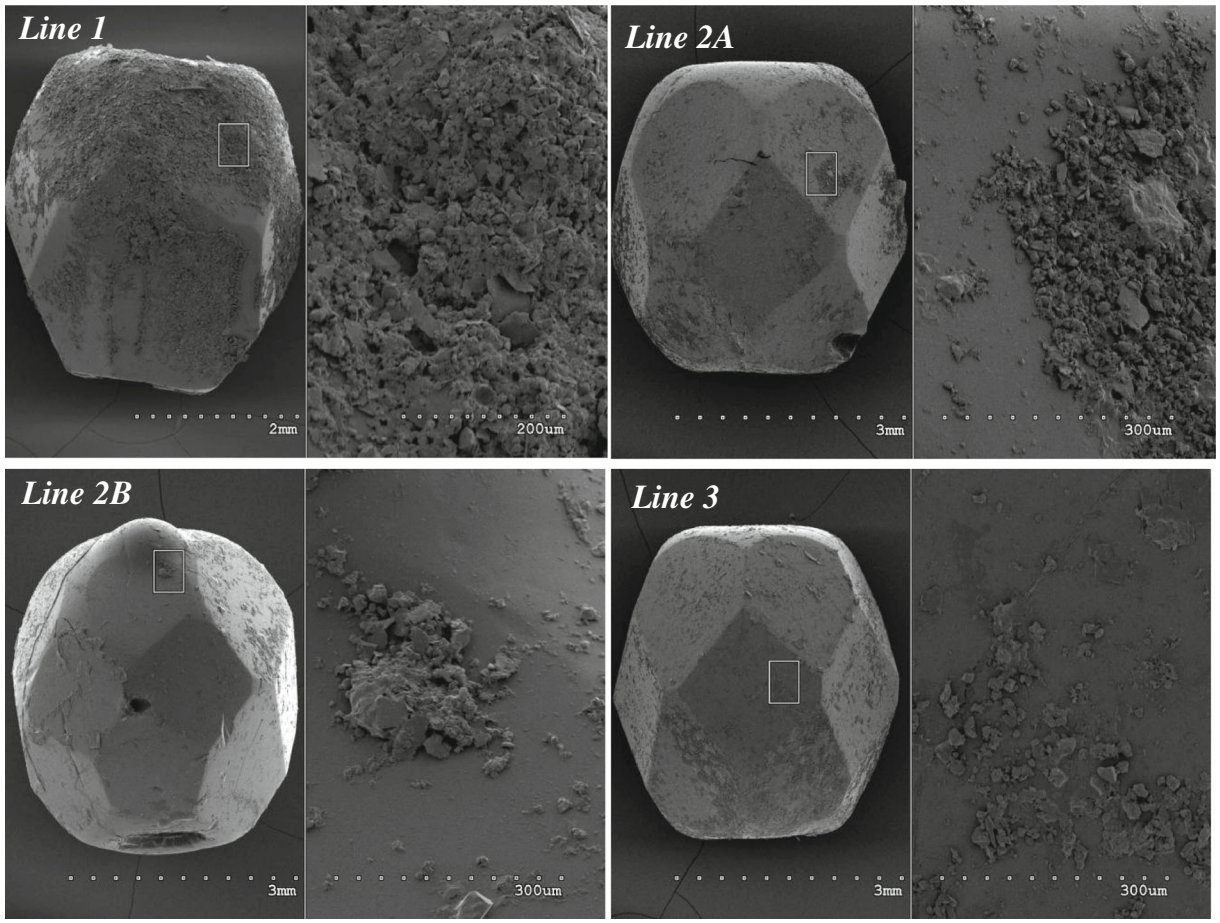

Fig. 5 SEM images of the beads and details of their surface, after 41 days of exposure to seawater (*line 1*), 0.2 mg L^{-1} ClO2 (*line 2A*), 0.4 mg L^{-1} ClO2 (*line 2B*) or 1 mg L^{-1} free Cl2 (*line 3*)

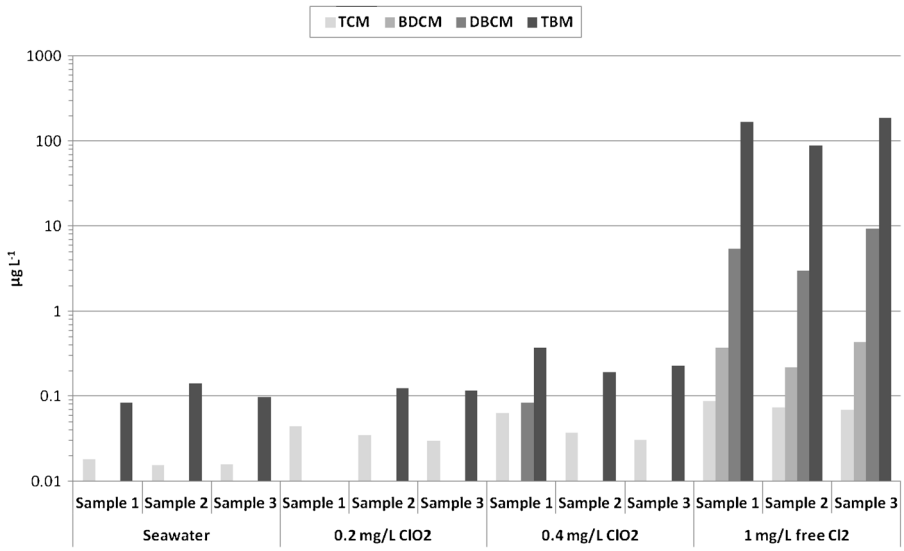

Fig. 6 THM occurrence and abundance for the three different sampling days. The treatments were seawater (line 1), 0.2 mg L^{-1} ClO2 (line 2A), 0.4 mg L^{-1} ClO2 (line 2B) and 1 mg L^{-1} free Cl2 (line 3)

a function of time. In this study, a surrogate BFR is calculated from the slope during the linear increase of the biomass produced at the surface of the beads over time. Note that the alternate BFR data obtained in this work can only be partially compared with those found in the bibliography (see Table 2) due to the different experimental conditions (flow conditions, test rigs, etc.).

According to Table 2, our coastal seawater had a moderate biofilm formation capacity, whereas that of seawater disinfected by either free chlorine or chlorine dioxide was comparable to that of ultrafiltered water. Furthermore, BFR values higher than 100–120 ng ATP cm^{-} day^{-1} indicate potentially high biofouling-related problems in the operating system, and BFR values below 1 ng ATP cm^{-2} day^{-1} are indicative of a rather inhibited biological activity that would enable stable operation for long periods (Vrouwenvelder et al. 1998, 2003; Vrouwenvelder and van der Kooij 2001).

3.2.2 Biofilm Observation by Scanning Electron Microscopy

The SEM micrographies of the beads' surface showed that the highest biomass developed in the control beads (line 1; see Fig. 5). Thus, the beads collected from the treated lines clearly showed less biological activity on their surface. Note that the surface of the control beads was unevenly colonised, probably because the beads were not perfect spheres, but polyhedral. Thus, the shear forces were stronger at the edges of the beads.

The matrix, composed of both organic and inorganic material, contained bacteria and other planktonic (diatoms, coccolithophorids, flagellates) and benthic (basically diatoms) microorganisms commonly found in the natural coastal seawater. They were probably entrapped and concentrated on the bead surface, which functioned as a microcosm nucleus where the microbial community develops at the expense of particulate and dissolved organic matter.

Table 3 Highest values of THMs in some international regulations (EPA 2001; EU Standards 1998; WHO 2008)

DBPs		WHO [µg L^{-1}]	EU standards [µg L^{-1}]	US EPA [µg L^{-1}]
THMs	TCM	200		
	BDCM	60		
	DBCM	100		
	TBM	100		
	THM_4[a]	–	100	80

WHO World Health Organization, *US EPA* United States Environmental Protection Agency

[a] THM_4=sum of the concentrations of $CHCl_3$, BDCM, DBCM and $CHBr_3$

3.3 THM Occurrence

The seawater disinfected by NaClO produced more THMs than the water disinfected by ClO_2 (either 0.2 or 0.4 mg L^{-1}). The types and abundance of THMs for each of the three samples (taken at different days) are detailed in Fig. 6.

When NaClO was used as a disinfectant, total THM account were 154±12 μg L^{-1}, whereas only up to 0.34± 0.03 μg L^{-1} were produced in the ClO_2 treatment. The low production of THMs was also because ClO_2 cannot oxidise bromide (Petrucci and Rosellini 2005). Therefore, at the ClO_2 range tested, THM production could be virtually reduced by 100 %. Among the compounds, TBM was the most abundant, representing the 77–96 % of the total THMs in disinfected samples and reaching 148 μg L^{-1} when NaClO was used. These findings are consistent with those of other seawater disinfection studies (Ali and Riley 1989; Allonier et al. 1999; el Din et al. 1991; Fabbricino and Korshin 2005; Ram et al. 1990; Sam Yang 2001), but the values are fairly low compared with the THM levels found in swimming pools from southeast France, due to the impact of bromide ions, organic carbon, temperature and also residual disinfectants (Parinet et al. 2012).

In conclusion, if disinfected seawater is meant to be used as drinking water, then the ClO_2 lines (lines 2A and 2B) would meet current legislation guidelines, whereas the NaClO line (line 3) would not (see Table 3).

4 Conclusions

Seawater can be disinfected by continuous treatment with either chlorine dioxide (residual concentration of 0.2–0.4 mg ClO_2 L^{-1}) or sodium hypochlorite (1 mg free Cl_2 L^{-1}). The two disinfecting agents show distinct effects on biological activity and cell counts, suggesting that they have different mechanisms of cell inactivation. Although chlorine dioxide reduces biological activity by cell disruption, sodium hypochlorite inactivates the cells' physiology and metabolism without bursting them.

The biofilm precursors are successfully depleted when seawater is disinfected by either chlorine dioxide or sodium hypochlorite at the concentrations tested. Indeed, both disinfectants reduced the biological activity on the biofilm from 700 to below 1 pg ATP cm^{-2} (at day 40). These findings were also confirmed by SEM micrographies.

Different DBP concentrations were produced by each disinfectant tested. Chlorine dioxide was able to virtually not generate any THMs, whereas sodium hypochlorite generated approximately 155 μg L^{-1} of mostly brominated compounds (77–96 % of the total) due to the high concentration of bromide in seawater.

Our overall results suggest that ClO_2 may be a useful alternative disinfectant to free chlorine due to its combined efficiency in biofilm precursor reduction and minimal DBP production. The present study was based on a continuous supply of the disinfectants. To identify the best disinfection strategy, further studies will address other possibilities, such as a shock or a discontinuous supply of the chemical and comparisons with other products (e.g., chloramines).

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