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Chlorine dioxide as an alternative antifouling biocide for cooling water systems: Toxicity to larval barnacle *Amphibalanus reticulatus* (Utinomi)

Srinivas Venkatnarayanan [a], P. Sriyutha Murthy [a,b], Ramalingam Kirubagaran [c], Vayalam P. Venugopalan [a,b,*]

[a] Biofouling and Biofilm Processes Section, Water & Steam Chemistry Division, Bhabha Atomic Research Centre, Kalpakkam 603 102, Tamil Nadu, India
[b] Homi Bhabha National Institute, Anushakti Nagar, Mumbai 400 094, India
[c] Marine Biotechnology, ESSO-National Institute of Ocean Technology, Chennai 600 100, India

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ABSTRACT

Chlorine dioxide (ClO_2) is seen as an effective alternative to chlorine, which is widely used as an antifouling biocide. However, data on its efficacy against marine macrofoulants is scanty. In this study, acute toxicity of ClO_2 to larval forms of the fouling barnacle *Amphibalanus reticulatus* was investigated. ClO_2 treatment at 0.1 mg/L for 20 min elicited 45–63% reduction in naupliar metamorphosis, 70% inhibition of cyprid settlement and 80% inhibition of metamorphosis to juveniles. Increase in concentration to 0.2 mg/L did not result in any significant difference in the settlement inhibition or metamorphosis. Treatment with 0.2 mg/L of ClO_2 elicited substantial reduction in the settlement of barnacle larvae compared to control. The study indicates the possibility of using ClO_2 as an alternative antifouling biocide in power plant cooling water systems. However, more work needs to be done on the environmental effects of such switchover, which we are currently undertaking.

1. Introduction

Cooling water systems (CWS) of coastal electric power generating stations draw large quantities of seawater for condenser cooling purposes (Langford, 1990; Lopez-Galindo et al., 2010; Rajagopal et al., 2012). The abstracted seawater contains meroplanktonic organisms, many of which tend to settle and grow in the CWS, resulting in biofouling. Conventionally, chlorine (either as gas or as hypochlorite) has been the biocide of choice for biofouling control in CWS due to its cost-effectiveness, easy handing and well studied chemistry in seawater (Rajagopal et al., 1995; Nair et al., 1997). However, recently, utilities are on the lookout for a more efficient alternate biocide, due to some drawbacks associated with chlorine: 1) chlorine is reactive to organic matter, leading to the formation of halogenated organic by-products such as trihalomethanes (THM), haloacetonitriles (HANs), halophenols (HPhs) and haloacetic acids (HAAs) (Allonier et al., 1999; Sabrina and Carlo, 2005; Rajamohan et al., 2007, 2014), 2) dissociation of hypochlorous acid (HOCl, the actual biocidal component produced during chlorination) is pH dependent and at high seawater pH of 8.2, its biocidal effect is reduced, 3) chlorine has limited ability to penetrate biofilms and is easily consumed and neutralized by the biofilm matrix, resulting in a quenching effect (LeChevallier et al., 1990; Simpson et al., 1993; De Beer et al., 1994; Pickrell et al., 1999).

Another problem encountered in the use of chlorine is that relatively high concentrations are required to achieve effective biofouling control (Rajagopal et al., 2003), thereby exceeding the prescribed environmental discharge limits. In-plant administered levels of chlorine (0.2–0.5 mg/L, TRO) did not prevent larval settlement in the barnacle *Amphibalanus reticulatus* (Venkatnarayanan et al., 2016). Despite chlorination, significant amount of fouling by barnacles has been observed in the cooling water conduits of Madras Atomic Power Station (MAPS) (Rajagopal et al., 1995; Murthy et al., 2011). Earlier studies on chlorination have also revealed that concentrations ranging from 0.5 to 1.0 mg/L are required to kill adult organisms such as *Balanus improvisus* (McLean, 1973), *Perna viridis* (Rajagopal et al., 1995, Gunasingh et al., 2002), Zebra mussel (Nakayama et al., 1997), *Mytilopsis leucophaeata* (Verween et al., 2009) and *Modiolus philippinarum* (Rajagopal et al., 2003), which is practically impossible to adopt in an operating plant. Therefore, there is a need to explore alternate biocide, which is effective at low concentrations and produces less toxic residues.

Chlorine dioxide is gaining importance as a broad spectrum industrial biocide due to the following reasons: 1) it is selective and has high oxidation potential (Agus et al., 2009), 2) it does not react with bromides, amines, ammonia and organics to form disinfection by-products (Petrucci and Rosellini, 2005), 3) it has more penetrative power on established biofilms and their EPS matrix (Junli et al., 1997) and 4) it is effective at a wide pH range (5.0 to 9.0). These unique features of

* Corresponding author at: Biofouling and Biofilm Processes Section, Water & Steam Chemistry Division, Bhabha Atomic Research Centre, Kalpakkam 603 102, Tamil Nadu, India.
E-mail address: vpvenu@barc.gov.in (V.P. Venugopalan).

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chlorine dioxide allow it to be effectively used in controlling biofilms in dairy industry (Oliver et al., 1989), dental unit water systems (Wirthlin and Marshall, 2001), drinking water systems (Junli et al., 1997), desalination units (Petrucci and Rosellini, 2005), paper and pulp industry (Nelson, 1982), fertilizer plants and petrochemical industry (Petrucci and Rosellini, 2005). Chlorine dioxide is known to be highly effective in treating biofilms; intermittent application at 0.6 to 1.0 mg/L has been known to remove established biofilms in cooling towers (Mayack et al., 1984). Studies by Junli et al. (1997) suggested that chlorine dioxide could penetrate through cellular membranes and kill and remove biofilms, suggesting that it had more cleansing effect than liquid chlorine. Similarly, Petrucci and Rosellini (2005) demonstrated very low concentrations (0.05 to 0.1 mg/L) of ClO_2 to be very effective in combating biofilms and macrofoulers in cooling water systems of power stations and petrochemical industries. However, extensive use of chlorine dioxide in marine systems is limited due to (1) its relatively high cost (it is about 2.5 times more costly than chlorine) and (2) its inherent instability that requires on-site generation (White, 1999).

In comparison to chlorine, very few studies exist on the toxicity of chlorine dioxide to barnacle larvae and concentrations required to prevent settlement of barnacle larvae are not known. In accordance with the Best Available Technology (BAT) for cooling water systems, it is important to generate data on the biocidal action of chlorine dioxide on larval organisms and its antifouling potential in industrial cooling water systems (CWS). *Amphibalanus reticulatus* (Utinomi, 1967) is a dominant fouling organism in the CWS of Madras Atomic Power Station (MAPS), located at Kalpakkam on the east coast of India, and is not effectively controlled by the in-plant concentrations (0.2–0.5 mg/L, TRO) of chlorine employed. The main objectives of this study were: (1) to assess the toxicological effects of chlorine dioxide on barnacle larval (pre-competent and competent larvae) forms and (2) to assess the effect brief exposures to chlorine dioxide on larval metamorphosis. It was anticipated that the data would be useful to both industries (for developing antifouling strategy) and regulatory authorities (to frame discharge criteria for effluents containing chlorine dioxide).

2. Materials and methods

2.1. Adult brood stock for larval culture

Amphibalanus reticulatus (Utinomi) adults were collected by suspending fibre re-inforced plastic (FRP) coupons from the jetty piers of Madras Atomic Power Station (MAPS) located at Kalpakkam on the East coast of India. The FRP coupons were brought to the laboratory and washed in running seawater to clear debris and other fouling organisms. The coupons were air-dried under shade for 2 h and then suspended in sterile 0.22 μm filtered seawater. Within a few hours, the adult barnacles began to release nauplius I (N-I) larvae. The nauplii were collected using a Pasteur pipette, after attracting them to a point source of light. The nauplius I larvae metamorphose to nauplius II (N-II) within 3 h after hatching. The nauplii II were maintained in 1.0 L beakers containing 0.22 μm filtered seawater at 26 ± 1 °C at a photoperiod of 12 h light: 12 h dark in an environmental chamber. The nauplii were fed with a diet of *Chaetoceros lorenzianus* (diatoms) cultured in *f/2* medium (Guillard and Ryther, 1962) at a concentration of ~2×10^5 cells/mL. The larval stock was regularly monitored under a stereomicroscope to determine the stage of development. The seawater was changed daily. Cypris larvae emerging from the culture beakers were collected and stored at 4 °C until used for experimentation.

2.2. Chlorine dioxide toxicity experiments

Seawater (ca 34 ppt salinity) for the experiments were collected from the coastal waters off Kalpakkam, filtered (0.22 μm Millipore) and stored in black carboys. Before the commencement of experiments, the chlorine dioxide demand of the seawater was assessed. After neutralization of the demand (0.3 ± 0.05 mg/L), the chlorine dioxide concentrations required for the toxicity experiments (0.1, 0.2 and 0.5 mg/L) were prepared. All the treatments were performed at room temperature 26 ± 1 °C. The biocide concentration (measured as total residual oxidants, TRO) was estimated using a HACH pocket colorimeter using DPD tablet NO 4 (Lovibond, USA) (White, 1972; Jolley and Carpenter, 1983). The HACH colorimeter had a detection range of 0.02 to 8.0 mg/L. The amount of biocide dosed and the residual obtained in each treatment are shown in Table.1.

Review of literature suggests that chlorine dioxide is 25 times more effective than conventional chlorine as it is effective at a wide range of pH (Zhang et al., 2008). The test concentrations (0.2 to 0.5 mg/L) were fixed on the basis of this presumption. Commercial chlorine dioxide (BIOX), a proprietary product was purchased from M/s. Scotmas Limited, UK. ClO_2 solution was prepared by acidification of sodium chlorite. This was done by mixing solution (A) sodium chlorite, the precursor, and solution (B) an acid activator, which is a mixture of hydrochloric acid and phosphoric acid, in an equal ratio (1:1). Immediately after mixing, the concentration of the stock solution was determined by Iodometric titration (White, 1972). From the stock solution, a separate working stock of 500 mg/L was prepared. Chlorine dioxide concentrations for toxicity experiments (0.1, 0.2 and 0.5 mg/L) were prepared by diluting appropriate volumes of working stock to 50 mL of demand-free seawater. The contact time for chlorine dioxide exposure used was 3, 10 and 20 min. The exposure times were fixed based on the logic that seawater abstracted for cooling in a typical power plant would take about 20 min to reach the outfall point. In the present study, two different experiments were conducted to 1) assess the chlorine dioxide toxicity on naupliar stages N-II, N-IV and N-VI and its impact on whole larval development and 2) study the effect of chlorine dioxide on cypris settlement and metamorphosis to juveniles. The experiments were conducted using two independent batches of larvae, with three replicates for each treatment (concentration and contact time). As the differences between the two batches were not significant, the data shown here is from one representative batch.

2.3. Effect of chlorine dioxide on discrete larval stages

In this experiment, independent naupliar stages N-II, N-IV and N-VI were exposed to different chlorine dioxide test concentrations for various contact times to assess the impact on larval development. The stock barnacle larval cultures were checked regularly (once every 12 h) under a stereomicroscope. The larvae that reached the desired stage were picked up and pooled together. Each of the treatment consisted of about 20 to 30 N-II, N-IV and N-VI nauplii. The larvae were dispersed in 50 mL Falcon tubes containing the prescribed chlorine dioxide concentrations (0.1, 0.2 and 0.5 mg/L) and were exposed to three different contact times 3, 10 and 20 min, respectively. After treatment, the larvae in the tubes were filtered through 100 μm Nylobolt silk and rinsed twice with filtered seawater to remove traces of chlorine dioxide. Similar kind of treatment was done for the controls also, but with no exposure to the biocide. The larvae were re-suspended in Falcon tubes with 30 mL of 0.22 μm filtered seawater and were fed daily with the diatom *Chaetoceros lorenzianus* at a final concentration of ~10^5 cells/mL. The seawater in the tubes was changed daily; and the larvae were reared under conditions described earlier. Larval survival and moulting were assessed every day using a stereomicroscope, until they reached the cypris stage. Percentage survival and metamorphosis at each stage were noted.

2.4. Effect of chlorine dioxide on cypris settlement and metamorphosis

The naupliar larvae of *A. reticulatus* were reared up to the cypris stage, under conditions mentioned earlier. About 10 to 15 cypris larvae were exposed for 3, 10 and 20 min to chlorine dioxide concentrations of 0.1, 0.2 and 0.5 mg/L (TRO). After exposure, the larvae were rinsed in

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