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Iron dependent degradation of an isothiazolone biocide (5-chloro-2-methyl-4-isothiazolin-3-one; CMI)

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An isothiazolone biocide, 5-chloro-2-methyl-4-isothiazolin-3-one (CMI), was degraded in the presence of iron. According to the Fe-dependent degradation of CMI, stoichiometric production of chloride was observed. Copper and stainless steel did not enhance the physico-chemical degradation of CMI, whilst phosphate inhibited the Fe-dependent degradation. Neither aerobic or anaerobic conditions influenced the Fe-dependent CMI degradation. Furthermore, FeO(OH)-powder and Fe_3O_4-powder did not lead to the physico-chemical degradation of CMI. Rapid disappearance of CMI was observed in an operating cooling water plant. CMI added into the cooling tower declined from 1.4 mg l^{-1} to below 0.1 mg l^{-1} in two days. This finding is important in optimizing use of CMI and combating resistance if encountered.

Keywords: *Biocide, isothiazolone, biofilm, fouling, degradation*

Introduction

The presence of biofilm on a metal surface, as well as its metabolic activities, can cause microbiologically influenced corrosion (MIC) (Cloete et al. 1992; George et al. 2003; Hamilton 2003; Kjellerup et al. 2005; 2006). In addition, biofilm increases the heat transfer resistance of process heat exchangers, fluid friction resistance and causes *Legionella*-related problems (Goh et al. 2005; Ishimatsu et al. 2001). Biofilm formation on the surface of heat exchange tubes reduces the heat transfer rate because the thermal conductivity of biofilm is significantly less than that of metal. The major economic impact caused by biofilm in cooling water systems is because of energy losses due to increase of fluid frictional resistance and increase of heat transfer resistance (Michael, 2003). Isothiazolone biocides are widely used in a variety of water treatment applications for control of microbial growth and biofouling (Williams, 2004).

Isothiazolone utilizes a two-step mechanism involving rapid inhibition (within minutes) of growth and metabolism, followed by irreversible cell damage resulting in loss of viability (Williams, 2006). Isothiazolones are stable under typical environmental conditions, but are degraded by strong nucleophiles, such as sulfide or alkanolamines (Williams, 2004). Biological degradation studies using high performance liquid chromatography (HPLC) and radiotracer techniques showed that isothiazolones rapidly biodegrade in aquatic environments with half-lives significantly less than 24 hours (Williams et al. 1999). In addition to these mechanisms of isothiazolone degradation, Fe-dependent degradation was investigated for the first time in this study. The correct use of a biocide is important if biofilm formation and enlargement, as well as reducing the total number of microbes in the bulk water, are to be controlled.

Materials and methods

Biocide

A commercial mixture of two isothiazolone compounds, 5-chloro-2-methyl-4-isothiazolin-3-one (CMI) 10% and 2-methyl-4-isothiazolin-3-one (MI) 3% was used. The isothiazolone biocides described in this paper were manufactured by Chemicrea Inc., Japan. The concentration of CMI does not include the MI component in this study.

Fe-dependent CMI degradation

Carbon steel (CS) coupons (10 mm × 20 mm) with a thickness of 0.35 mm were cut from a sheet. The specified composition of the carbon steel coupons was in wt% 99.71 Fe, 0.03 C, 0.19 Mn, 0.017 S, 0.013 P, and 0.01 Si. Stainless steel (SUS304) and copper (99.994 wt%) coupons were also used. The surface was wet polished with an 800-grit polishing paper. The polished coupons were cleaned ultrasonically in acetone for 15 min, air-dried, and placed in a glass vial (125 ml) with a CMI solution (20 mg l^{-1}, 40 ml). As a control, CMI was incubated without any additives. Incubation was conducted by shaking the vial at 120 rpm in 25 °C. The CMI solution was sampled once a day to analyze the concentration of CMI and chloride. CMI concentration was determined by high performance liquid chromatography (LC-10AT, Shimadzu) equipped with reversed phase chromatography column (TSKgel ODS-80Ts, Tosho Co., Japan). Solvent (MeOH 40%, Acetic acid 60%) was run at 0.5 ml min^{-1} and 40˚C through the column. Chloride concentration was measured by ion-chromatography (LC-10AD; Shimadzu) equipped with a guard column (Slim-pack IC-GA3, Shimazu Co., Japan) and a measurement column (Slim-pack IC-A3, Shimazu Co., Japan). Solvent (8mM p-hydroxybezoic acid, 3.2 mM Bis-Tris, 50 mM

Boric acid) was run at 1.2 ml min^{-1} and 40˚C through the column. Experiments were conducted at 25˚C.

The effect of phosphate and two ferrous compounds, Iron (III) hydroxide oxide (FeO(OH)) and triiron tetroxide (Fe_3O_4), on Fe-dependent CMI decomposition was also investigated. CS coupons (10 mm × 20 mm) were incubated in CMI solution (20 mg l^{-1}, 40 ml) with and without sodium phosphate (PO_4^{3-}: 500 mg l^{-1}). The same size of CS coupon was also incubated in CMI solution (20 mg l^{-1}, 40 ml) with iron powder (0.56 g), FeO(OH)-powder (0.89 g), and Fe_3O_4-powder (0.79 g). The weight of each powder was prepared to obtain the same weight of iron in the coupon and each metal-powder respectively. The incubation condition was same as described above.

Aerobic and anaerobic experiments

To investigate the effect of dissolved oxygen on Fe-dependent CMI degradation, CS coupons (10 mm × 20 mm) were incubated in CMI solution (20 mg l^{-1}, 40 ml) in glass vials (125 ml) under aerobic and anaerobic condition at 25˚C, respectively. Aerobic condition were achieved by aerating the solution for 10 min. Anaerobic conditions were produced by bubbling the solution with pure nitrogen gas (99.9995 V%) for 10 min and sealing hermetically with butyl rubber. The incubation was conducted for two days and the CMI concentration in the solution was analyzed. Incubation was also conducted without CS coupons under aerobic and anaerobic conditions, respectively.

Fate of CMI in the operating plant

To investigate the fate of CMI in the operating plant, the change in the concentration of CMI in the cooling water system was analyzed. The main specifications of the plant are shown in Table 1.

The system consisted mainly of a cooling tower equipped with a heat-exchanger on the roof of the building. The main material used in for the heat-exchanger was copper; minor parts were of carbon steel. The water sample was filtered through a membrane filter (0.22-μm pore size; Millipore), transported to the laboratory in a cooler-box, and the concentration of CMI analyzed.

Results

Fe-dependent CMI degradation

In cooling water systems, carbon steel, copper and stainless steel are commonly used for heat exchanger pipage and wall material. The same size of carbon steel, copper and stainless steel coupons (10 mm × 20 mm) were suspended in the CMI solution and the change in CMI concentration in the glass vial analyzed over four days (see Figure 1). The CMI concentration in the solution without metal coupon did not change (see Figure 1A). On the other hand, the CMI concentration decreased in the presence of CS coupon. Phosphate is a corrosion inhibitor that is frequently used to control corrosion. The high affinity of phosphate anions for corroded surface metals leads to the formation of a stable phosphate-metal complexes, which limits further corrosion (Hiemstra & Van Riemsdijk, 1996; Persson et al. 1996). The addition of phosphate reduced the decrease of CMI concentration in the presence of CS coupons. Stainless steel and copper coupons did not influence the change in CMI concentration (see Figure 1B).

To identify the degradation of CMI, the concentration of chloride, which is one of the byproducts of CMI degradation, was analyzed. After 4d incubation under three different conditions, the chloride concentrations in the CMI solution were compared (see Figure 1C). When the CMI solution was incubated with the CS coupon, about 6 mg l^{-1} chloride was detected in the solution. The theoretical concentration of chloride when the CMI (initial concentration: 22.7 mg l^{-1}) is completely decomposed is 5.4 mg l^{-1}. Therefore, the decrease of CMI concentration in the presence of the CS coupon was not due to the adsorption of CMI to the CS coupon, but due to complete chemical decomposition. This result indicated that Fe-dependent degradation of CMI did not produce organic bound chlorine or lose biocidal activity.

To investigate the effect of iron derivatives on CMI degradation, fine powders of Fe, FeO(OH) and Fe_3O_4 respectively were incubated in the CMI solution (see Figure 2). The mean diameter of these fine powders was not available from the data sheet of the supplier. However, microscopic observation showed the size of the Fe powder granules was <100 μm. Neither the presence of FeO(OH) nor Fe_3O_4 influenced CMI degradation. On the other hand, the presence of Fe powder enhanced CMI degradation. The weight of Fe powder (0.56 g) was the same as that of CS coupon (10 mm × 20 mm × 0.35^t mm). The degradation rate of CMI in the presence of Fe powder was higher than that in the presence of the CS coupon.

Aerobic and anaerobic experiments

Since oxidative degradation of CMI was thought to be one of the possible mechanisms, the influence of aerobic and anaerobic conditions on the Fe-dependent CMI degradation was investigated. Without CS coupons, bubbling with nitrogen did not influence the CMI concentration (see Figure 3). On the other hand, in the presence of CS coupons, the CMI concentration decreased below 5 mg l^{-1} after 2d incubation under both aerobic and anaerobic conditions. This result indicates that dissolved oxygen was not involved in Fe-dependent CMI degradation. Fe-dependent CMI degradation was not due to an oxidative reaction.

Fate of CMI in the operating plant

CMI is commonly used as a biocide in cooling water plants in Japan. The cooling water plant used in this study was in an office building in the Tokyo area. The cooling tower was located on the roof and the heat-exchanger inside the building. Carbon steel pipes are used to transport circulating water between the cooling tower and the heat-exchanger. CMI was poured into the cooling tower to a final concentration of 2.0 mg l^{-1} as recommended by the supplier. However, the initial measured concentration of CMI was 1.4 mg l^{-1}. The CMI concentration was monitored for one week. Although the concentration of CMI in the circulating water was scattered during the initial several hours after addition due to the uneven distribution of CMI in the circulating water (see Figure 4) the concentration decreased rapidly and reached below 0.1 mg l^{-1} after 2d.

Discussion

The present study was motivated by observations made in an earlier study (Tanji et al. 2002). While investigating the effect of CMI on biofilm formed on the carbon steel, the rapid decrease of CMI concentration in the test vessel was also observed. Several possible causes of this decrease of CMI concentration were considered possible, including physico-chemical adsorption to the biofilm or carbon steel and biological degradation by biofilm microorganisms. The experimental results suggested that physico-chemical adsorption was the likely cause. A decrease in CMI was observed when CMI (20 mg l^{-1}) was incubated for 5 d under three separate conditions: in the presence of bare carbon steel, biofilm and rust formed on carbon steel, and activated sludge obtained from a municipal wastewater treatment plant. It was observed that the decrease in CMI of activated sludge was smaller than that in the bare carbon steel and biofilm formed on carbon steel conditions. The CMI decrease in the presence of bare carbon steel was the almost same as when biofilm formed on the carbon steel.

Carbon steel, stainless steel and copper are the main materials used in cooling water plants. Copper and stainless steel did not influence the physico-chemical degradation or adsorption of CMI (see Figure 1B). The main component of stainless steel is iron. For instance, SUS304, one of the most commonly used stainless steels, consists of Cr-18%, Ni-8%, and the rest is Fe. The surface of the stainless steel is covered with a thin chromic passive film which blocks direct contact between the iron and CMI, and therefore it does not influence Fe-dependent CMI degradation. Phosphates are corrosion inhibitors that are frequently used to control corrosion in drinking water distribution systems (Magali et al. 2003). Phosphate leads to the formation of a protective film at the carbon steel surface. Therefore, the addition of a high concentration of phosphate inhibited the Fe-dependent CMI degradation in a similar fashion to stainless steel (see Figure 1A). Two typical iron compounds, FeO(OH) and Fe_3O_4, known as red and black rust, respectively, were also examined with regard to their influence on CMI degradation. However, no substantial effect of these compounds on CMI degradation was shown (see Figure 2). These results indicated that the direct contact of CMI with bare iron is essential for Fe-dependent CMI degradation. However, further studies are necessary to identify the mechanism of Fe-dependent CMI degradation.

The lowest concentration of biocide that inhibits the growth of the microbes is defined as the minimum inhibitory concentration (MIC). MIC (mg l^{-1}) of MCMI (mixture of CMI and MI) is reported as 1.3 for bacteria, 0.35 for algae and 1.7 for fungi (Williams, 2004). CMI added to the operating plant disappeared rapidly, and reached the lower limit of detection in 3d (see Figure 4). Therefore, for the control of bacteria in the system, continuous addition and monitoring of CMI might be needed. Elucidation of the Fe-dependent CMI degradation mechanism and chemical modification of CMI to make CMI resistant to Fe-dependent degradation might improve its value as a biocide.

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Figure legends

Figure 1. Fe-dependent CMI degradation. The estimated initial concentration of CMI was 20 mg l^{-1}. (A) Change of CMI concentration. CMI solution without any additive (solid cycle), with a CS coupon (10 mm × 20 mm) (triangle), and with a CS coupon and sodium phosphate (PO_4^{3-} : 500 mg l^{-1}) (square). (B) Change of CMI concentration. CMI solution with a stainless steel coupon (10 mm × 20 mm) (solid cycle) and with a copper coupon (10 mm × 20 mm) (triangle), (C) Production of chloride ion by Fe-dependent CMI degradation. Bars indicate standard errors in triplicate.

Figure 2. Fe-dependent CMI degradation. CMI solution (20 mg l^{-1}, 40 ml) was incubated with iron powder (0.56 g, solid cycle), FeO(OH)-powder (0.89 g, triangle), and Fe_3O_4-powder (0.79 g, square). Bars indicate standard errors in triplicate.

Figure 3. Influence of aerobic and anaerobic condition on Fe-dependent CMI (20 mg l^{-1}) degradation. Bars indicate standard errors in triplicate.

Figure 4. Fate of CMI in the operating plant. The estimated initial concentration of CMI was 2.0 mg l^{-1}.

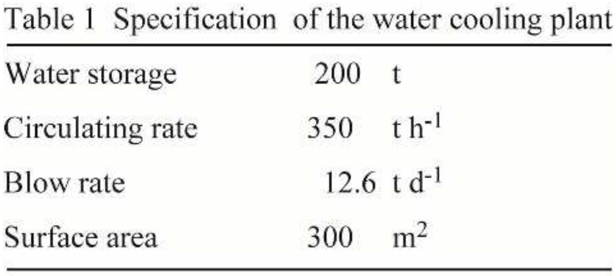
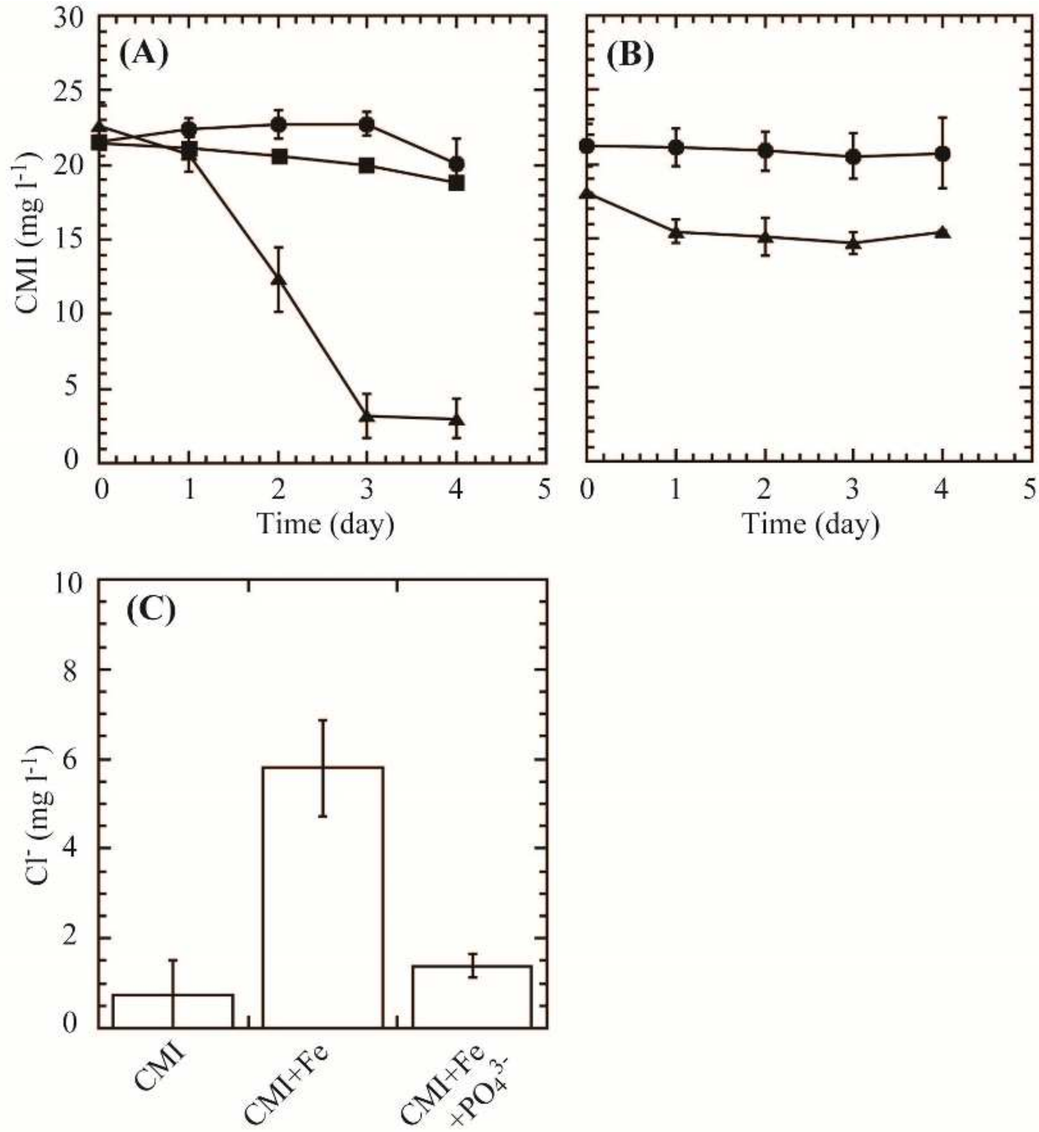


Table 1 Specification of the water cooling plant

Water storage	200	t
Circulating rate	350	t h^{-1}
Blow rate	12.6	t d^{-1}
Surface area	300	m^2



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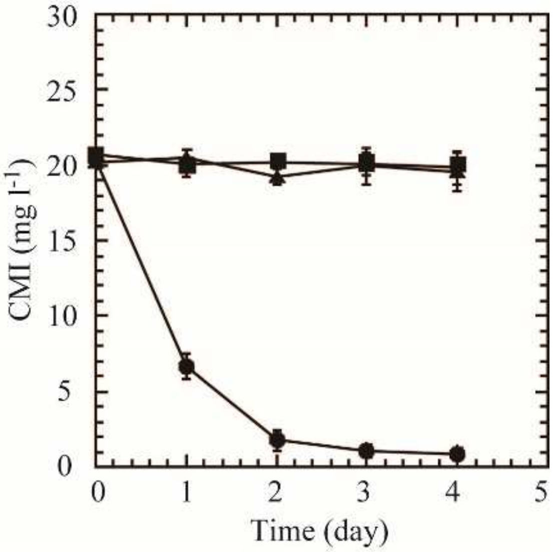

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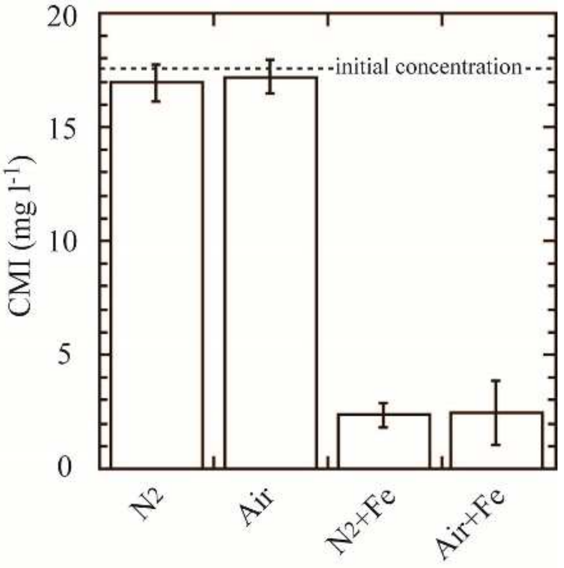

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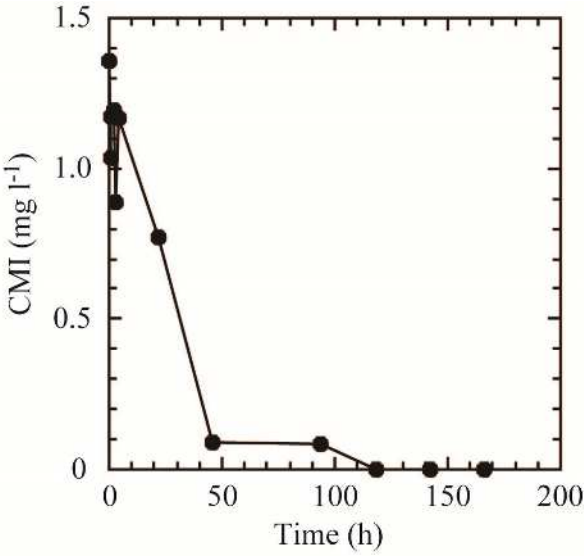

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