SCIENCE

Effect of Chlorine and Chlorine/Bromine Biocide Treatments on the Number and Activity of Biofilm Bacteria and on Carbon Steel Corrosion ☆

M.J. Franklin, D.E. Nivens, A.A. Vass, M.W. Mittelman, R.F. Jack, N.J.E. Dowling, and D.C. White*

ABSTRACT

The efficacy of NaOCl and a mixture of NaOCl and NaBr as biocides at reducing the numbers and metabolic activity of sessile bacteria on carbon steel in a moderately alkaline test medium were evaluated. The polarization resistances, measured by electrochemical impedance spectroscopy (EIS) of the carbon steel coupons, were evaluated prior to and during biocide treatments. Corrosion tubercles were observed on the carbon steel samples four days after inoculation with a consortia of five different physiological types of bacteria. NaOCl or NaOCl/NaBr was then added to the vessels containing the steel samples. Exposure to 2 ppm of residual chlorine or residual chlorine/bromine for two hours had little effect on the numbers of sessile bacteria or the bacterial metabolic activity, as measured by ^{14}C-acetate incorporation into total lipids. Treatment for two hours with 16 ppm of residual chlorine or residual chlorine/bromine, followed by a 24-h treatment with 2 ppm of residual biocide, decreased sessile aerobic bacteria by two orders of magnitude and decreased sessile sulfate-reducing bacteria (SRB) by three orders of magnitude. Bacterial metabolic activity was decreased to the levels of the sterile controls following the 16 ppm treatment. Bacteria in the bulk solution were reduced from approximately 5 × 10^7 cells/mL to less than 1 cell/mL following the 16 ppm treatments. The carbon steel admittance increased when samples were treated with the high concentrations of biocides, compared to untreated controls. These results indicated that low levels of halogen biocide treatments may be ineffective at reducing sessile bacterial populations in moderately alkaline systems. Treatments with high concentrations of halogen biocide, though effective at reducing bacterial numbers and activities, increased the corrosion rate of the carbon steel.

KEY WORDS: ^{14}C-acetate incorporation, CFU, electrochemical impedance spectroscopy, microbial activity, microbiologically influenced corrosion, most probable number

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INTRODUCTION

Biofouling and biodeterioration by microorganisms can lead to premature failures of metallic pipelines.[1] As a result, biocide treatments are often used as countermeasures to decrease the biofouling and microbially influenced corrosion (MIC) of steel pipes. Evaluation of biocide efficacy against sessile bacteria at industrial plants is often a difficult task. Often only the planktonic bacterial populations are evaluated since planktonic bacteria are more easily sampled than sessile bacteria. Sessile bacteria tend to be more resistant to biocide treatment than are planktonic bacteria.[2,3,4]

In addition to the difficulties in microbiological evaluation of biocide efficacy, the effect of the biocide on the metal corrosion is also difficult to assess in industrial plants. Halogen biocides, such as hypochlorous acid and hypobromous acid, are strong oxidizing agents and can be corrosive to metals.[5] Low-level use of these biocides is often recommended for treatment of cooling water systems in order to minimize the corrosive effects of these biocides.[6] Since low-level treatment may not be effective against the sessile bacteria, evaluation of the effectiveness of these treatments based on planktonic cells may provide a false sense of security.

Often biocide efficacy studies are performed on monocultures rather than consortia of bacteria. It is possible that biocide efficacy is not only dependent upon number and biomass parameters, but also on consortial makeup. In the present study, the biocidal activities of NaOCl and a mixture of NaOCl and NaBr against a consortium of five different physiological types of bacteria, which were isolated from failed steel pipes, were evaluated. The rate of corrosion influenced by bacteria appears to be dependent on the metabolic activities of the microbes, not merely on the amount of microbial biomass.[7,8] Therefore, in addition to evaluation of the numbers of sessile bacteria present during biocide treatments, the activity of the biofilm, as determined by ^{14}C-acetate incorporation into lipids, was evaluated.

Primarily due to high photosynthetic activity, the pH of lake water can rise. The acid form of halogen biocides is more effective than the salt.[4] Hypobromous acid is considered to be more effective in alkaline environments than is hypochlorous acid, since the pKa of hypobromous acid, 8.8, is higher than hypochlorous acid, 7.2. The medium used in the present study was a pH 8.5 dilute flowing medium. A dilute flowing system was used, since flowing

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systems better simulate *in situ* environments than do batch culture systems.

In the present study, a test system was developed to evaluate the biocidal effects of biocide treatments against sessile bacteria and the effects of the biocide on the carbon steel corrosion rates.

EXPERIMENTAL PROCEDURES

Media

The basal salts used for all media contained (per liter of deionized water): 50 mg NH_4Cl, 50 mg $MgSO_4 \cdot 7H_2O$, 5 mg $CaCl \cdot 2H_2O$, 27 mg KH_2PO_4, 142 mg Na_2SO_4, 121 mg Tris-HCl buffer, and 1 mL Hutner's mineral.[8] The pH of all media was adjusted to 8.5 with NaOH. The following carbon sources were added to the media used in the flowing system (per liter of deionized water): 20 mg glucose, 10 mg calcium lactate, 10 mg sodium citrate, and 5 mg yeast extract. For analyses of colony-forming units (CFUs), 15 g/L agar was added to the media and the concentrations of the carbon sources used in the solid media were increased 10-fold to allow visual detection of colonies. Most probable number (MPN) tubes for sulfate-reducing bacteria (SRB) contained (per liter of basal salts) 100 mg calcium lactate, 100 mg yeast extract, and 1 g ferric ammonium sulfate. MPN tube media were made anaerobic by boiling under a stream of O_2-free nitrogen. Anaerobic media were dispensed into rubber stopper-sealed pressure tubes as described by Balch et al.[9] Sulfate reduction was assayed by the production of black FeS after a one-week incubation period.

Bacteria

The bacteria used in this study were obtained from a laboratory culture collection of bacteria isolated from tubercles on failed steel pipes. These bacteria were analyzed by the Hewlett-Packard[(1)] microbial identification system, which compares the profile of membrane fatty acids with those contained in a computer library.[10] The bacteria selected for this study were two physiologically different *Pseudomonas* spp., a *Bacillus* sp., an *Erwinia* sp., an *Acinetobacter* sp., and a lactate-using sulfate-reducing bacterium. Sterile microbiological medium was allowed to flow into the flasks for one day prior to inoculation with bacteria. Bacteria were incubated for one to two days at 24°C prior to inoculation into test flasks. Two milliliters, approximately 10^9 cells, of each culture was used as the inoculum.

Test System

Two flasks were used for each treatment, one flask for performing electrochemical analysis and one flask for performing microbiological analysis. The complete experimental system was described.[11] This arrangement was used so that the electrochemical measurements would not be disturbed during the removal of coupons for microbiological evaluation. The working electrodes were C1020 (UNS[(2)] G10200) carbon steel coupons embedded in epoxy.[(3)] Electrical leads were soldered to the back of the samples. The composition of the steel by weight percent was C, 0.17; Mn, 0.42; P, 0.009; and S, 0.006. The electrochemical flasks also contained a salt bridge for connecting a saturated calomel reference electrode (SCE) to the solution and a titanium counterelectrode. The flasks had medium inlet and outlet ports. The liquid volume of the flasks was 500 mL and the dilution rate for the medium was 0.1 per hour. The medium was constantly stirred using magnetic stir bars.

The flasks for microbiological analyses contained 10 to 16 epoxy embedded C1020 carbon steel coupons per flask. Stainless steel hooks were used to suspend the embedded coupons in the medium. All carbon steel samples were finished to 600 grit with silicon carbide finishing paper. Flasks were sterilized by ethylene oxide sterilization at the University of Tennessee Medical Center. All experiments were performed under aeseptic conditions in a Class 100 laminar-flow hood.

Biocide Additions

Biofilms were allowed to develop on the metal surfaces for four days prior to biocide addition. NaOCl was prepared immediately prior to use as a 100x stock solution. The solution was added to the medium until the appropriate level of total residual biocide was obtained, as measured by the DPD Cl_2 test.[12] For studies using the continuous treatment of NaOCl and NaBr, 4 mg/L NaBr was added to the medium reservoir and a stock solution of NaOCl was continuously pumped into the flasks using peristaltic pumps.[(4)] The total residual biocide was monitored over the course of the experiments to allow adjustment of the biocide level to the appropriate levels.

Evaluation of Bacterial Populations

Coupons were removed from the flasks and flanged tubes containing O-ring seals were clamped to the coupons. One milliliter of anaerobic basal salts solution was added to each tube. The solution was point-sonicated 3 times in 3-second pulses to remove bacteria from the surface. Prior tests comparing total cell counts using acridine orange direct counts (AODC) and viable counts using CFUs showed that this sonication treatment had no significant effect on bacterial viability. The suspension was serially diluted in anaerobic basal salts and the dilutents were plated on agar media or inoculated into MPN tubes. The number of aerobic and facultatively anaerobic bacteria, associated with the steel surfaces, were determined by aerobic plate counts of CFUs. The number of sulfate reducing bacteria was determined by MPN analysis of anaerobic broth tubes. The CFU data represent the average of duplicate plates from individual coupons. Three-tube MPNs were used to obtain values for sulfate reducers.

Evaluation of Bacterial Metabolic Activity

Test coupons were removed from the flask and a modified separatory funnel with an O-ring base was clamped onto each coupon.[13] One-half milliliter of basal salts solution, containing 2.5 μCi ^{14}C-acetate (55 mCi·mmole^{-1}) was added to each separatory funnel. After 30-min incubation, 5 mL chloroform, 5 mL methanol, and 4 mL of pH 7.4 phosphate buffer were added to the separatory funnels. After two hours, the solutions were transferred to separatory funnels using two washes of 2.5 mL chloroform each. Five milliliters of 18 MΩ-cm deionized water was added to each solution to cause phase separation. The chloroform phase, containing the lipid fraction, was removed and dried under a stream of N_2. This lipid fraction was then transfered to a scintillation vial by three washes of chloroform. The samples were again dried under N_2 and 7 mL Instagel[(5)] scintillation cocktail was added. Radioactivity was counted in a RackBeta[(6)] model 1212 liquid scintillation counter.

Scanning Electron Microscopy

Metal coupons were removed and fixed for 30 min in 0.1 M sodium cacodylate buffer, pH 7.4, containing 2% (v/v) glutaraldehyde. The samples were then dehydrated by exchanging the solution with increasing concentrations of acetone (50, 75, 100 percent). The samples were critical-point dried and sputtercoated with

(1)Hewlett-Packard Co., Precision Instruments, Palo Alto, CA.

(2)UNS numbers are listed in *Metals and Alloys in the Unified Numbering System*, published by the Society of Automotive Engineers (SAE) and cosponsored by the American Society for Testing and Materials (ASTM).

(3)Metal Samples Co., Munford, AL.

(4)Cole-Parmer Co., Chicago, IL.

(5)Packard Instruments Co., Meriden, CT.

(6)LKB Wallac, Gaithersburg, MD.

gold/palladium. The samples were then analyzed using an ETEC[7] autoscan scanning electron microscope.

Electrochemical Impedance Spectroscopy

A Solartron[8] 1250 frequency response analyzer and a Solartron 1091 electrochemical interface controlled by a Hewlett-Packard series 300 computer were used to generate signals and analyze the magnitude and phase shift of the resulting currents. Sinusoidal potentials of 5 mV rms, ranging in frequency from 5 mHz to 10 Hz, were applied to the working electrode. The resulting impedances were plotted using the Nyquist format. A single depressed semicircle was obtained for most of the impedance analyses. Therefore, estimates of solution resistance (R_s), polarization resistance (R_p), and double-layer capacitance (C_{dl}) could be obtained.[14,15]

RESULTS

Bacterial Colonization of Carbon Steel

Bacteria colonized the steel surface for four days prior to biocide treatment. Loosely adhered corrosion products, due to localized corrosion, formed on the metal surfaces over this time. Figure 1(a) shows several morphological types of bacteria as well as dehydrated bacterial extracellular polymer associated with the corrosion products in the a nontreated metal sample. When the biofilm and the corrosion products were removed from the metal surface, localized corrosion of the surface was observed (Figure 1[b]).

Effects of 2 ppm Biocide Treatments on Numbers and Activities of Sessile Bacteria

After four days of biofilm growth, 2 ppm of residual chlorine or a total of 2 ppm of residual chlorine/bromine (3:1), was maintained in flasks for two hours. Treatment was stopped for 22 h, then continued for two additional hours. Figure 2 shows the CFUs of heterotrophic bacteria for the two 2 ppm treatments. No significant decrease was observed in the chlorine- or the chlorine/bromine-treated samples. The scatter in the data is likely due to sampling variation, since the nontreated control shows a slight decrease in CFUs and the treated samples show a slight increase after the first 2 ppm treatment. In addition to the CFUs, no significant decrease in the numbers of SRB or the lipid biosynthetic activity were observed for either treatment compared to the nontreated controls. Approximately 10^4 sulfate-reducers/cm^2 were observed after the 2 ppm treatments and for the nontreated controls. The lipid biosynthetic activity remained above 50 pmole ^{14}C-acetate incorporation/cm^2 for all samples.

Figure 3 shows a scanning electron micrograph following the final 2 ppm treatment with NaOCl. The micrograph shows similar microbial biomass to that of the untreated control. As with the untreated control, dehydrated extracellular polymer could be seen in association with the biofilm.

16 ppm Biocide Treatment Followed by 2 ppm Treatment

Since the 2 ppm biocide treatments showed no decrease in bacterial numbers or metabolic activity, large biocide doses followed by continuous low doses were evaluated. As with the previous experiments, the biofilm was allowed to develop for four days prior to treatment. The 16 ppm residual biocide was injected into the flasks for a contact time of two hours. Biocide was then continually pumped into the vessels for 24 h, to maintain a 2 ppm residual biocide. In order to prevent dilution of the medium with the biocide, and to prevent mixing of the NaBr with the NaOCl prior to addition to the flasks in the chlorine/bromine experiments, 4 ppm

[7]ETEC, Hayward, CA.

[8]Solartron Instrumentation Group, Sangamo Weston Inc., Irvine, CA.

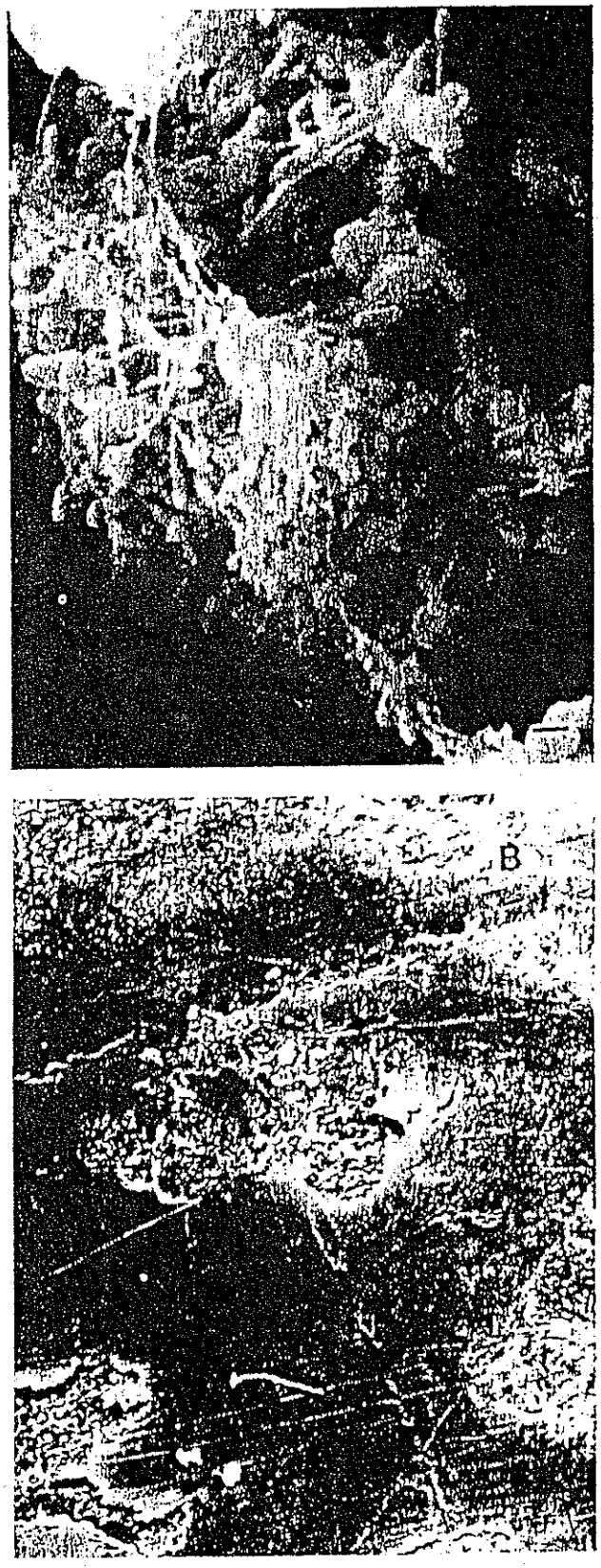

FIGURE 1. *(a) Scanning electron micrograph showing a consortium of bacteria and their extracellular polymer associated with the corrosion products of a carbon steel sample. Bar represents 1 μm. (b) Scanning electron micrograph of the surface of the sample after the biofilm and corrosion products were removed. Bar represents 10 μm.*

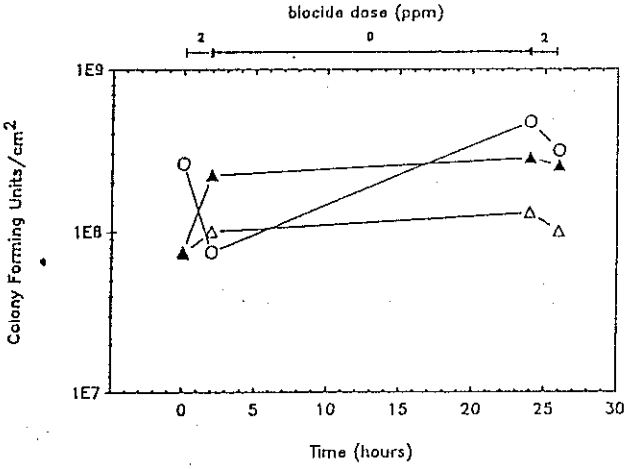

FIGURE 2. *Total CFUs of heterotrophic bacteria per cm². Values represent the mean of two CFU platings. Time zero is after 4 days of biofilm development: (O) no biocide treatment, (Δ) 2 ppm NaOCl treatments, (▲) 2 ppm NaOCl/NaBr treatments.*

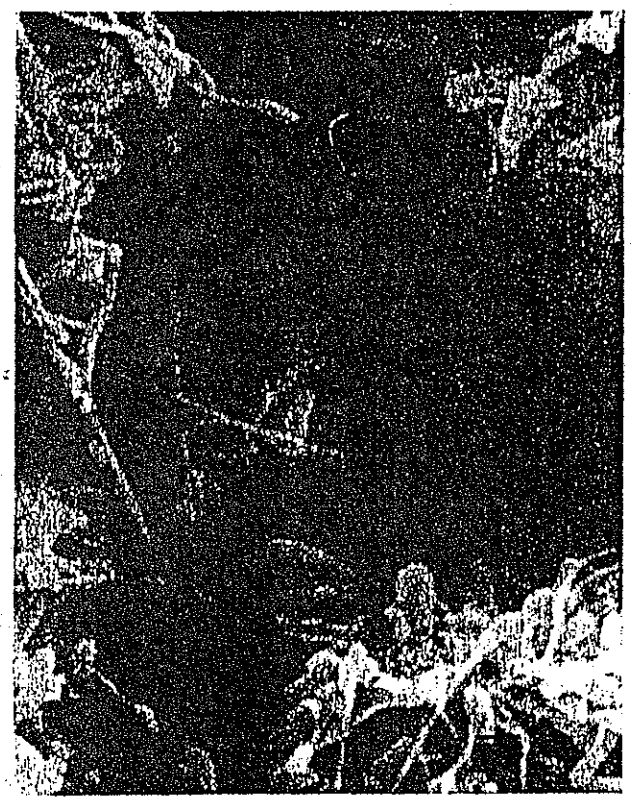

FIGURE 3. *Scanning electron micrograph showing the biofilm associated with the carbon steel sample after the second treatment of 2 ppm NaOCl for 2 h. Bar represents 1 μm.*

NaBr was added directly to one medium reservoir. From the previous experiment, the biocide demand of the flasks was estimated to be 8 ppm. A stock solution containing 37 ppm NaOCl was prepared. The stock solution was pumped into the treated flasks at a rate of 8.3×10^{-4} L/min for the 24-h period. The level of biocide was assayed every 30 minutes during the 16 ppm treatment and additional biocide was injected when the level of free residual chlorine or chlorine/bromine fell below 16 ppm. The level of biocide was assayed five times throughout the 24-h 2 ppm treatment. Additional biocide was added when the residual biocide fell below 2 ppm. The residual biocide level ranged from 0.5 ppm to 3.0 ppm throughout the remainder of the experiment.

Figure 4 shows the effects of the 16 ppm followed by the 2 ppm treatments on the numbers and activities of the suface associated bacteria. The two-hour 16 ppm treatment with NaOCl resulted in a decrease in aerobic heterotrophs by two orders of magnitude (Figure 4[a]). The NaOCl/NaBr treatment resulted in a decrease in aerobic heterotrophs by one order of magnitude. Continuous treatment with 2 ppm of either biocide resulted in approximately 10^5 cells/cm² following 24-h treatment. The bacteria rapidly increased to the original numbers when the treatment was discontinued.

After four days of biofilm growth, 10^5 to 10^6 sulfate-reducers/cm² could be recovered from the surface (Figure 4[b]). The 16 ppm treatment of either biocide reduced the level of sulfate reducers by three orders of magnitude. The continuous 2 ppm treatment maintained the SRB level at 10^3 cells/cm².

The lipid biosynthetic activity was reduced to the level of the sterile controls following treatment with 16 ppm of either biocide (Figure 4[c]). This level was maintained by the continuous treatment with 2 ppm biocide. The activity rapidly rebounded following cessation of the treatment. Figure 5 shows an electron micrograph of the biofilm following treatment with NaOCl/NaBr. Few bacteria were observed associated with the corrosion products. Electron micrographs following the NaOCl treatment also showed few bacteria.

Bulk Phase Effects

The 16 ppm treatment with NaOCl reduced the number of aerobic heterotrophs from approximately 10^7 cells/mL to less than 1 cell/mL. Treatment with NaOCl/NaBr reduced the number of planktonic aerobic heterotrophs to 10^3 cells/mL. After 26-h treatment with 2 ppm of either biocide, no bacteria could be cultured from the solution.

Effect of Treatments on the Corrosion Rates

The impedance, which is inversely proportional to the corrosion rate, of the C1020 carbon steel coupons was analyzed over the course of the experiment by EIS. Figure 6 shows the impedance in the form of the Nyquist format for one sample prior to treatment with NaOCl (Figure 6[a]), after 2-h treatment with 16 ppm NaOCl (Figure 6[b]), and after 24-h treatment with 2 ppm treated with NaOCl. The units for the real and imaginary axes are in ohms. The impedance analyses reveal a single depressed semicircle from which the polarization resistance could be determined. Figures 6(a) through 6(c) demonstrate that the polarization resistance of the carbon steel decreased following treatment with NaOCl, indicating an increase in the corrosion rate. A similar decrease in polarization resistance was observed for the NaOCl/NaBr treatment.

The inverse of the polarization resistance, the admittance, is summarized in Table 1. Initially, the admittance was two- to three-fold greater in the presence of the biofilm than in the sterile control. The treatments with 16 ppm biocide followed by the continuous treatment with 2 ppm biocide showed an increase in the admittance in both the inoculated flasks and the sterile controls. The admittance increased in the inoculated flasks from 1.6 to 5.0 mho/cm² after the 16 ppm followed by the 2 ppm treatment with biocides. The sterile samples increased from 0.6 to 2.5 mho/cm² after the NaOCl treatment, and from 0.4 to 4.0 mho/cm² after the NaOCl/NaBr treatment. The nontreated controls increased from 0.4 to 0.5 mho/cm² for the sterile samples, and 1.2 to 1.6 mho/cm² in the biofilm containing samples. The two-hour 2 ppm treatments with either biocide showed no effect on the corrosion rate of the metal, compared to the nontreated controls.

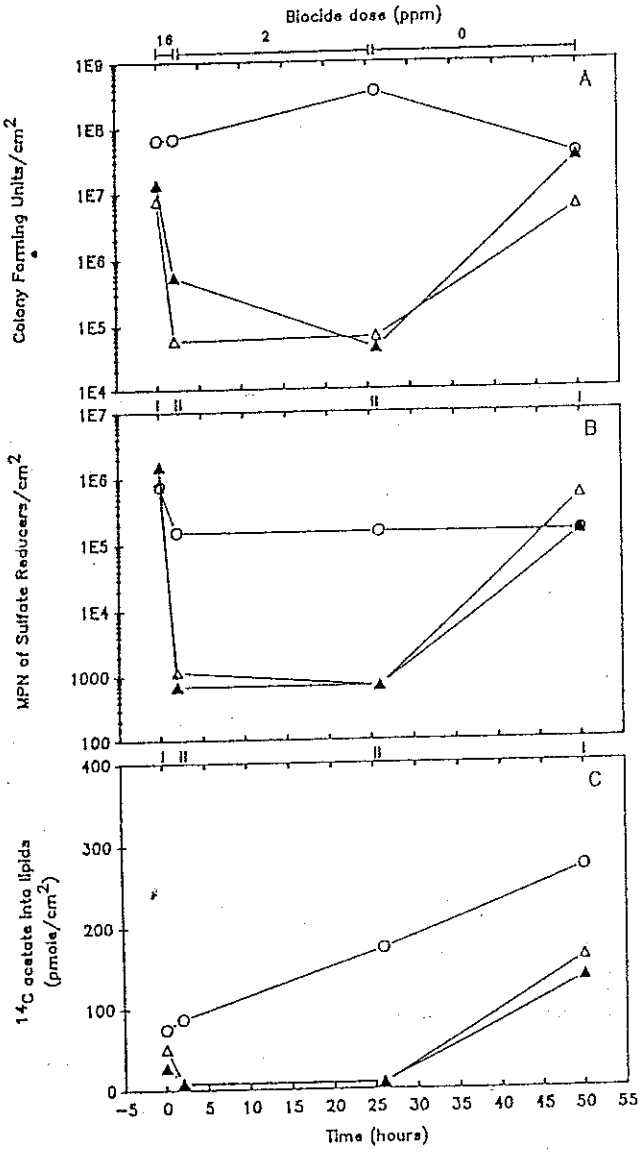

FIGURE 4. *(a) Total CFUs of heterotrophic bacteria per cm². Values represent the mean of two CFU platings. (b) Three-tube MPNs of SRB per cm². (c) Bacterial metabolic activity as measured by ¹⁴C-acetate incorporation into total lipids. Values represent the means of three samples. Time zero is after 4 days of biofilm development: (○) no biocide treatment, (△): 16 ppm followed by 2 ppm NaOCl treatment, (▲) 2-h 16 ppm followed by 2 ppm NaOCl/NaBr treatment.*

DISCUSSION

Test System

In this study, a test system was developed to study microbial fouling and microbial influenced corrosion simultaneously over time. This system was used to examine the efficacy of certain countermeasures. Parameters that appear to be important in microbial influenced corrosion include microbial biomass, including numbers of sulfate-reducing bacteria, and microbial metabolic activity.[7] Therefore, the system was developed to examine these parameters over time. In addition, electrochemical impedance spectroscopy was used to examine corrosion rates over time, since this technique does not appear to alter the progression of corrosion.[16]

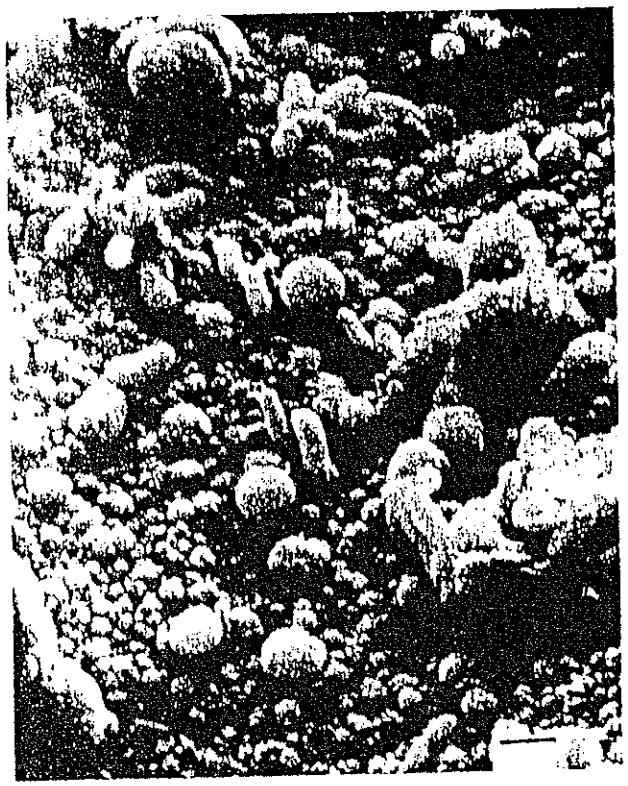

FIGURE 5. *Scanning electron micrograph of biofilm on carbon steel coupon after treatment with 16 ppm NaOCl/NaBr for 2 h and 2 ppm NaOCl/NaBr for 24 h. Few rod-shaped bacterial cells were seen. Bar represents 1 μm.*

Flowing media were used in these experiments. Flowing systems have several advantages over batch culture conditions. First, flowing systems with low nutrient input are more characteristic of environmental conditions than are batch culture systems. Second, the influence of environmental factors, such as nutrient limitation and bacterial end-product accumulation, become less significant in flowing systems than in batch culture systems. For example, Figures 2 and 4 show little change in the number of viable bacteria over time in the untreated control. Under batch culture conditions, the cell numbers would likely decline as nutrients became limiting and as toxic bacterial end products accumulated. Third, the flowing system allows the dilution of corrosion products, which can affect the corrosion rates.

Sulfate-Reducing Bacteria in an Aerobic System

Sulfate-reducing bacteria are often found associated with aerobic bacteria within biofilms. Sulfate-reducing bacteria in biofilms enhance the corrosion rate of carbon steel, compared to biofilms without sulfate reducers.[7] Therefore, in addition to total aerobic bacteria, the numbers of SRB are also determined in these experiments. As many as 10^6 SRB/cm² were found in these biofilms, even though the bulk solution was aerobic. These results indicated that the SRBs were able to inhabit and reproduce in anaerobic environments within biofilms in aerobic systems.

The number of SRBs increased following cessation of the 16 ppm followed by the 2 ppm biocide treatments. No SRBs could be cultured from the bulk fluid after the 26-h treatments. However, sessile SRBs survived to recolonize the metal surface after cessation of the treatment. These results indicated that sessile SRBs may be able to survive exposure to oxygen and halogen biocides for at least 26 h.

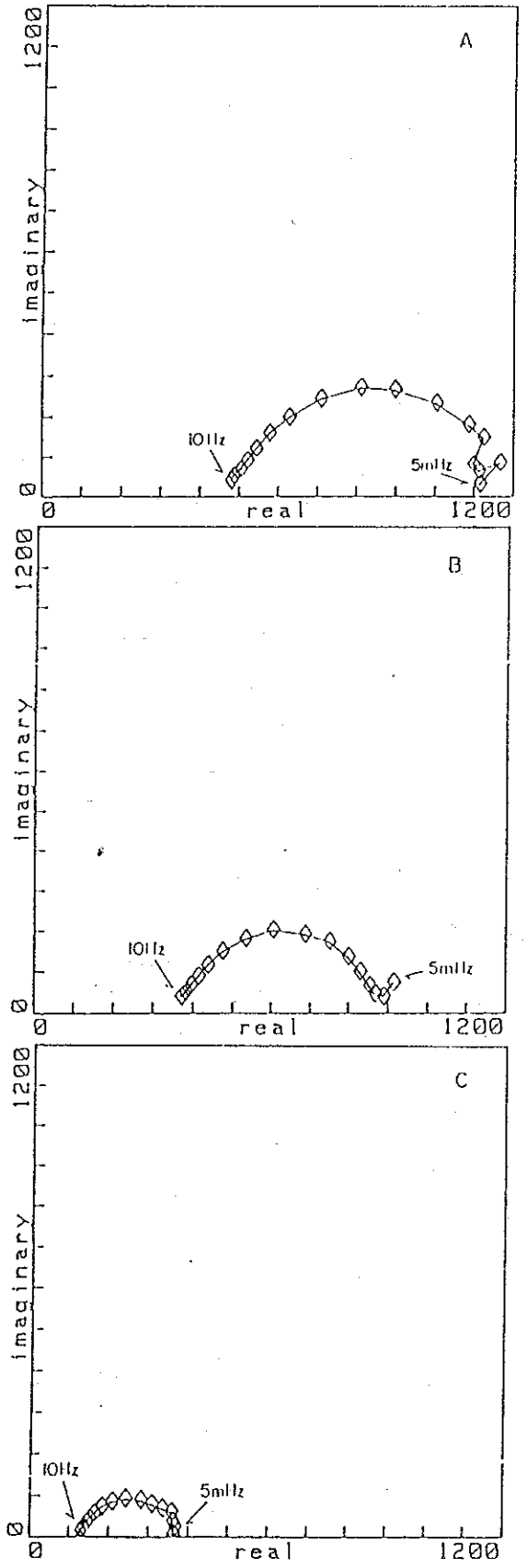

FIGURE 6. *EIS analysis of carbon steel electrode in the Nyquist format. The units for the real and imaginary axes are ohms: (a) EIS of the sample after biofilm was allowed to form for 4 days. (b) EIS after exposure of the same sample to 16 ppm for 2 h. (c) EIS of the sample after additional exposure of the sample to 2 ppm for 24 h.*

TABLE 1
Carbon Steel Admittance for Samples Treated with 2-h 16 ppm Biocides Followed by 24-h 2 ppm Biocides

Treatment		Polarization Resistance[A] (mho/cm²) Pretreatment	After 2 h 16 ppm	After 24 h 2 ppm
Sterile	no biocide	0.4	0.4	0.5
Inoculated	no biocide	1.2	1.2	1.6
Sterile	NaOCl	0.6	0.7	2.5
Inoculated	NaOCl	1.7	2.0	a5.0
Sterile	NaOCl/NaBr	0.4	0.4	4.0
Inoculated	NaOCl/NaBr	1.6	0.9	5.0

[A]Polarization resistance values were obtained by electrochemical impedance spectroscopy.

Bacterial Metabolic Activity

The metabolic activities of bacteria are important components in MIC. In addition to the hydrogenase and sulfate-reduction activities of SRBs, other bacterial metabolic activities have been implicated in MIC. These activities have included iron reduction,[17] acid production,[18] differential oxygen utilization, and exopolymer production.[19] Bacteria capable of each of these activities were used in this study. Bacterial metabolic activity was measured as lipid biosynthetic activity. Lipid synthesis occurs in all living cells, and acetate is used as a precursor of lipid synthesis. Therefore, ^{14}C-acetate incorporation into lipids has been used in a number of studies to analyze bacterial activities in complex ecosystems.[20] In this study, the effects of biocide treatments on the lipid synthetic activity of a sessial bacterial consortium were demonstrated. The results indicated that the two-hour 2 ppm treatments had little effect on the consortial lipid synthetic activity. The two-hour 16 ppm treatment was effective at inhibiting metabolic activity, and continuous 2 ppm treatment was effective at maintaining the inhibition. These results suggest that a continuous low dose of biocide is effective at inhibiting the membrane synthesis and recovery of cells previously treated with high doses of biocides.

Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy (EIS) was used in these studies because it has several advantages over direct current techniques. First, EIS enables the evaluation of corrosion rates over time, before and after the biocide treatments. EIS requires application of 5 mV sinusoidal potentials, which are less destructive to the bacteria and to the metal samples than are Tafel polarizations.[16] In addition, EIS can be used to distingulsh solution resistances from the polarization resistances. This was advantageous in this system, since the two resistances were similar. Thus, direct current measurements may have led to an underestimation of the corrosion rates.

Evaluation of the admittance for the two-hour 2 ppm treatments showed little effect of the biocides on the polarization resistances. Since the biofilm was not affected by these treatments, the lack of change in the admittance was probably due to the lack of penetration of the biocide through the biofilm. The NaOCl and the NaOCl/NaBr 16 ppm treatments followed by the 2 ppm treatments showed an increase in the corrosion rates of both the sterile and the inoculated systems. These results indicated that halogenated biocides were corrosive toward the carbon steel.

Biocide Treatments

In industrial plants where lake or river water is transported through pipes, 2 ppm biocide treatments for one to two hours are often used to bulk water.[6] Studies have shown that hypochlorous acid at 2 ppm residual is effective at reducing planktonic bacteria, but sessial bacteria can be resistant to this concentration of biocides.[4] The results presented here demonstrate that the 2 ppm treatment of hypochlorite in a pH 8.5 medium was ineffective at inhibiting sessial bacteria. In addition, the chlorine/bromine treatment was also ineffective, even though the pKa of hypobromous acid is higher than that of hypochlorous acid, and thus should be more effective at alkaline pH.

The 16 ppm followed by the 2 ppm treatments was effective at reducing the number of bacteria and inhibiting the metabolic activity of the bacteria. However, the treatments resulted in an increase in the corrosion rates of the carbon steel, as measured by EIS. Ventura et al.[5] demonstrated the effects of hypochlorite on stainless steel corrosion in flowing seawater. Their results indicated that hypochlorite treatment increased the short-term propensity for pitting of stainless steel, but decreased the long-term corrosion rates. The results of this study indicated an increase in the short-term corrosion rates of carbon steel by the addition of high doses of biocides.

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