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Electrochemical Prevention of Marine Biofouling with a Carbon-Chloroprene Sheet

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A carbon-chloroprene sheet (CCS) electrode was used for the electrochemical disinfection of the marine gram-negative bacterium *Vibrio alginolyticus*. When the electrode was incubated in seawater containing 10^5 cells per ml for 90 min, the amount of adsorbed cells was 4.5×10^3 cells per cm^2. When a potential of 1.2 V versus a saturated calomel electrode was applied to the CCS for 20 min, 67% of adsorbed cells were killed. This disinfection was due to the direct electrochemical oxidation of cells and not to a change in pH or to the generation of toxic substances, such as chlorine. In a 1-year field experiment, marine biofouling of a CCS-coated cooling pipe caused by attachment of bacteria and invertebrates was considerably reduced by application of a potential of 1.2 V versus a saturated calomel electrode. Since this method requires low potential electrical energy, use of a CCS coating appears to be a suitable method for the clean prevention of marine biofouling.

Biofouling of marine infrastructures such as water-cooling pipes or ship hulls is economically undesirable. The accumulation of biomass on these surfaces causes energy inefficiencies which are due to increased fluid frictional resistance in the case of ship hulls and to decreased efficiency of heat transfer in the case of water-cooling pipes (9). One of the initial stages in the biofouling process is usually the attachment of bacteria (4, 5, 14, 16), although attachment of invertebrates is not necessarily directly dependent on the presence of bacteria. In the past, biofouling has been prevented by toxic chemical agents such as copper (1) and organotin (7). However, this approach is not entirely successful because of the inherent heavy metal resistance of certain bacteria which can selectively colonize surfaces treated with antifouling paints (6). These types of paint are also not acceptable because of the leaching of the heavy metal ions into the marine environment and their subsequent incorporation into food chains.

Recently, we have reported a new disinfection method based on the electrochemical oxidation of intracellular coenzyme A (8, 10–13, 15), which is different from other electrochemical methods which generate toxic substances (2, 17–21). Since this method is known to reduce the presence of microbial biofilms in the laboratory, clean prevention of biofouling in the environment might be reduced by electrochemical disinfection of attaching organisms. Previously, carbon electrodes have been used (10, 11, 13) for sterilization because they are stable at positive potential. However, a carbon electrode is not suitable for coating a large surface. Therefore, plastics containing carbon powders have been prepared for prevention of biofouling on solid surfaces. Previously, marine bacteria adsorbed onto a graphite-silicone electrode have been electrochemically disinfected (15). The graphite-silicone electrode is a paint-based electrode which takes about 1 day to harden.

In this paper, we describe the prevention of marine biofouling by electrochemical disinfection with a newly developed carbon-chloroprene electrode. This electrode was made from carbon and chloroprene rubber and is a sheet-type electrode which may readily be used for coating large surfaces.

MATERIALS AND METHODS

Materials. Marine broth 2216 was purchased from Difco Laboratories (Detroit, Mich.). Other reagents for residual chlorine determination were obtained from Orion Research Co. (Boston, Mass.). Water was purified with an automatic water distillation apparatus (model Aquarius; Advantec Co., Tokyo, Japan) and used within a few hours. Seawater, from Miura Peninsula (Japan), was used after sterilization by autoclaving for 10 min and filtration through a sterile 0.2-μm-pore-size membrane filter (cellulose nitrate filter; Advantec Co.). The sterilized seawater was pH 8.0.

Preparation of CCS. The carbon-chloroprene sheet (CCS), which consisted of carbon black (16.5%, by weight) and graphite (11.0%) as the electrically conductive material and chloroprene rubber (72.5%) as the binder, was obtained from Daiki Engineering Co., Ltd., Chiba, Japan. This mixture was shaped into a 3-mm-thick sheet with a mechanical press. The resistivity of the CCS was 11.9 Ω per cm. The CCS was used to coat titanium plates with insulated surfaces, which were used as working electrodes for laboratory experiments. A freshly prepared CCS electrode was used for each laboratory experiment. Laboratory experiments were performed three times unless otherwise stated.

Laboratory experiments. (i) Culture of *Vibrio alginolyticus*. *V. alginolyticus* ATCC 17749 was cultured aerobically at 25°C for 10 h in 10 ml of marine broth after preculture for 12 h under the same conditions. Cells were centrifuged at 1,600 × *g* at room temperature for 10 min, washed, and suspended in sterilized seawater. The cell concentration was determined with a hemocytometer.

(ii) Electrolysis of seawater by the CCS. The CCS electrode (electrode area, 10.6 cm^2) was immersed in 50 ml of sterile seawater which was mixed with a magnetic stirrer at 150 rpm. A constant potential was applied to the CCS electrode

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with a potentiostat (model HA-151; Hokuto Denko Co.). A saturated calomel electrode (SCE) was used as a reference electrode, carbon fiber was used as a counter electrode, and the CCS was used as a working electrode connected with a salt bridge (13, 15). After 30 min, the chlorine concentration and pH of the electrolyzed seawater were measured as described below.

(iii) Measurement of chlorine concentration and pH. Residual chlorine concentration was measured with a chlorine electrode (model 97-70; Orion Research Co.) at room temperature. A 0.1-ml volume of acid reagent and 0.1 ml of iodide reagent were added to 10 ml of seawater after electrolysis, and the mixture was incubated for 2 min. A 1-ppm chlorine solution was used as a standard. pH was measured with a pH electrode (type 6155; DKK Co., Tokyo, Japan) at room temperature.

(iv) Electrochemical killing of bacteria adsorbed onto the CCS. The CCS electrode was immersed in 200 ml of seawater containing 10^4 to 10^7 *V. alginolyticus* cells per ml and incubated for 90 min at room temperature with stirring at 350 rpm. During this period, bacteria adsorbed onto the electrode surface. The CCS electrode was then removed from the cell suspension, and the surface was washed briefly by immersing the electrode in 50 ml of sterile seawater. This step removed unadsorbed cells. The CCS electrode was then immersed in a fresh beaker of sterile seawater. The effect of applied potential on the number of viable cells attached to the CCS electrode surface was then examined after applying each potential for 20 min. The effect of applying a potential of 1.5 V versus the SCE for various times was also examined. We confirmed that cells were being killed rather than being detached from the electrode by performing plate count assays on the sterile seawater in which the CCS electrode was incubated during application of potential. If cells were detached from the electrode but still viable after application of a potential, they could be detected by the plate count method by measuring the number of cells in the suspension. However, cells were not detected in the sterile seawater after electrochemical killing. Other marine bacteria, such as *Pseudomonas* species and *Alteromonas haloplanktis*, could be electrochemically killed with a basal-plane graphite electrode in a way similar to the killing of *V. alginolyticus* with the CCS. When CCS electrodes were sampled, replicate samples were employed.

(v) Determination of number of viable cells attached to the CCS. After a constant potential was applied to the CCS, the electrode was removed from the seawater and bacterial cells which had adsorbed onto the CCS surface were removed by vigorously washing the electrode surface for 2 min with 50 ml of sterilized seawater with a pipette. Cells were removed by repeatedly pipetting 1 ml of the seawater against the working surface of the electrode. Bacterial cells which adsorbed onto the CCS surface during the control experiment, in which no potential was applied, were also removed in this way. The number of viable cells on the CCS electrode was then determined by plating suitably diluted samples. Colonies which appeared on marine broth agar (0.7%) plates after 24 h of incubation at 25°C were used to estimate the number of viable cells. In addition, the efficiency of cell removal by pipetting was determined by overlaying the washed electrode with marine broth agar and incubating it for 24 h at 25°C. No bacteria were detected on the surface of the electrode after washing. However, the possibility exists that some bacteria may remain, since longer incubation times were not tested.

Field experiments. Figure 1A is a schematic diagram of the electrochemical system used for prevention of marine biofouling of a water-cooling pipe containing seawater (East China Sea, West Kyushu, Japan). Seawater was passed through the pipe at a flow rate of about 0.53 m/s after passage through a 5-mm-mesh filter. The cooling pipe was 10 cm in diameter, and the internal face of the pipe was coated with a CCS 5 mm thick, which was used as a working electrode. A stainless steel ring (internal diameter, 10 cm; electrode area, ca. 150 cm^2) was used as a counter electrode, and an Ag/AgCl electrode was used as a reference electrode. The potential applied to the CCS was controlled by using a potentiostat (model HA-151; Hokuto Denko Co.). A constant potential of 1.2 V versus the SCE was applied to the CCS electrode, and a separate control experiment in which no potential was applied was carried out. Potential was applied for an experimental period of about 1 year, from 1 August 1990 until 7 August 1991. To determine the effect of applied potential on the extent of biofouling on the pipe surface, biomass attached to the pipe was removed from 314 cm^2 of the CCS surface and weighed. Small organisms lightly attached to the CCS were dislodged by washing the inside surface of the tube section with filtered seawater. Larger organisms, more strongly attached, were removed with a knife. Dates of sampling and sections of the pipe sampled are shown in Fig. 1B. After the attached invertebrates were removed from the CCS-coated pipe surface, the three most commonly occurring species were identified as *Mytilus edulis*, *Hormomya mutabilis*, and *Balanus trigonus*. Wet weights of removed biomass for each species were determined after 1 year. Unidentified biomass, organisms less than 2 mm in size and organisms which did not belong to the above-mentioned three species, were weighed as "other" attached organisms.

RESULTS

Laboratory experiments. (i) Effect of applied potential on chlorine concentration and pH in the seawater. pH change and residual chlorine concentration were determined after each constant potential was applied to the CCS (electrode area, 10.6 cm^2) for 30 min, with stirring at 150 rpm in 50 ml of seawater. The electrolysis experiments were run three times and were reproducible with an average relative error of 7%. Chlorine was not detected below an applied potential of +1.5 V versus the SCE (detection limit, 0.02 ppm). Above 1.7 V versus the SCE, the chlorine concentration increased sharply because of electrochemical oxidation of the Cl^- ion to Cl_2. At 2.0 V versus the SCE, chlorine concentration was 0.5 ppm. A change in pH was not observed in the range of −0.6 to +1.2 V versus the SCE. At potential higher than 1.2 V versus the SCE, the pH decreased, and this may have been due to the anodic reaction of H_2O converted to $1/2\ O_2$ and $2H^+$. Below −0.6 V versus the SCE, the pH increased, probably because of the cathodic reaction generating H_2 from $2H^+$. For further experiments, the applied potential was used in the range of −0.6 to 1.2 V versus the SCE since electrolysis of seawater did not occur and no toxic substances were detected.

(ii) Electrochemical disinfection of marine bacteria attached to the CCS. Figure 2A shows the effect of applied potential on the number of viable cells of *V. alginolyticus* attached to the CCS when various potentials were applied for 20 min. Disinfection experiments were usually run three times and were reproducible with an average relative error of 10%. The CCS was immersed in a suspension of *V. alginolyticus* (1.0×10^5 cells per ml) for 90 min. During this time, 4.5×10^3 cells

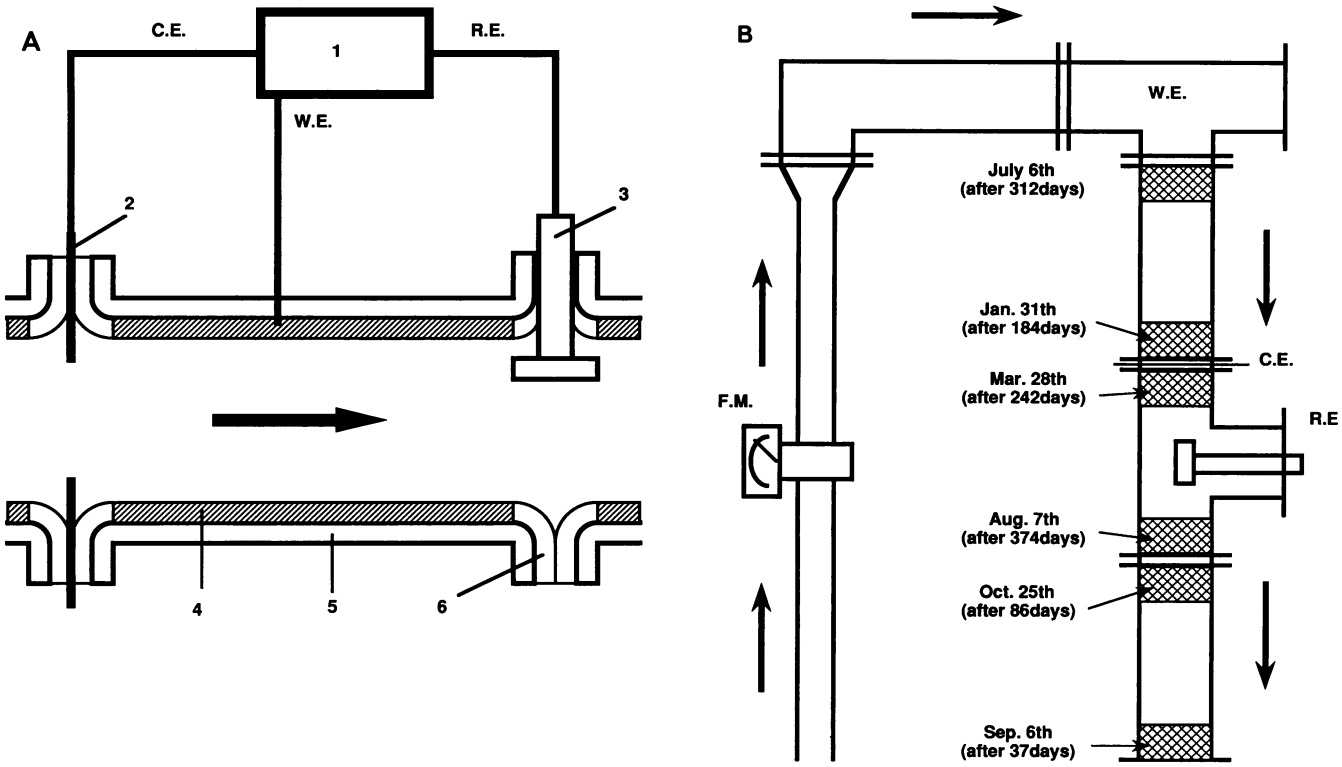

FIG. 1. Schematic diagram of the equipment for the electrochemical inhibition of biofouling in pipes. (A) Numbers: 1, potentiostat; 2, stainless steel; 3, Ag/AgCl electrode; 4, CCS; 5, cooling pipe; 6, insulator. Abbreviations: C.E., counter electrode; R.E., reference electrode; W.E., working electrode. (B) Schematic diagram of the complete experimental pipe system. The hatched regions represent sections of the pipe from which biomass was sampled, and dates indicate the dates of sampling. F.M., flow meter; other abbreviations are as for panel A.

per cm^2 attached to the CCS. The potential was then applied, and the viable cell number did not change below 0.9 V versus the SCE. The viable cell number decreased to 2.1×10^3 (47%) and 8.0×10^2 (18%) cells per cm^2, respectively, when potentials of 1.2 and 1.5 V versus the SCE were applied to the CCS. At 1.2 and 1.5 V versus the SCE, cells on the CCS were killed without chlorine since generation of chlorine was not observed at this potential.

Figure 2B shows the time course of the viable cell number when a constant potential of 1.2 V versus the SCE was applied to the CCS. Disinfection experiments were usually run three times and were reproducible with an average

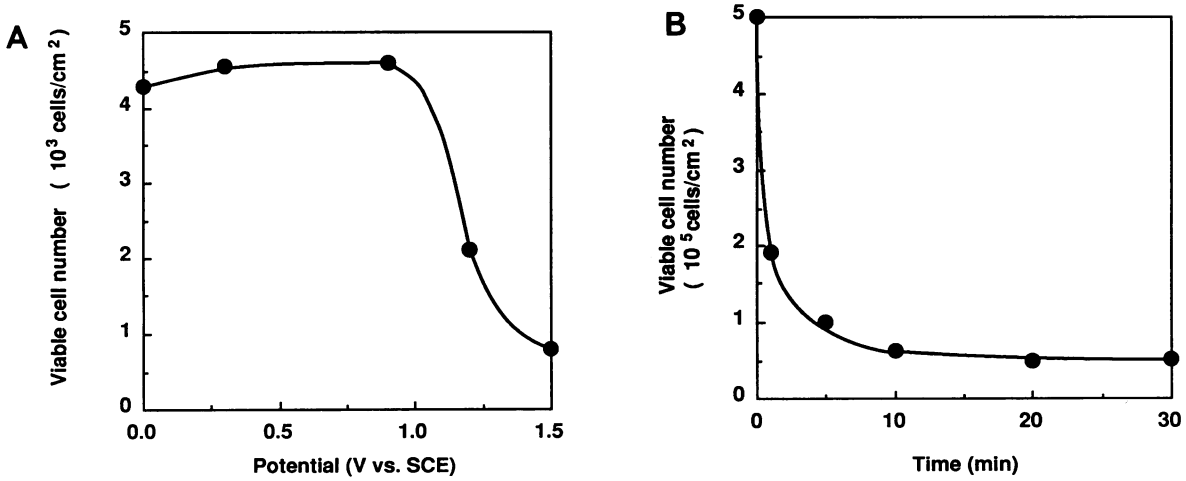

FIG. 2. Electrochemical disinfection of bacteria attached to the CCS. (A) Effect of potential on viable cell numbers of *V. alginolyticus* attached to the CCS. Electrode area (CCS), 10.6 cm^2; electrolysis time, 20 min. (B) Effect of reaction time on viable cell numbers of *V. alginolyticus* attached to the CCS. Electrode area (CCS), 10.6 cm^2; potential, 1.2 V versus the SCE.

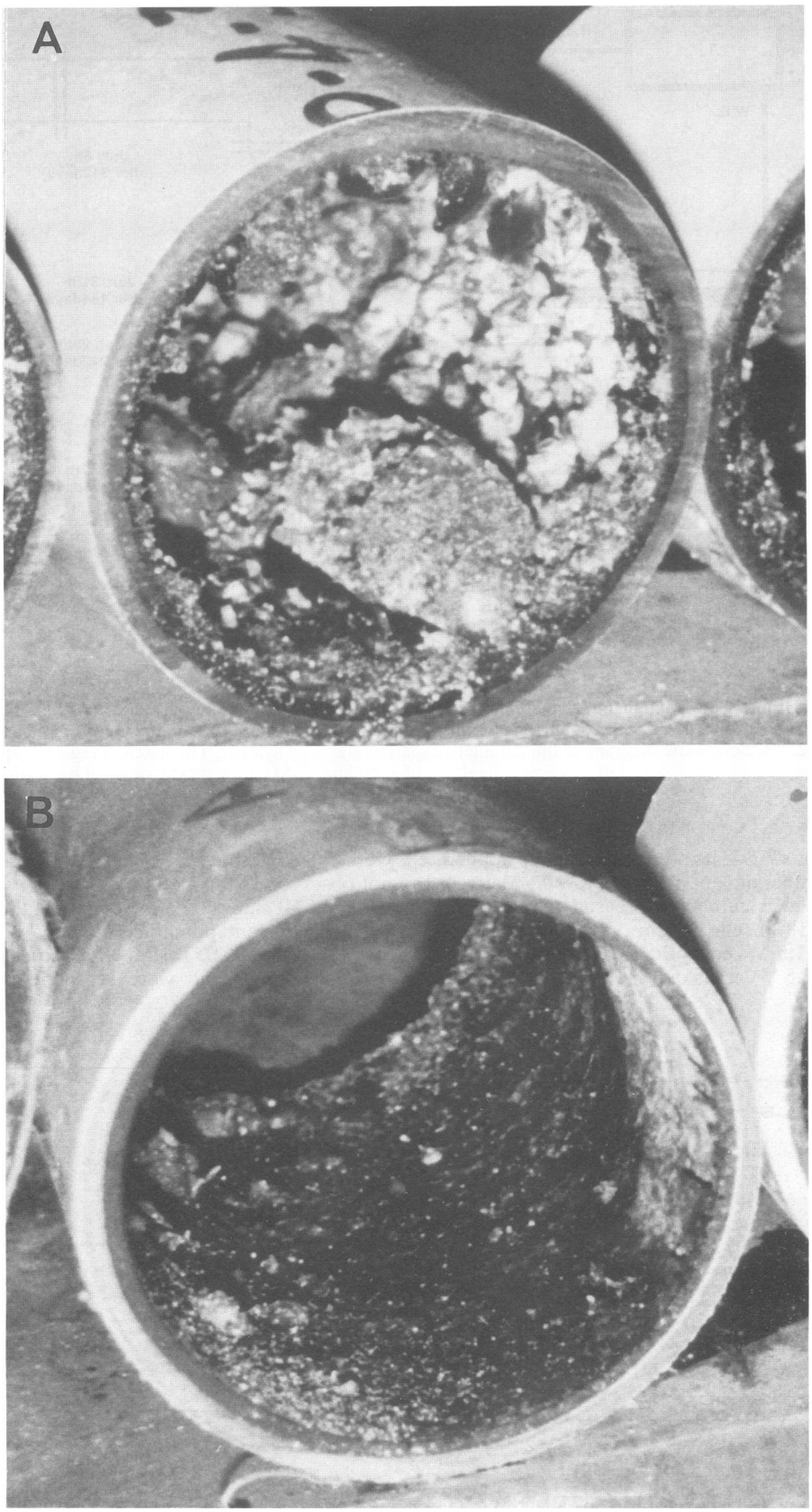

FIG. 3. Pipes coated by CCS after 374 days with no applied potential (A) and with a potential of 1.2 V versus the SCE (B). The experimental period was 1 August 1990 to 7 August 1991, and the seawater flow rate was 0.53 m/s.

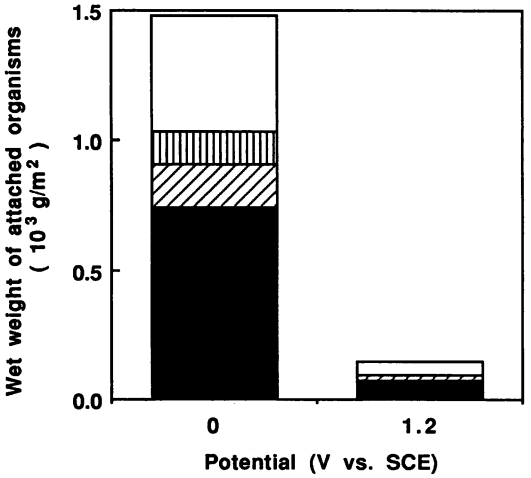

FIG. 4. Effect of potential on wet weight of biofouling organisms with time, with no applied potential and with a potential of 1.2 V versus the SCE. The experimental period was 1 August 1990 to 7 August 1991, and the seawater flow rate was 0.53 m/s. Solid bar, *B. trigonus*; diagonal lines, *H. mutabilis*; vertical lines, *M. edulis*; no shading, other organisms.

relative error of 10%. Before the application of potential, 5.0×10^5 *V. alginolyticus* cells per cm^2 were adsorbed onto the CCS when the CCS was immersed in a cell suspension containing 1.0×10^7 cells per ml for 90 min. The viable cell number decreased sharply at the applied potential, and 90% of the attached *V. alginolyticus* cells were killed after 20 min. The number of viable cells attached to the CCS became constant at 10% of the initial value. This value may represent viable bacteria which remained attached to insulated chloroprene areas of the CCS.

Field experiments. Electrochemical prevention of marine biofouling on the surface of the cooling pipe is shown in Fig. 3. Pipe sections are shown after 1 year of exposure to seawater. Attached organisms grew on the CCS coating when no potential was applied (Fig. 3A). These organisms were mainly barnacles (52%, by weight) and mussels (39%). When a potential of 1.2 V versus the SCE was applied to the CCS on the pipe surface, attachment of organisms was inhibited (Fig. 3B). Inhibition of attachment was not species specific, since attachment of all organisms was inhibited.

The effect of potential on wet weight of biofouling organisms with time is shown in Fig. 4. Dates and locations of sampling of these organisms on the pipe are shown in Fig. 1B. Organisms attached to the pipe in the absence of applied potential and grew only during the summer. Application of a constant potential of 1.2 V versus the SCE very significantly reduced biofouling of the pipe surface for 1 year. This suggests that the inhibition of biofouling may be caused by the prevention of attachment or metamorphosis of larvae and may not be due to removal of grown invertebrates.

After 1 year, the mass of organisms attached to the CCS at the pipe center was 1,478 g (wet weight) per m^2. The masses of *B. trigonus*, *H. mutabilis*, *M. edulis*, and other organisms were 740 (50%, by weight), 166 (11%), 128 (9%), and 444 (30%) g (wet weight) per m^2, respectively. At an applied potential of 1.2 V versus the SCE, attachment of organisms did not increase during the summer and was kept below 146 g (wet weight) per m^2. The masses of attached *B. trigonus*, *H. mutabilis*, *M. edulis*, and other organisms were 73 (50%, by weight), 18 (12%), 0 (0%), and 55 (38%) g (wet weight) per m^2, respectively. Growth of organisms at the applied potential was 10% of that which occurred during the control experiment.

DISCUSSION

The CCS is a sheet-type plastic consisting of graphite, carbon black, and chloroprene rubber which can be more readily used to coat the inner surfaces of cooling pipes than a graphite-silicone electrode can and is suitable for electrochemical disinfection because it is stable at positive potential. *V. alginolyticus* cells adsorbed onto the CCS were killed above 1.2 V versus the SCE. Electrochemical disinfection with the CCS required a higher potential than that with the basal-plane graphite electrode because the electrical resistance of the CCS was higher than that of the basal-plane graphite. In the potential range of −0.6 to 1.2 V versus the SCE, residual toxicity, from substances such as chlorine, or a change in pH was not detected. These results suggest that the disinfection is based on direct electron transfer between the cell and the CCS. Since this electrochemical disinfection method is based on intracellular coenzyme A oxidation and has been shown to be effective for killing several types of bacteria, this method may be generally useful for prevention of growth of marine bacteria by application of an electrical potential. However, a more thorough survey of the effectiveness of the CCS for disinfection of other types of bacteria remains to be performed.

When no potential was applied to the CCS-coated pipe during field experiments, microbial fouling was observed and attachment of invertebrates occurred. In this experiment, attachment of larger organisms may be affected by the presence of a microbial biofilm since development of microbial biofilms is known to affect the settlement of some types of marine invertebrates (3, 16, 22). Laboratory experiments showed that below an applied potential of 0.9 V versus the SCE, marine bacteria were not killed (Fig. 2A). Thus, attachment of organisms to the CCS-coated pipe at potentials below 0.9 V versus the SCE would not be expected to reduce biofouling. At 1.2 V versus the SCE, marine bacteria on the CCS were also killed and biofouling could be significantly reduced. Thus, growth of many types of biofouling organisms on the CCS may be inhibited by application of a potential to the CCS surface.

Significant reduction of macrofouling in the environment may be due to inhibition of bacterial growth followed by a decrease in microfouling by attached organisms. In laboratory experiments, applied potential did not directly inhibit attachment and metamorphosis of invertebrates. We carried out an inhibition assay to determine the effect of an applied potential on attachment of adult mussels (*M. edulis*) to the CCS by production of byssus thread (data not shown). Attachment was not inhibited at an applied potential of 1.2 V versus the SCE. However, the effect of an applied potential on larval settlement onto the CCS and subsequent metamorphosis was not investigated. Growth of some types of bacteria on solid surfaces is known to induce the settlement and metamorphosis of some larvae. Therefore, the electrochemical prevention of invertebrate attachment may be due to the initial prevention of a microbial biofilm. The absence of such a biofilm would reduce the settlement of higher organisms, leading to a reduction in macrofouling. In addition, a direct inhibitory effect of an applied potential on attachment and/or metamorphosis of larvae on the CCS is

possible, since larvae can attach to surfaces not covered by a microbial biofilm.

After a 1-year field experiment, some cracks could be observed on the CCS surface by scanning electron microscopy. In addition, the electrical conductivity of the CCS was reduced. Therefore, a binding polymer which is more stable in seawater should be used if more effective and longer-term prevention is to be carried out.

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