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Inactivation of *Vibrio parahaemolyticus* in Effluent Seawater by Alternating-Current Treatment

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***Vibrio parahaemolyticus*, the cause of gastroenteritis in humans, was inactivated by alternating low-amperage electricity. In this study, the application of alternating low-amperage electric treatment to effluent seawater was investigated for the large-scale disinfection of seawater. This method was able to overcome the problem of chlorine generation that results from treatment with continuous direct current. In conclusion, our results showed that alternating-current treatment inactivates *V. parahaemolyticus* in effluent seawater while minimizing the generation of chlorine and that this alternating-current treatment is therefore suitable for practical industrial applications.**

Vibrio parahaemolyticus is a gram-negative, halophilic bacterium that naturally inhabits marine and estuarine environments and causes three major syndromes of clinical illness: gastroenteritis (the most common syndrome), wound infections, and septicemia (11). *V. parahaemolyticus* has been recognized as a common cause of food-borne illness in the world (6, 16). During the past 10 years in the United States, *V. parahaemolyticus* has been the most common *Vibrio* species isolated from humans, as well as the most frequent cause of *Vibrio*-associated gastroenteritis (9). Recent *V. parahaemolyticus* outbreaks in the United States have been associated with consumption of raw or undercooked shellfish (1, 4, 5). Thus, seawater used in mariculture requires a sterile process to prevent the propagation of bacteria. As an alternative to conventional techniques of water sterilization, such as antibiotics (8), heat sterilization (10), ozone (15), UV light (3), and pulsed electric field (14), low-amperage electric treatment of bacteria in seawater was developed in a previous study (13). The method as proposed above was potentially much simpler and cheaper than any other methods for disinfection of seawater. In the present study, we evaluate the improvements and practical applications of the method for the large-scale sterilization of seawater.

To apply to large-scale treatment, we employed a flow-simulating fluidic conduit made of a titanium pipe and rod, connected to a feeding pump (NP-2; Nakasa, Japan) and an electric power supply (model 525C; Metronix Corp., Tokyo, Japan) (Fig. 1). The pipe and rod were coated with a 1-μm-thickness of platinum to function as direct electrodes. In passing through the space between the pipe and rod, the seawater containing *V. parahaemolyticus* flowed at the speed of 850 ml/min while being treated with a certain electric current. In the experiments involving static conditions, an electrolysis vessel used in previous study (13) was also employed. In this case, each experiment was applied to a stationary quantity of 5 ml of seawater.

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The natural seawater used in this study was collected from the seashore of In-cheon in Korea. *V. parahaemolyticus* ATCC 17802 (American Type Culture Collection) was grown in nutrient broth (Difco, Detroit, Mich.) containing 3% (wt/vol) NaCl at 37°C for 18 h until the end of the exponential growth phase and resuspended in filtered seawater to a density of 10^4 cells/ml. CFU counting determined the number of viable microorganisms in the treated seawater as follows. After the electric treatment, 100 μl of treated seawater was spread on nutrient agar (Difco) plates containing 3% NaCl, and the plates were incubated at 37°C for 48 h. Therefore, the detection limit of this procedure was 10 CFU/ml.

To compare the sterilizing efficiencies between static seawater and effluent seawater, we have converted the effluent speed into the treatment duration under static conditions. Consequently, the effluent speed of 850 ml/min was converted to the approximate duration of 350 ms in accordance with the 5-ml quantity of the previous static seawater experiment. Although treatment for 350 ms was sufficient to completely inactivate *V. parahaemolyticus* in seawater under static conditions (13), the effectiveness of the comparable sterilizing action under the effluent condition was not certain. In addition, the continuative electrolysis of seawater under the effluent conditions produces an excessive concentration of Cl_2 due to the approximately 19,000-ppm concentration of Cl^- in natural seawater (2). Chlorine has been used to disinfect drinking water and swimming pool water and to control bacteria and odors in the food industry, but chlorine has a pungent and disagreeable odor at concentrations above 2 mg/liter in distilled water (7, 12). Therefore, we have also studied the quantitative analysis of chlorine in seawater treated with direct current (DC) under static conditions in advance. During treatment with 12 V of DC for 10, 30, 100, and 1,000 ms with 1 or 3 A, the concentration

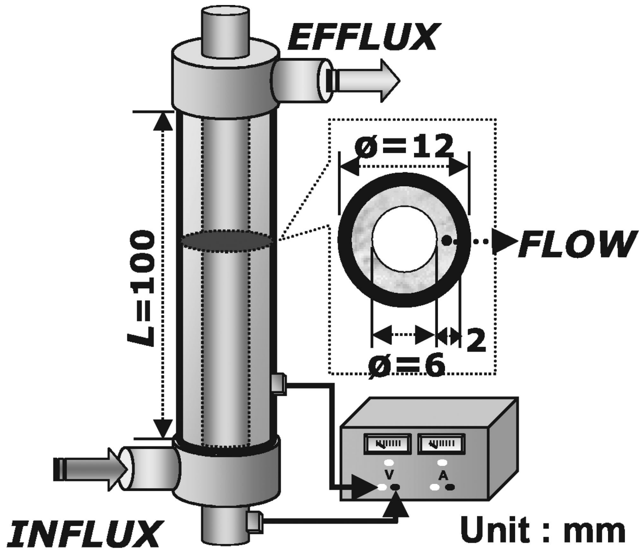

FIG. 1. Prototypical fluidic conduit made of titanium pipe and rod coated with a 1-μm thickness of platinum. While passing through the space between the pipe and rod, the seawater flowed at the speed of 850 ml/min and was treated with a certain electric current.

of the Cl_2 product in treated seawater was checked with a digital Cl_2 meter (UC-5; Central Kagaku Corp., Tokyo, Japan).

Under the static conditions, *V. parahaemolyticus* in seawater treated with DC was fully inactivated after more than 30 ms (Fig. 2A). However, the generation of Cl_2 in seawater with 12 V of DC was proportional to the duration of treatment with a concentration peaking at 212 mg/liter with treatment for 1,000 ms (Fig. 2B). This Cl_2 level in water was sufficient to cause itching and burning of the eyes, nose, throat, and respiratory tract. Bronchitis and accumulation of fluid in the lungs (chemical pneumonia) could occur within hours of exposure to high levels (*Material Safety Data Sheet*, *Chlorine*, Westlake Chemical Corp., Houston, Tex., 1999). Therefore, these results suggested that electric sterilization with DC would not be suitable for large-scale treatment of seawater because of the generation of chlorine gas.

To reduce the generation of chlorine, alternating current (AC) was employed instead of DC. Because the AC polarity alternates periodically, the seawater undergoes less electrolysis, which results in reduced generation of chlorine. Therefore, we previously evaluated the sterilizing effect of AC treatment and checked the concentration of chlorine in seawater treated with AC under static conditions. The experimental conditions were the same as for DC treatment, except for the duration of treatment, which was fixed to 350 ms to simulate the effluent conditions, and the frequency of AC, which was set at 5, 16, and 50 Hz instead. These frequencies of AC were considered to be relatively similar to durations of 100, 30, and 10 ms in the DC experiment.

As shown in Fig. 3A, *V. parahaemolyticus* in the seawater treated with AC at 3 A was fully inactivated, but in the cases of treatment at 16 and 50 Hz with 1 A, *V. parahaemolyticus* in seawater was not entirely inactivated. These results suggested that AC treatment is able to fully inactivate *V. parahaemolyticus* in static seawater only under more severe conditions, such as higher amperage and lower frequency of AC. Because the lower frequency was related to the longer duration of transmitting an electric current, the lower frequency of AC inactivated *V. parahaemolyticus* more effectively in seawater. In brief, the sterilizing power of AC treatment decreased as the AC frequency increased. The concentration of Cl_2 in the AC-treated seawater was also considerably less than that in seawater treated with DC (Fig. 3B). However, the treatment at a higher AC frequency is needed to prevent chlorine generation, because chlorine was generated in the seawater at a concentration as much as 50 mg/liter in the case of the 5-Hz treatment. Therefore, AC treatment with 3 A at 5 Hz or 1 A at 50 Hz is considered to be an adequate condition to achieve satisfactory sterilizing power without excessive chlorine generation.

Based on the data from the previous experiments under the static conditions, the sterilizing effect of AC current using the fluidic conduit was actually evaluated under the effluent con-

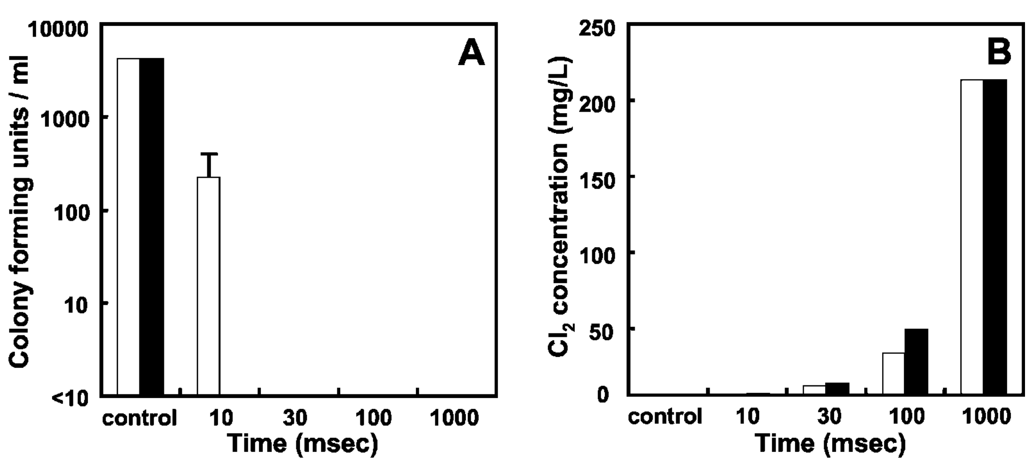

FIG. 2. (A) Effects of DC treatment on viability of *V. parahaemolyticus* in static seawater. The treatment voltages are 1 A (white bars) and 3 A (black bars). The results are means based on data from three experiments, and the error bars indicate standard deviations. (B) Detection of Cl_2 concentration in static seawater treated with DC.

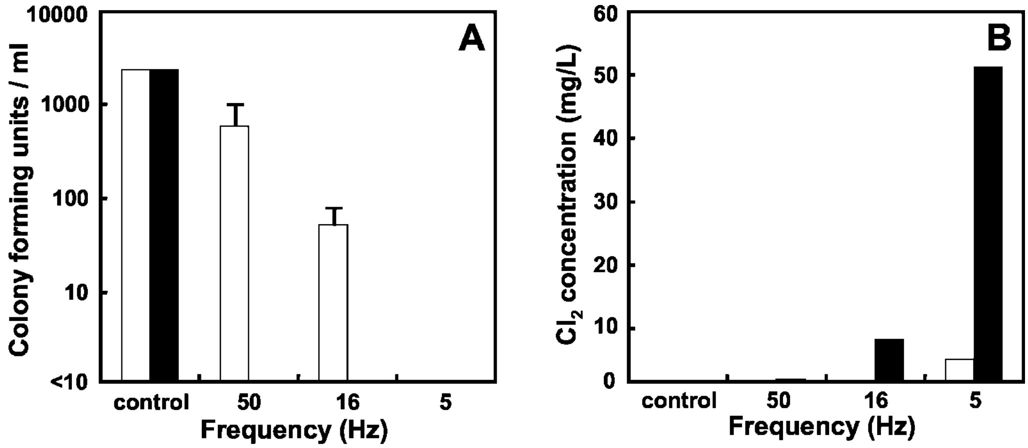

FIG. 3. (A) Effects of AC treatment on viability of *V. parahaemolyticus* in static seawater. The treatment voltages are 1 A (white bars) and 3 A (black bars). The results are means based on data from three experiments, and the error bars indicate standard deviations. (B) Detection of Cl_2 concentration in static seawater treated with AC.

ditions. As shown in Fig. 4, the population of *V. parahaemolyticus* in seawater was eradicated with AC treatment of 5 Hz, 12 V, and 1 A under the effluent conditions. At 3 A, *V. parahaemolyticus* in seawater was fully inactivated at all frequencies.

In conclusion, this study showed that low-amperage AC treatment inactivated *V. parahaemolyticus* with a sterilizing efficiency comparable to that of DC treatment in the static and effluent seawater. Moreover, AC treatment was able to dramatically reduce the generation of harmful chlorine compared to DC treatment. Therefore, this AC treatment, which disinfects seawater and resolves the problem of chlorine generation, is more suitable than DC treatment for practical industrial applications.

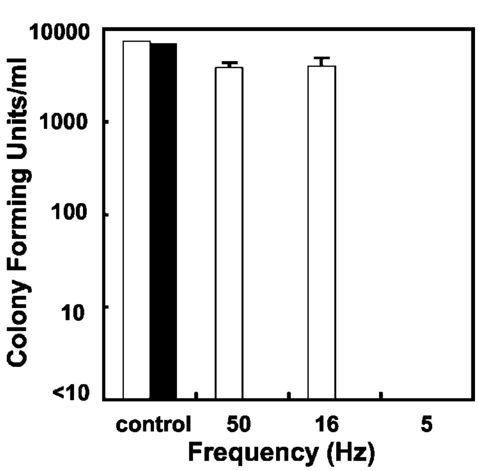

FIG. 4. Effects of AC treatment on viability of *V. parahaemolyticus* in effluent seawater. The treatment voltages are 1 A (white bars) and 3 A (black bar). The results are means based on data from three experiments, and the error bars indicate standard deviations.

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