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Disinfection of Microorganisms by Use of Electrochemically Regenerated Periodate

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A new method for disinfection of microorganisms by electrochemically regenerated periodate was developed. Oxidation of iodate to periodate was observed at 1.25 V versus a silver/silver chloride electrode in a cyclic voltammogram of potassium iodate. When 1.25 V was applied in 1.0 mM potassium iodate, approximately 4-log inactivation of *Escherichia coli* was observed in 30 min.

Iodine-based disinfectant has been used for many years in the medical field and for potable water disinfection where municipal water treatment is not reliable. The mechanism of antimicrobial disinfection is based on the fact that iodine is a strong oxidant (1, 6, 7, 17). Iodine-based disinfectants are more stable chemically and have a more acceptable taste than chlorine-based disinfectants, which makes them useful for disinfection in field work. Euthyroid individuals can be treated safely with iodine-containing solutions at a residual concentration less than 1.0 mg/liter, even for a long period of time (4, 5). However, excess iodine ingestion can be deleterious to human health. The major health effect of excess iodine ingestion is the development of thyroid diseases (2). Therefore, removal of excess iodine using activated carbon is required to ensure safe levels of iodine while efficient disinfection activity is maintained.

In studies evaluating the efficacy of electrochemical disinfection methods to prevent biofilm formation and biofouling, workers have used a potential of approximately 1.0 V (12, 15, 16). A potential of 0.74 V versus a saturated calomel electrode applied to *Saccharomyces cerevisiae* resulted in decreased respiratory activity and cell death (12). This method has been

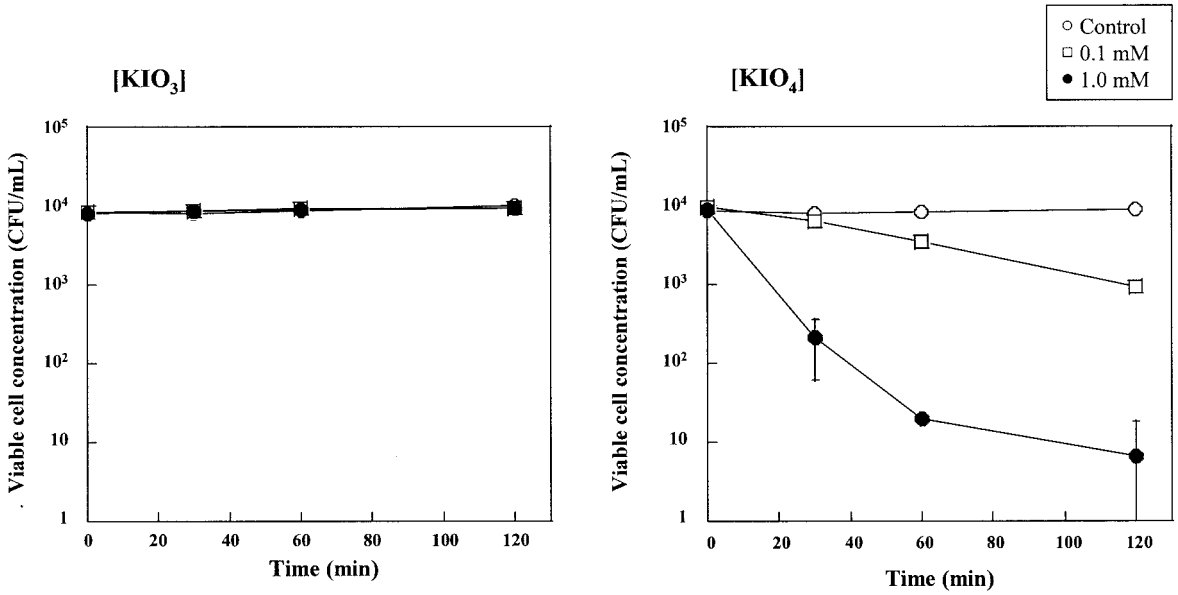

FIG. 1. Time course of disinfection by potassium iodate and potassium periodate. Potassium iodate or potassium periodate (dissolved in 0.1 M phosphate buffer, pH 7.0) at concentrations of 0 mM (control), 0.1 mM, and 1.0 mM were incubated with an *E. coli* cell suspension (initial cell concentration; 1.0×10^4 cells/ml).

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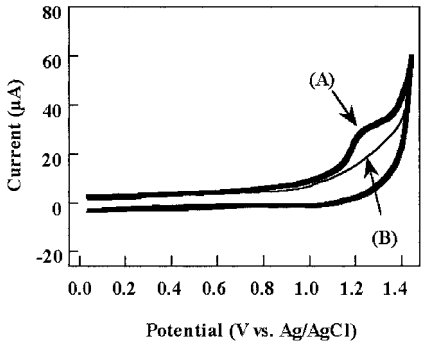

FIG. 2. Cyclic voltammogram of 0.1 M potassium iodate dissolved in 0.1 M phosphate buffer at pH 7.0 (line A). A reference line for a preparation without iodate was also obtained (line B). Basal-plane pyrolytic graphite (electrode area, 0.2 cm^2), Pt wire, and Ag/AgCl were used as the working electrode, the counter electrode, and the reference electrode, respectively. The scan rate was 100 mV/s.

applied to disinfection of drinking water utilizing granular activated carbon (10), carbon-cloth sheets (11), activated carbon fibers (9, 14), and titanium nitride mesh (13) as electrodes. Disinfection and chlorine removal were carried out using activated carbon fiber reactors by applying 0.8 to 1.0 V versus a saturated calomel electrode. A distinct advantage of this methodology is that bacterial regrowth on electrode surfaces can be controlled continuously without the production of hazardous by-products.

In the present study, a new method for disinfection using electrochemically generated periodate was developed. Periodate is also a strong oxidizing agent (3) and can be used for disinfection (8). A combination of electrochemical treatment and iodine-based disinfectant could result in effective disinfection.

Potassium periodate (KIO_4) and potassium iodate (KIO_3) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Cyclic voltammetry was carried out in 0.1 M KIO_3 at room temperature using an electroanalytical system (model CS-1090; Cypress System Inc., Kansas City, MO). The potential was referenced against a silver/silver chloride electrode (Ag/AgCl), and a coiled platinum wire was used as a counter electrode. A basal-plane pyrolytic graphite electrode (0.2 cm^2) was employed as the working electrode.

Escherichia coli strain DH5α (obtained from Toyobo Co. Ltd., Osaka, Japan) was cultured aerobically at 37°C for 12 h in Luria-Bertani medium (pH 7.0). The cells were centrifuged at 1,700 × *g* for 10 min, washed, and resuspended in 0.1 M phosphate buffer (pH 7.0). The cell concentration was determined using a cell counting chamber for bacteria (chamber area, 0.0025 mm^2; depth, 0.02 mm). Potassium periodate or potassium iodate was added to cell suspensions (10^4 cells/ml, 30 ml) to final concentrations of 0.1 and 1.0 mM. Cell mixtures were stirred continuously using a magnetic stirring bar, and 1.25 V was applied to the electrode using a potentiostat (model HA-151; Hokuto Denko Co., Tokyo, Japan). An Ag/AgCl reference electrode and a Pt wire counter electrode were used. The number of viable cells remaining after disinfection was determined by plating 50-μl aliquots of the samples on Luria-Bertani medium with 0.7% agar. Colonies that appeared after 24 h of incubation at 37°C were counted.

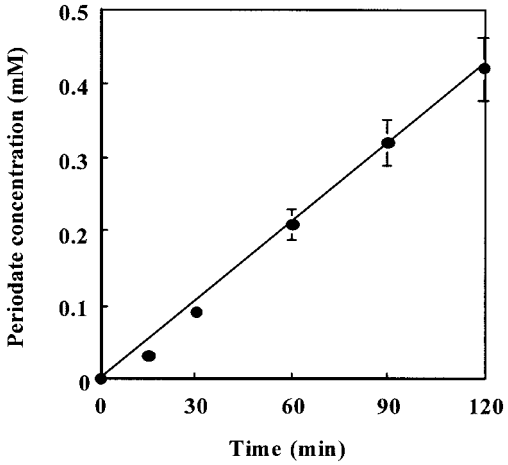

FIG. 3. Time course of electrochemically generated periodate when a potential of 1.25 V versus Ag/AgCl was applied in 10 mM potassium iodate using a graphite electrode (4.0 cm^2).

The effect of iodate or periodate on the concentration of viable *E. coli* cells was investigated at room temperature. When the cell suspension (1.0×10^4 cells/ml) was incubated in 0.1 mM or 1.0 mM KIO_3, a decrease in the concentration of viable cells was not observed (Fig. 1). In contrast, when the cell suspension was incubated with 1.0 mM KIO_4, an almost 2-log decrease in the concentration of viable cells was observed after 30 min, and a 3-log decrease was observed after 2 h (Fig. 1). *E. coli* cells were clearly killed by the addition of periodate. After incubation of the cultures with 0.1 mM periodate, the concentration of iodate (the reduced product of periodate) was measured by high-performance liquid chromatography. During 2 h of incubation, the iodate concentration increased gradually to 0.04 mM as analyzed by an anion-exchange column (Shim-pack IC-A1; Shimadzu Co., Ltd., Kyoto, Japan) with 2.4 mM Tris-HCl buffer containing 2.5 mM phthalic acid as the mobile

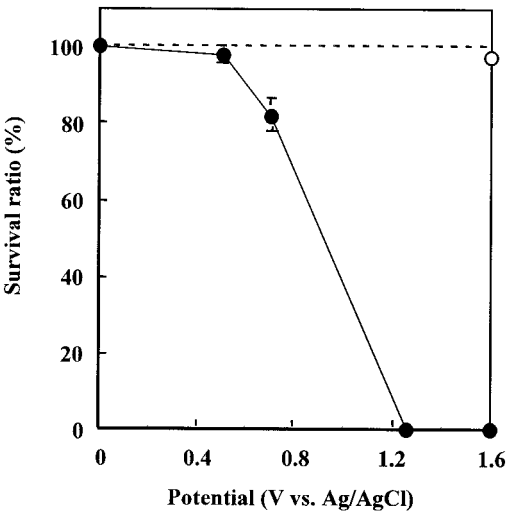

FIG. 4. Effect of applied potential on the survival ratio of *E. coli* in 1.0 mM potassium iodate (●) and 0.1 M phosphate buffer (○). Potentials were applied for 60 min. The initial cell concentration was 1.4×10^4 cells/ml.

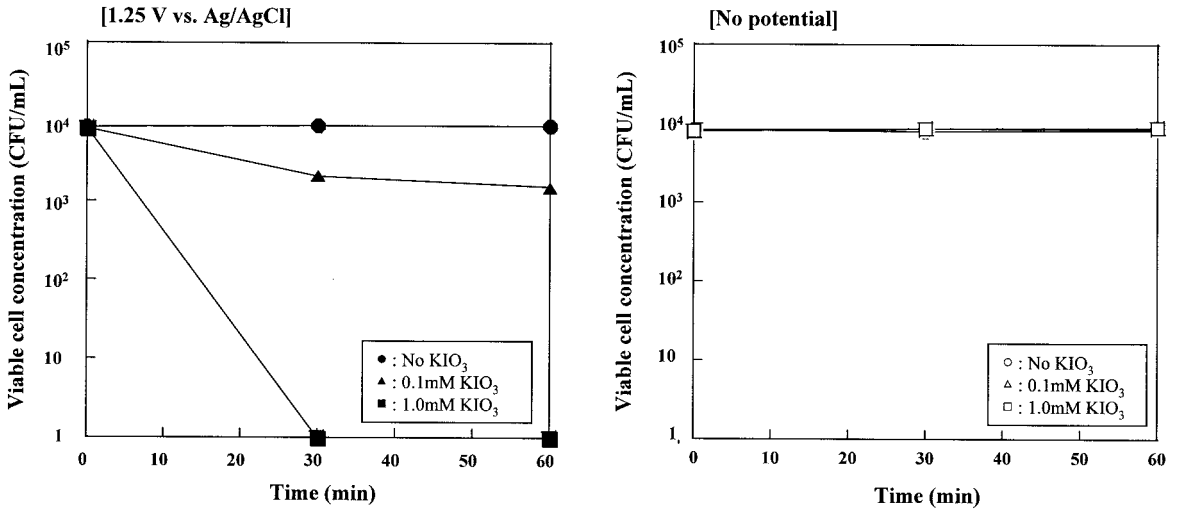

FIG. 5. Time course for the concentration of viable cells of *E. coli* when a potential of 1.25 V versus Ag/AgCl was applied in 0 mM, 0.1 mM, or 1.0 mM potassium iodate. The open symbols indicate the results for control experiments without potentials applied.

phase (data not shown). These results indicate that periodate was reduced to iodate during the disinfection process.

Figure 2 shows the cyclic voltammogram obtained at a scan rate of 100 mV · s^{-1} in 0.1 M KIO_3. An anodic peak appeared at approximately 1.25 V, and the peak current was 30 μA. In contrast, the corresponding peak did not appear when cyclic voltammetry was performed in 0.1 mM phosphate buffer without potassium iodate. It was found that iodate was electrochemically oxidized at a potential of 1.25 V. In order to confirm that periodate was formed by the oxidation of iodate, the periodate concentration was measured after 1.25 V was applied in 1.0 mM KIO_3. The periodate concentration was measured as follows. One milliliter of a periodate sample, 10 ml of Britton-Robinson buffer (pH 4.0), 5 ml of distilled water, and 5 ml of tetramethylammonium iodide (0.5%, wt/vol) were added sequentially and mixed. The solution was extracted twice with 2.5 ml of chloroform and then dehydrated with anhydrous sodium sulfate (1.0 g). The absorbance at 509 nm was measured using a spectrophotometer (model UV2400 PC; Shimadzu Co. Ltd.). When 1.25 V was applied in 1.0 mM KIO_3, the periodate concentration increased linearly with time (Fig. 3). Therefore, these results demonstrated that iodate is electrochemically converted to periodate when a potential of 1.25 V is applied.

The effect of the applied potential on the concentration of viable *E. coli* cells in 1.0 mM KIO_3 was investigated. When potentials between 0 and 1.6 V were applied for 60 min, the survival ratio was more than 90% in the absence of iodate. In contrast, when potentials were applied to cell suspensions containing 1.0 mM KIO_3, the concentration of viable cells decreased with increasing potential. The survival ratio was 0% at 1.25 V (Fig. 4).

Next, the time course of the concentration of viable cells when 1.25 V was applied in 0.1 mM or 1.0 mM KIO_3 was investigated (Fig. 5). When a potential was not applied, a decrease in the concentration of viable cells was not observed, as shown in Fig. 1. In contrast, when 1.25 V was applied in 1.0 mM KIO_3, an approximately 4-log decrease in the concentration of viable cells was observed in 30 min. Furthermore, when 1.25 V was applied in 0.1 mM KIO_3, the concentration of viable cells also decreased to 18% and 6% after 60 min and 120 min, respectively. In addition, a gram-positive bacterium, *Bacillus subtilis*, and a eukaryote, *Saccharomyces cerevisiae*, were also examined for inactivation. When 1.25 V was applied for 30 min in 0.1 mM KIO_4, the survival ratios of *B. subtilis* and *S. cerevisiae* at an initial cell concentration of 1.4×10^4 cells/ml decreased to 3% and 17%, respectively. With 1.0 mM KIO_4, more than 99% of the cells were inactivated. These results demonstrate that when a potential of 1.25 V was applied, iodate was electrochemically converted to periodate, which could be used as an efficient disinfectant.

Periodate is a well-known oxidizing agent that has been used for disinfection for a long time. Here we describe disinfection utilizing electrochemically regenerated periodate. By regeneration of periodate, disinfection can be conducted efficiently at low concentrations. Therefore, since iodine is electrochemically oxidized to iodate and consequently to periodate, it might be possible to reduce periodate electrochemically to iodate or iodine, compounds that are less toxic after disinfection.

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