# Electrochemical inactivation kinetics of boron-doped diamond electrode on waterborne pathogens

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## ABSTRACT

A boron-doped diamond (BDD) electrode was constructed as a water disinfector for the inactivation of water borne pathogens. The bactericidal effect of the disinfector was evaluated on artificially contaminated waters containing, respectively, *Escherichia coli*, *Pseudomonas aeruginosa* and *Legionella pneumophila* at high density. By treating the bacterial suspensions with 4 V of constant voltage between the BDD and the counter-electrode for 50 min, the population of *E. coli* and *P. aeruginosa* decreased from (10E + 7–8 colony-forming unit $mL^{-1}$) to below the detection limits of the colony-formation method. Meanwhile, *L. pneumophila* were reduced to virtually zero when analyzed by fluorescence-based staining. The influences of production parameters (voltage, NaCl concentration and flow rate) on the disinfection kinetics of the BDD disinfector were examined with respect to operational conditions. Voltage was the most significant factor for adjusting the extent of electrolysis, followed by NaCl concentration and flow rate, to influence the disinfection efficiency. The disinfection of natural river water samples containing numerous microbes was performed for a practicability investigation of the BDD electrode. Approximately 99.99% bactericidal efficiency was confirmed by viability detection for *E. coli* and common germs in treated water. The results showed that the BDD electrode is a promising tool for various wastewater disinfections to combat waterborne diseases.

**Key words** | boron-doped diamond electrode, electrochemical disinfection kinetics, *Escherichia coli*, *Legionella pneumophila*, practical water disinfection, *Pseudomonas aeruginosa*

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## INTRODUCTION

Water contamination is a major cause of water borne diseases and seriously threatens public health. These problems occur in developing (Egboka *et al.* 1989; Tibbetts 2000) as well as advanced countries. From 1990 to 2000, at least 10 cryptosporidiosis outbreaks associated with contaminated drinking water were reported in the United States (Moore *et al.* 1993; Kramer *et al.* 1996; Levy *et al.* 1998; Barwick *et al.* 2000). Various alternatives, including bleach with bromide, non-oxidizing biocides, ozone, ultraviolet light, chlorine dioxide, sodium chlorite, chloramines, copper–silver ionization and thermal disinfection, have been explored for disinfecting bacteria-contaminated water. Unfortunately, these methodologies have shortcomings that are difficult to resolve, such as the emergence of toxic disinfection by-products, resulting in potentially high levels of Cu in the surface waters and accumulation of Cu in the sediments or high operating costs, etc.

The choice of a wastewater treatment method, including disinfection, depends on treatment (bactericidal) efficiency, ease of control, running cost-effectiveness, as well as low burden on the environment. Electrochemical technology

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as a new approach for water treatment has attracted a great deal of attention in recent decades, because it possesses nearly all of these characteristics. In particular, electrochemical oxidation has been used for the removal of biorefractory organic pollutants or microtoxic substances from several specific types of industrial wastewater (Motheo & Pinhedo 2000; Körbahti & Tanyolacü 2003; Fernandes *et al*. 2004). Also, the use of electrolytic methodology to eliminate microorganisms in natural water for drinking water production has been well documented (Matsunaga *et al*. 1994). In addition, the demonstration of this usage has several excellent features, including non-selectivity of bacterial strains, non-generation of resistant bacteria and little adverse impact on the environment, because it does not require the use of potentially hazardous chemicals. Although numerous studies have proposed application modes of electrochemical water disinfection, more breakthroughs in technology are still required for economically practical application (Matsunaga *et al*. 1992; Velizarov 1999; Drogui *et al*. 2001).

Our previous report has shown that the boron-doped diamond (BDD) electrode has excellent electrochemical stability and high overpotential for the generation of the usual water electrolysis products ($H_2$ and $O_2$), and these properties were revealed to be superior to those of platinum (Pt) or glassy carbon electrodes (Zhi *et al*. 2003). Similar findings for anodic oxidation with a BDD electrode for the removal of chemical oxygen demand (COD) was also reported by Kraft *et al*. (2003). Due to these advantageous properties, employing the BDD electrode in combination with photocatalytic titanium dioxide materials to develop a high-performance, environmentally friendly water treatment system for practical use is our further objective (Ochiai *et al*. 2009).

Here, as a spearhead test of the development described, we applied a BDD electrode as a water disinfector to examine the kinetics of the disinfection of waterborne pathogens. *E. coli* was used as the main test bacteria and microbial indicator of water quality to assess the bactericidal efficiency. *P. aeruginosa* and *L. pneumophila* were also used as model microorganisms in experiments, because these are typical pathogens causing waterborne diseases (Lee *et al*. 2002; Steinert *et al*. 2002). Viable bacteria were detected by the microbial heterotrophic plate-counting method. Also, bacterial fluorescence staining was applied for rapid identification of live or dead cells of *L. pneumophila*. The influential operating parameters (voltage, NaCl concentration and flow rate) on bactericidal activation were investigated to identify the controllability of the BDD electrode. In order to examine whether the BDD electrode disinfector can be scaled up to a practical natural water disinfection process, the bactericidal effect of the BDD electrode disinfector was confirmed by treating river water samples containing a large number of viable microbes, including *E. coli*, without any additional electrolytes.

## MATERIALS AND METHODS

### Preparation of BDD electrode reactor

The BDD electrode used as a water disinfector to sterilize bacteria-contaminated water is illustrated in Figure 1. It was configured with a BDD electrode cell (DIACHEM® W; Condias GmbH, Itzehoe, Germany), a direct-current power source, a peristaltic pump and a flow rate controller. In order to ensure the feasibility of using lower-cost materials, the electrode was produced by coating BDD

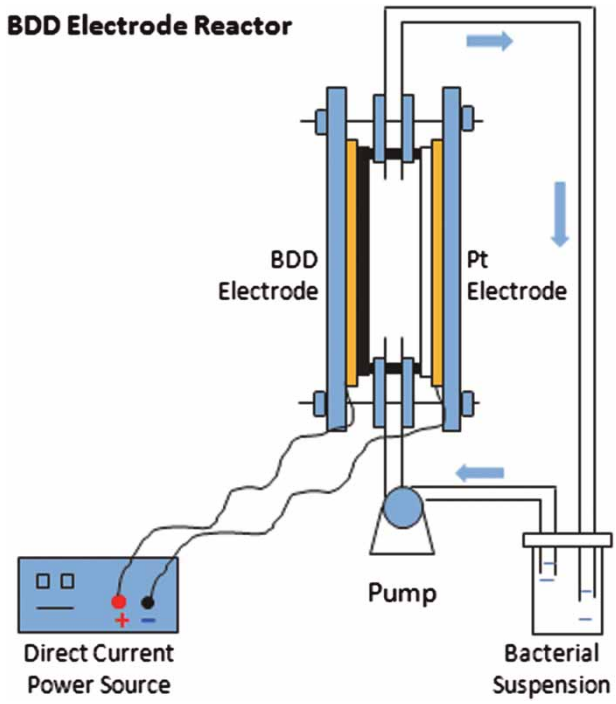


**Figure 1** | Illustration of the BDD electrode reactor.

onto a 2 mm thick niobium substrate plate using a hot filament chemical vapor deposition method (Matsumoto *et al.* 1982). A titanium (Ti) plate coated with a thin Pt film was used as the cathode. Both electrodes possessed a geometric area of 77.4 $cm^2$.

## Preparation of water samples artificially contaminated with bacteria and natural river water samples

*Escherichia coli* (NBRC 12713) used in this study was obtained from the Biological Resource Center of the National Institute of Technology and Evaluation (NITE-BRC), *P. aeruginosa* (IID 1700) was obtained from the Institute of Medical Science, University of Tokyo, and *L. pneumophila* (GTC/GIFU 00296) was received from the Department of Microbiology, Gifu University Graduate School of Medicine.

*Escherichia coli* were consecutively cultivated twice in 200 mL of nutrient broth (NB) solution (Becton Dickinson, Franklin Lakes, NJ, USA) at 35 °C for 18–20 h with continuous shaking at 180 rpm; then, the live cells were collected by centrifugation at 3000 rpm for 10 min at 4 °C. After washing the pellet with phosphate-buffered saline (PBS), it was re-suspended in 2 l of 0.1% of NaCl solution at a bacterial density of approximately 10E + 7–8 colony-forming units (CFU) $mL^{-1}$. The bacterial suspension was then used as the artificially contaminated water sample. The *P. aeruginosa* and *L. pneumophila* suspensions were prepared following a similar method, except that *L. pneumophila* was pre-cultured with a charcoal yeast extract (CYE) medium containing alpha-ketoglutarate (Becton Dickinson) at 37 °C for 72 h.

The natural river water samples were collected from the headwaters and the downstream waters of Turumi River (Kanagawa, Japan) and were stored at 4 °C. The Tsurumi River was the third worst in the Japanese river water ranking of 2006, reported by the Ministry of Land, Infrastructure, Transport and Tourism. The environmental water quality standard categories for the Turumi River sample was rank C and its COD value was 106.1 mg $dm^{-3}$. The electrical conductivity was 411 mS $cm^{-1}$ in the headwaters and 27 mS $cm^{-1}$ in the downstream region. The disinfection studies of the river water samples were implemented as soon as possible to acquire stable, accurate microbiological analysis data. The detection of microorganisms, including *E. coli*, was carried out according to the Japanese National Standard methods.

## Treatment of artificially contaminated water and natural river water

Artificially contaminated water samples were transferred into the BDD electrode disinfector with a peristaltic pump at a flow rate of 250 mL $min^{-1}$ and were treated with a constant voltage. During the water treatment process, the sampling was conducted with two cycles of treatment (approximately 16 min interval). Various constant voltages (3–5 V) and flow rates (100, 250 and 500 mL $min^{-1}$), as well as NaCl concentrations (0.1, 0.45 and 0.85%), were used for investigating the influence of production parameters on the bactericidal kinetics. Referring to the results observed from these experiments, the natural river water samples were treated under an optimum condition of 4 V and 250 mL $min^{-1}$ flow rate for 24 min, without any additional electrolyte. All inactivation tests were performed at room temperature (20 ± 2 °C) to simulate actual water treatment conditions.

The bactericidal studies were repeated a minimum of three times for each strain to ensure accuracy.

## Bacteriological viability assessment and data analysis

The viability of *E. coli* and *P. aeruginosa* in the artificially contaminated water samples was analyzed with NB agar medium (Becton Dickinson). For the natural river water, the *E. coli* were detected with XM-G agar medium (Nissui, Japan) and other microbial contaminants were analyzed with NB agar. The water samples from all sampling points were diluted with PBS in a tenfold dilution series. One milliliter aliquots of each dilution stage of the water samples were mixed with 14.0 mL of XM-G or NB agar medium at 45 °C in a 10 cm Petri dish and were allowed to cool to room temperature. After the medium solidified, the dishes were incubated at 35 °C for 24–48 h to determine the number of CFUs. The latter were counted in three parallel analyses for each sample and the bacterial survival rate was calculated as:

$$\text{Bacterial survival rate } (\alpha \text{ min}) = \text{CFU } (\alpha \text{ min}) \times 100/\text{CFU } (0 \text{ min}).$$

The survival of *L. pneumophila* cells in water samples taken at each sampling point was detected by bacterial fluorescence staining and by colony forming on a prepared CYE medium with added alpha-ketoglutarate. The fluorescent staining procedure was performed in accordance with the manufacturer's instructions (*Bac*Light Bacterial Viability kit, Invitrogen, Carlsbad, CA, USA) with some adjustments. Briefly, 10 mL of an *L. pneumophila*-contaminated water sample from each sampling point was centrifuged and the bacterial pellet was re-suspended in 90 μL of PBS buffer. Then, 10 μL of the 100 μM bacterial stain solutions (green or red) were added to the bacterial suspension and incubated at room temperature for 10–15 min. The stained bacteria were viewed by fluorescence microscopy (AxioImagerA1 SP; Carl Zeiss, Tokyo, Japan) and the bacterial cell death rate was quantified using an image analysis system comprising a digital counter (AxioCamMRc SP; Carl Zeiss) and software (Scion Image, freeware; http://www.scioncorp.com/pages/scion_image_windows.htm). The *Bac*Light Bacterial Viability kits employed two nucleic acid stains: a green-fluorescent SYTO 9 stain and a red-fluorescent propidium iodide stain. SYTO 9 stain labels both live and dead bacteria, whereas propidium iodide penetrates only bacteria with damaged membranes and SYTO 9 fluorescence is reduced when both dyes are present. Thus, live bacteria with intact membranes were fluorescent green, whereas dead bacteria with damaged membranes were fluorescent red. The *L. pneumophila* death rate was calculated as:

$$\text{Bacterial death rate } (\alpha \text{ min}) = \text{red fluorescent cells } (\alpha \text{ min} - 0 \text{ min}) \times 100/\text{green fluorescent cells } (0 \text{ min}).$$

In order to verify the results observed in the staining experiment, the culturable count of *L. pneumophila* cells in the water samples were also detected by the colony formation method with CYE medium with added alpha-ketoglutarate.

## RESULTS AND DISCUSSION

### Selection of electrochemical treatment condition and artificially contaminated water disinfection

Taking into account that the aim of a water treatment system is the purification of the water environment, it should be eco-friendly. Thus, we sought to use low voltages and low concentrations of NaCl solution to achieve the sterilization of the water samples. Electrolyzed NaCl water solutions involving high and low free-chlorine concentrations are abbreviated as electrolyzed strong acid water (ESW)-H and ESW-L, respectively. The higher bactericidal kinetics of ESW-H on various microorganisms has been well documented (Iwasawa & Nakamura 1993; Abe *et al.* 1994). However, ESW-H contains high concentrations of free chlorine and its byproducts may have potential cytotoxicity and genotoxicity, as reported by Knasmüller *et al.* (1996) and Daniel *et al.* (1993). Moreover, ESW-H has a corrosive activity against instruments. ESW-L was prepared by the electrolysis of an NaCl solution at low concentrations and was confirmed to be effective against blood borne pathogenic viruses (Morita *et al.* 2000; Tagawa *et al.* 2000). Although the effect was slightly weaker than that of ESW-H, it is acceptable as a disinfectant, as stated by Kiura *et al.* (2002). Thus, we investigated the bactericidal kinetics of the BDD electrode disinfector by using electrolysis of 0.1% NaCl solutions, which exhibited a conductivity of 1.9 mS $cm^{-1}$. To select a possible low-voltage value, we investigated the electrolytic bactericidal effect with a mini-BDD electrode (1 $cm^2$) at various voltages in preliminary tests. Using 2 V (0.015 mA) in 0.1% NaCl solution was not at all effective during 2 h, since the *E. coli* concentration was the same as at the beginning (approximately 1.2E + 9 cells $mL^{-1}$). In contrast, 3 V already after 30 min destroyed all *E. coli* cells to less than the detection limit (which is 1 cell in 1 mL). Simultaneously, we found that the current increased drastically at 3 V (4.5 mA), which suggested that some special reactions began to occur in the electrolytic process. According to the theory given in *Diamond Electrochemistry*, edited by Fujishima *et al.* (Cho *et al.* 2005; Varistas *et al.* 2005), as shown in Equations (1) and (2), these phenomena could be attributed to the fact that the amounts of ozone produced by the electrolysis of the solution at 3 V were larger than those at 1.8 V:

$$H_2O + O_2 \Rightarrow O_3 + 2H^+ + 2e^- \quad E^0 = +2.07\,V \tag{1}$$

$$3H_2O \Rightarrow O_3 + 6H^+ + 6e^- \quad E^0 = +1.51\,V. \tag{2}$$

It has been documented that the electrolysis of water containing chlorides can yield hydroxyl radicals and

hypochlorite, as well as other species (Comninellis 1994; Israilides *et al.* 1997; Szpyrkowicz *et al.* 2001), as given in Equations (3)–(5):

$$H_2O \Rightarrow OH^- + H^+ + e^- \quad (3)$$

$$H_2O + Cl^- \Rightarrow HOCl + H^+ + 2e^- \quad (4)$$

$$2Cl^- \Rightarrow Cl_2 + 2e^-. \quad (5)$$

It should further be noted that the $Cl_2$ and $OH^-$ produced can react as follows:

$$Cl_2 + 2OH^- \Rightarrow ClO^- + Cl^- + H_2O \quad (6)$$

$$Cl_2 + OH^- \Rightarrow HOCl + Cl^-. \quad (7)$$

At the same time, chlorine dioxide can be found among the oxidants produced from the electrolysis of solutions, according to Equation (8):

$$H_2O + HClO + Cl_2 \Rightarrow ClO_2 + 3H^+ + 2Cl^- + e^-. \quad (8)$$

In addition, $H_2O_2$ is formed at the cathode, as described by Equation (9):

$$H_2O + O_2 + 2e^- \Rightarrow H_2O_2 + 2OH^- \quad (9)$$

There are various explanations for the mechanism of microbial inactivation by electrochemical processes. Most studies stated that the excellent electrochemical sterilization could be ascribed to bactericidal factors such as pH, electrochemically produced active species, including hydrogen peroxide, ozone and free chlorine, and the corresponding high oxidation–reduction potential (ORP) (Venczel *et al.* 1997; Venkitanarayana *et al.* 1999; Kim *et al.* 2000). The oxidants produced according to Equations (1)–(9) have extremely strong oxidation potentials. They could attack the bacterial cell wall and membrane, and disrupt the membrane integrity, or electrolyze the molecules in the cell surface, which would bring about cell death and lysis (Liu *et al.* 1997; Diao *et al.* 2004). The fact that no bactericidal effect was observed at 1.8 V in our preliminary tests revealed that the oxidants produced in this instance were very low, and they were not able to exert a sufficiently strong oxidation effect on the bacteria that possess a certain degree of antioxidant capacity.

Referring to the results obtained in the preliminary tests, the subsequent examination of the bactericidal kinetics of the flow-type BDD electrode (77.4 $cm^2$) disinfector was performed by electrolyzing a 0.1% NaCl solution at 3 V. The result shows that, by treatment at 3 V and a flow rate of 250 mL $min^{-1}$ for 96 min, the survival rate of *E. coli* in the artificially contaminated water was decreased from approximately 10E + 7–8 CFU $mL^{-1}$ to below the detection limits of the colony-forming method (Figure 2(a), round symbols). A longer treatment time (more than 150 min) was required for

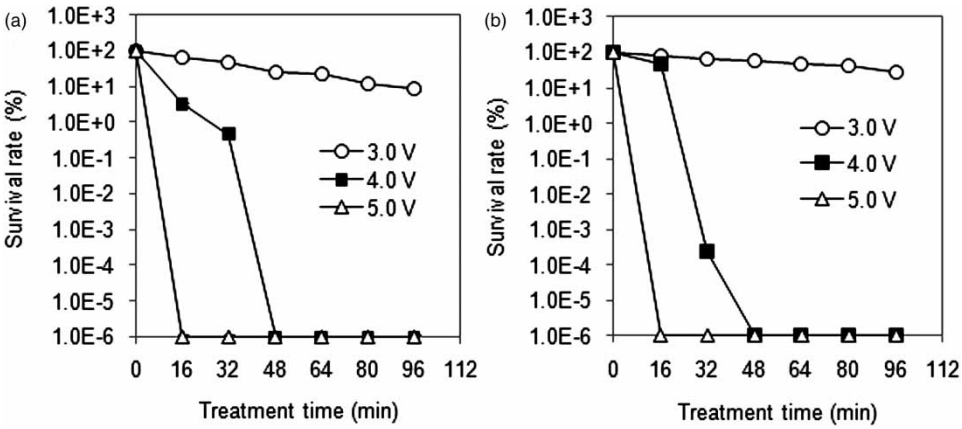


**Figure 2** | Effects of voltage on the bactericidal properties of the BDD disinfector against (a) *E. coli* and (b) *P. aerigonosa*. The flow rate was fixed at 250 mL $min^{-1}$ and 0.1% NaCl solution was used as the electrolyte in each test.

inactivation of *P. aeruginosa* to achieve a similar efficiency (Figure 2(b), round symbols). The different sensitivities to the electrochemical treatment between the two bacterial strains may be due to the outer membrane permeability of *P. aeruginosa*, which is 12–100 times lower than that of *E. coli*, as reported by Nikaido & Hancock (1986). It has been clarified that, in a variety of antibiotic resistance mechanisms of *P. aeruginosa*, a low outer membrane permeability posed by its mucous membranes is an important feature (Hancock & Speert 1996).

## Influence of operating parameters on electrochemically induced bactericidal kinetics

The influence of operating parameters, including voltage, electrolyte concentration and flow rate, on electrochemically induced bactericidal activity was found to be significant. When the constant voltage was fixed at 4.0 V, the growth of *E. coli* and *P. aeruginosa* was completely inhibited within a short treatment time (48 min). After application of 5.0 V for 16 min, no bacterial colonies were formed on the cultured agar plate from the treated water samples (Figure 2(a) and (b)). As shown in Figure 3, increased NaCl concentration also enhanced the bactericidal efficiency of the BDD disinfector; however, this influence was much less than that caused by increased voltage.

It is remarkable that the influence of flow rate on the bactericidal kinetics of the BDD disinfector was significant when the concentration of electrolyte was fixed. When water disinfection was performed with a flow rate of 100 mL $min^{-1}$, the inactivation activity was greatly reduced. Referring to the mechanism of electrochemical inactivation described above, it is clear that the disinfection efficiency depends on the dose of various electrochemically produced reactive oxidants in the BDD cells. The production rate of various reactive oxidants under a fixed constant voltage is proportional to the dose of electrolyte provided to the unit area of BDD electrode in unit time. Thus, the inactivation efficiency in 100 mL $min^{-1}$ was lower than in 250 mL $min^{-1}$, which can be ascribed to the lower production rate of reactive oxidants of 100 mL $min^{-1}$.

Interestingly, there was no inactivation of *E. coli* when the flow rate was increased to 500 mL $min^{-1}$, as shown in Figure 4. We consider that excessive electrolyte flow rate led to the extremely short contact time between the electrolyte and BDD electrode to produce very few oxidants. It can also disturb the diffusion of produced oxidants to the bacteria in the aqueous solution. The result is consistent with several studies that examined the effects of voltage, flow rate, temperature and salt concentration on the chemical and physical properties of electrolyzed water. As Eneike & Hung (2004) and Hsu (2005) have demonstrated, the higher voltage and NaCl concentration resulted in higher ORP and residual chlorine of the electrolyzed water. They also found that increasing the flow rate produced electrolyzed water with lower ORP and

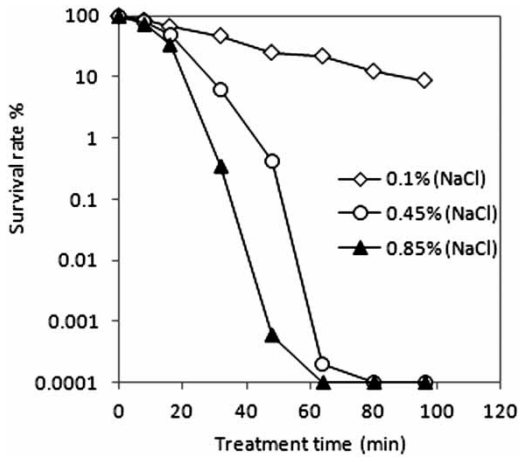


**Figure 3** | Effects of NaCl concentration on the bactericidal properties of the BDD disinfector against *E. coli*. The water flow rate was fixed at 250 mL $min^{-1}$ and a voltge of 3 V was used in the electrolytic process.

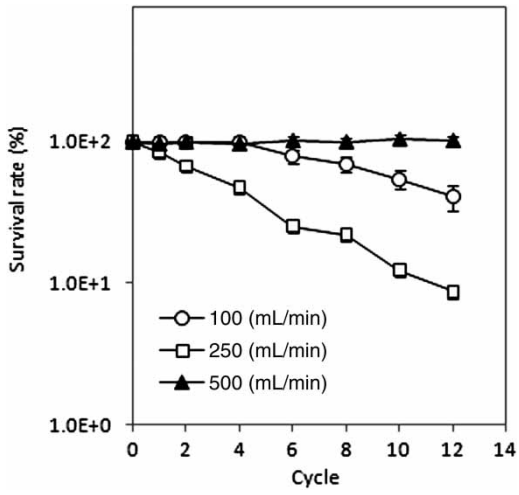


**Figure 4** | Effects of flow rate on the bactericidal properties of the BDD reactor against *E. coli*. The concentration of NaCl solution was controlled at 0.1% and a voltage of 3 V was used in the electrolytic process.

residual chlorine due to the shorter residence time of the electrolyte in the electrolytic cell. From these findings, the message is clear that finding an optimal flow rate is vital to obtain a satisfying bactericidal efficiency by using electrolysis at a specific electrolyte concentration and voltage conditions.

We also note that there were no temperature changes observed through the whole range of experimental settings used, even for the 5.0 V (15 mA $cm^{-2}$) treatment for 96 min. We ponder that electrochemical treatment did not change the temperature, but temperature together with electrolytic treatment might have a common effect.

## Changes in pH value of solutions

Production of $H^+$ and $OH^-$ by the electrolysis of water may generate modifications in the pH of bacterial suspensions. The results of measurements of pH in *E. coli* suspensions are shown in Figure 5, which indicates that little change in pH was induced by electrolysis at higher voltages (4 and 5 V). In general, the reduction of water in the cathode compartment of an electrochemical cell can increases the pH due to the consequent hydrogen evolution and the formation of hydroxyl anions. On the other hand, the reactions that include oxygen evolution, chlorine evolution and organic oxidation occur on the anode surface. In contrast to the hydroxyl formation on the cathodic side, there is generation of protons ($H^+$) on the anodic side, which may decrease the pH. When the products of the two electrodes mix together in an electrolytic cell without a unidirectionally permeable membrane, as with the present BDD electrode cell, the pH value was only slightly increased at higher voltages, such as 4 V and 5 V. Guillou & Murr (2002) have reported that no lethal effect of pH in the 5–8.5 range was observed on the inactivation of yeast *Saccharomyces cerevisiae* by electrolysis. Therefore, it can be suggested that the influence of pH from 6.5 to 8.5 shown in Table 1 is relatively small and did not contribute to the inactivation activity of the bacteria in our electrolysis process.

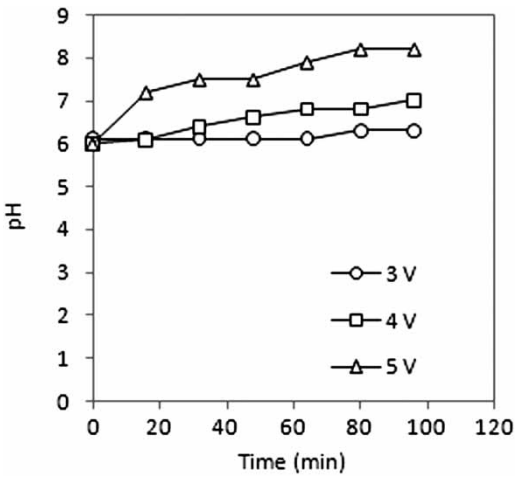


**Figure 5** | Changes of pH in electrolyzed artificial wastewater containing 0.1% NaCl.

On the basis of the results described above, it can be summarized that the cell density of both bacterial strains decreased proportionally to the voltage value, electrolyte concentration, oxidant production rate and detention time. For bactericidal efficiency, running cost and water environmental safety consideration, searching for the optimum operating conditions corresponding to the electrolytic system applied is very important. For the whole range of experimental settings used in this study, we conclude that a voltage of 4 V and a flow rate of 250 mL $min^{-1}$ are optimum treatment conditions for the BDD electrode disinfector, when the electrolyte was fixed at 0.1% NaCl.

## Practicability of BDD electrode

We used 4 V and a 250 mL $min^{-1}$ flow rate to treat *L. pneumophila* suspensions at a density of approximately $10 \times 10^7$ cells $mL^{-1}$ and found that the viable *L. pneumophila* in the treated water samples decreased to virtually zero within 64 min when analyzed by fluorescence-based bacterial staining (Figure 6(a)–(c)). This result was verified by the microbial

**Table 1** | Bactericidal effect on *E. coli* and other microbial contaminants in river water samples

| Bacteria name | Treatment time (min) | Surviving population in water sample (log CFU $mL^{-1}$) Headwaters | Downstream |
|---|---|---|---|
| *E. coli* | 0 | $540 \pm 10$ | $495 \pm 10$ |
| | 8 | 0 | 0 |
| | 16 | 0 | 0 |
| Other microbial contaminants | 0 | $1 \times 10^5$ | $8 \times 10^4$ |
| | 8 | $2 \times 10^2$ | $1 \times 10^1$ |
| | 16 | 0 | 0 |

The river water samples were obtained from the Turumi River (Kanagawa, Japan). The bactericidal tests were performed with a 4 V voltage and a 250 mL $min^{-1}$ flow rate using the BDD electrode reactor.

culture method (Figure 6(d)). As is already well known, *E. coli, P. aeruginosa* and *L. pneumophila* are typical pathogens that cause waterborne infections in a wide range of water sources. The strong bactericidal action of the BDD electrode against these infectious pathogens provides a highly promising approach to the prevention of waterborne diseases. Finally, we treated natural river water samples by the BDD electrode disinfector with the enumerated optimum conditions. As stated in the materials section, the electrical conductivities in the headwater and downstream regions are relatively high and sufficient for the electrochemical oxidation treatment without adding any electrolyte. Table 1 shows that there were $540 \pm 10$ cells $mL^{-1}$ of *E. coli* in the headwaters and $495 \pm 10$ cells $mL^{-1}$ of *E. coli* in the downstream region before the treatment. After the water samples were exposed to the disinfector at the enumerated optimum conditions for only 16 min, the microorganisms, including *E. coli*, were reduced to a non-detectable level.

Complementary, in our further study with a combined BDD electrode and $TiO_2$ photocatalyst unit, the COD values of the Tsurumi River water samples were reduced from 106.1 mg $dm^{-3}$ to less than 1.0 mg $dm^{-3}$, i.e., an environmental water quality standard category rank of AA for the sample (Ochiai *et al*. 2009).

## CONCLUSIONS

A BDD electrode was employed as a water disinfector due to its electrochemical, mechanical and thermal stability, as well as its large overpotential for gas evolution. The strong bactericidal activity of the disinfector was confirmed on wastewater

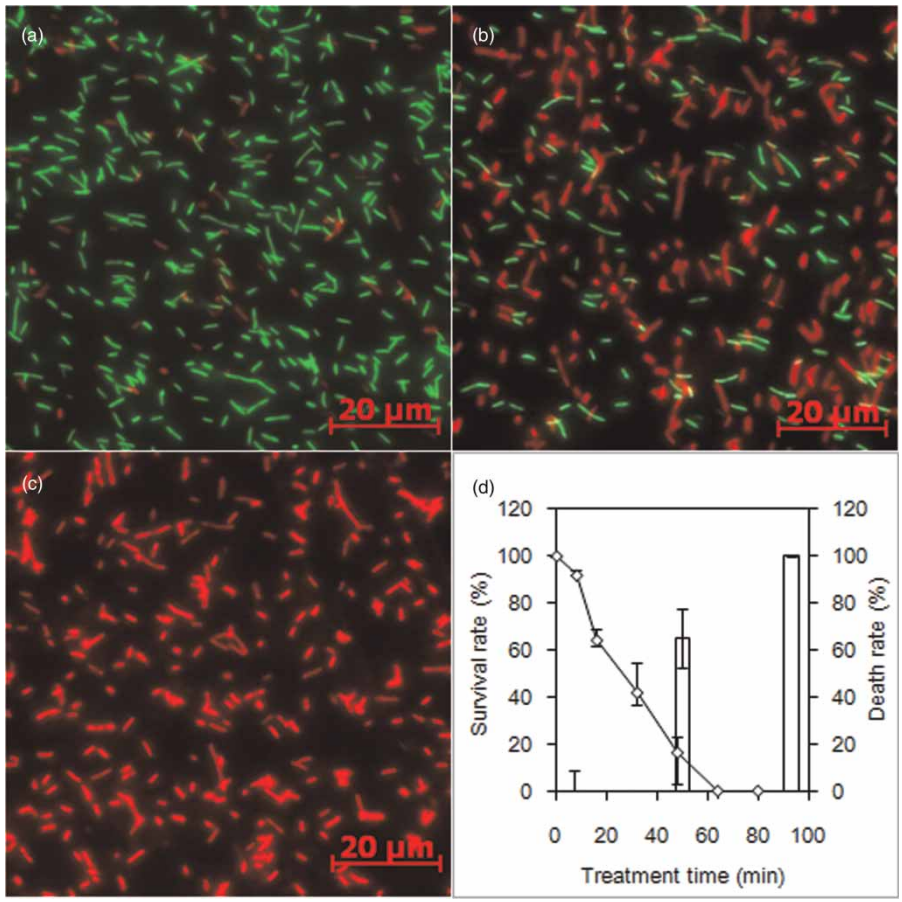


**Figure 6** | Bactericidal effect of the BDD reactor on *L. pneumophila* in water samples treated with a 4.0 V voltage and a 250 mL $min^{-1}$ flow rate. (a), (b) and (c) indicate the representative fluorescence microscopy from 0, 32 and 80 min of treatment, respectively. (d) Shows the survival and death rates of *L. pneumophila* obtained by (◇) the colony formation method and (□) quantitative fluorescence microscopy analysis.

artificially contaminated with *E. coli, P. aeruginosa* and *L. pneumophila*. The bactericidal kinetics was significantly enhanced by increasing the voltage and electrolyte (NaCl) concentration. The optimum flow rate of electrolyte through the BDD electrode cell is required to obtain the expected bactericidal efficiency at a certain voltage. Using the BDD electrode, a large number of viable microorganisms including *E. coli* in the natural river water samples can be effectively destroyed to less than the detection limit (which is 1 cell in 1 mL) without any additional electrolyte. The results indicated that the BDD electrode can be applied in a variety of different designs of water treatment systems in response to various wastewater treatments, such as medical and industrial purposes. With reference to the results of this study, practically, we have applied the BDD electrode in a novel electrochemical–photocatalytic sequential water treatment system (Ochiai *et al*. 2009). This system may be useful for supplying higher quality water during a disaster due to its high-performance water treatment, which is totally driven by solar energy.

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