Killing of *Escherichia coli* using the gas diffusion electrode system

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ABSTRACT

To be best of our knowledge, this study is one of the first investigations to be performed into the potential benefits of gas diffusion electrode (GDE) system in controlling inactivation of *E. coli*. This study mainly focused on the dual electrodes disinfection with gas diffusion cathode, using *Escherichia coli* as the indicator microorganisms. The effects of Pt load \(W_{Pt}\) and the pore-forming agent content \(W_{NH_4HCO_3}\) in GDE, operating conditions such as pH value, oxygen flow rate \(Q_{O_2}\), salt content and current density on the disinfection were investigated, respectively. The experimental results showed that the disinfection improved with increasing Pt load \(W_{Pt}\), but its efficiency at Pt load of 3‰ was equivalent to that at Pt load of 4‰. Addition of the pore-forming agent in the appropriate amount improved the disinfection while drop of pH value resulted in the rapid rise of the germicidal efficacy and the disinfection shortened with increasing oxygen flow rate \(Q_{O_2}\). The system is more suitable for highly salt water. The germicidal efficacy increased with current density. However, the accelerating rate was different: it first increased with the current density, then decreased, and reached a maximum at current density of 6.7–8.3 mA/cm². The germicidal efficacy in the cathode compartment was about the same as in the anode compartment indicating the contribution of direct oxidation and indirect treatment of *E. coli* by the hydroxyl radical was similar to the oxidative indirect effect of the generated H\(_2\)O\(_2\). This technology is expensive in operating cost, further research is required to advance the understanding and reduce the operating cost of this technology.

**Key words** | cathode, *Escherichia coli*, gas diffusion electrode, operating cost, oxygen reduction

INTRODUCTION

Pathogenic microbes are contaminants of major concern in drinking water, thus disinfection of water is very important to provide a sanitary environment and maintain human health. However, the commonly used disinfection methods, such as chlorination, ozonation, and ultraviolet (UV) irradiation, have been found to have potentially adverse effects on human health and the environment, or they may be ineffective in the presence of organic matter. Chlorinating generates hazardous and carcinogenic compounds (*Jolley et al. 1982*). Additionally, several processes are involved in bacterial resistance/adaptation to chlorine stress and together they form a barrier against external damages caused by chemical disinfection (*Abu-Shkara et al. 1998*). Ozone and ultraviolet light are efficient, but they are more expensive (*Christopher & Richard 1995*); moreover, ultraviolet light has no remnant effect.

Research of alternative oxidants is actually carried out to identify inexpensive techniques, with remnant effect, producing harmless substances. Electrochemical (EC) disinfection has been proven effective and with remnant effect, it has been reported that EC disinfection can destroy a wide variety of microorganisms from viruses through bacteria and algae to larger species, such as *Euglena* (*Grahl & Markl 1996; Li et al. 2002; Park et al. 2003*). Therefore, its use is
well established in the disinfection of potable and swimming pool waters. Direct passage of current through aqueous solution leads to the anodic generation of disinfecting substances and direct killing of micro-organisms at the anodes or in the vicinity of the anodes. Generally speaking, the free available chlorine is by far the most important disinfecting substance generated in water, therefore, like chlorination, its use has the main drawback of possible formation of organochlorinated compounds (Panizza & Cerisola 2003; Panizza & Cerisola 2009).

In contrast, hydrogen peroxide is an environmentally friendly chemical that leaves no hazardous residuals since it decomposes only to water and oxygen. Hydrogen peroxide is a metastable molecule of high redox potential (1.77 V), its oxidizing properties result from a direct molecular action, but, mostly, from free radicals formed by catalysis. Along with hydrogen evolution, hydrogen peroxide can be electrogenerated in acidic solutions by two-electron reduction of oxygen on the cathode surface:

\[
O_2 + 2H^+ + 2e^- \rightarrow H_2O_2
\]  

(1)

or in an alkaline solution by the reaction:

\[
O_2 + H_2O + 2e^- \rightarrow HO_2^- + OH^- 
\]  

(2)

Disinfecting by electroperoxidation combines the direct effect of electrolysis to the indirect oxidative effect of hydrogen peroxide, which allows reducing the operation cost. \( H_2O_2 \) electrosynthesis is also of interest because of the cost and risks associated with transportation, storing and handling of concentrated hydrogen peroxide. Oxygen consuming cathode was used for the electrolytic disinfection of drinking water. However, the current efficiency of electroperoxidation is generally lower than for other electrochemical processes; this is because \( H_2O_2 \) generated at the oxygen consuming cathode can react with free available chlorine according to the reaction expressed by Equation (3), leading to elimination of the latter, the most important anodically formed disinfectant:

\[
H_2O_2 + HClO \rightarrow H_2O + HCl + O_2
\]  

(3)

Another major disadvantage of oxygen consuming cathodes is the fact that they can only be used to reduce the oxygen dissolved in aqueous solution to \( H_2O_2 \).

However, the solubility of oxygen in aqueous solution is typically lower than 10 mg/L at atmospheric pressure. Even when pure oxygen is aerated, DO in water will be lower than 25 mg/L. In other words, the generated \( H_2O_2 \) is of high energy consumption, low yield, and can hardly be used for drinking water and wastewater disinfection. For this reason, oxygen consuming cathode for water disinfection has not been widely applied.

More recently, three-dimensional carbon-based gas diffusion electrodes (GDE) have demonstrated to be promising electrode materials for the electrogeneration of \( H_2O_2 \) from oxygen reduction (Harrington & Fletcher 1999; Qiang et al. 2002; Agladze et al. 2007b; Forti et al. 2007). Many studies have reported the application of \( H_2O_2 \) electrogenerated at GDE, fed with pure oxygen, for the treatment of wastewater containing organic pollutants, especially in the presence of \( Fe^{2+} \) ions (electro-Fenton process) (Do & Chen 1993; Do & Chen 1994; Oturan et al. 2000; Brillas et al. 2004; Hanna et al. 2005; Agladze et al. 2007a; Brillas et al. 2007; Liu et al. 2007a, b; Sires et al. 2007) or with \( Fe^{2+} \) and UV irradiation (photoelectro-Fenton process) (Flox et al. 2006; Brillas et al. 2007; Flox et al. 2007). Shen et al. (2005) used a porous gas diffusion electrode (GDE) to reduce oxygen to hydrogen peroxide, which could oxidize acid red B in the cathodic chamber.

Achievements have been made in research on electroperoxidation. However, since little attention was paid to the application in water treatment, most of the systems for electroperoxidation are plagued by problems such as narrow pH coverage, separation of an electrolytic cell by membrane to avoid transportation of \( H_2O_2 \) to anode, need for gas passing through a chamber to get to cathode.

Aiming at these problems, study on the electrochemical disinfection with the \( H_2O_2 \) generated at the GDE from active carbon/ poly-tetrafluoroethylene (PTFE) was performed by our research group recently in the non-membrane cell. The experimental results showed good efficiency in inactivation of bacterial cultures inoculated in the secondary effluent from wastewater treatment plant. These microbes can be completely inactivated within 30 min or less by the system depending on the type of GDE used. The system was proven to have wide pH coverage; additionally, the need for gas chamber was eliminated. Similar experiments were performed in this
work, and bactericidal activity and major disinfecting substances were investigated using *Escherichia coli* as an indicator bacterium.

**MATERIALS AND METHODS**

**Reagent and instruments**

Active carbon powder with a diameter of 200 nm (Shanghai Hainuo Carbon Co. Ltd.), the PTFE suspension (60%) and Pt/C catalyst with W_{Pt} of 40% (Shanghai Hesen Electric Co Ltd.) were used to fabricate the gas diffusion electrode (GDE). The remaining reagents applied in the process of the experiments were analytical grade (AR) materials.

The homemade electrolytic cell with a volume of 1 L from 0.5 cm thick acrylic glass, a laboratory constant current DC power supplier (RXN-6010D, Wuxi Qiaobo Power Supplier Co. Ltd., China) having the maximum output of 10A, 60V, and the gas flow meter with a range of 5 L/min (Dawn Instrument Co. Ltd. in Suzhou Industrial Park, China) were used to perform batch disinfection experiments.

**Fabrication of gas diffusion electrode**

A Pt/C gas diffusion electrode (GDE) was fabricated and used as a cathode fed with oxygen to produce H_{2}O_{2}. For the fabrication of the Pt/C GDEs, a homogeneous suspension was prepared by mixing and stirring the above mentioned active carbon, Pt/C catalyst, pore-forming agent NH_{4}HCO_{3}, with an appropriate amount of PTFE suspension and ethanol as solvent in an ultrasonic bath at room temperature for 30 min. The mixing ratio of ethanol/active carbon/PTFE was 12/10/5 by weight, the mixing ratio of catalyst/active carbon W_{Pt} was <5% by weight, and the mixing ratio of NH_{4}HCO_{3}/active carbon W_{NH_{4}HCO_{3}} was <70% by weight. The mixture was dried at T = 80°C for >2 h until a uniform paste was obtained.

The paste was hot-pressed at T = 95°C for 2 min under 15 MPa to form a GDE. The image of the fabricated GDE is shown in **Figure 1**.

The prepared GDE lay soaked in fresh acetone for 24 h, then was flushed repeatedly with distilled water and dried. The GDEs with the pore-forming agent lay soaked in the boiling deionized water for 30 min afterwards.

SEM of the GDEs has been obtained to study the morphology of the prepared diffusion layer, as shown in **Figure 1**. It seems that the PTFE polymer covers a certain surface of the diffusion catalyst layer, which brings about enough hydrophobicity. The white grains can be identified as PTFE (*Shen et al.* 2005). Also macropores of the layer are visible. The macroporosity of the diffusion active layer influences the electrode performance as the rate of mass transport of reactant gas on catalytic sites increases.

**Preparation and analysis of *E. coli***

*E. coli* was inoculated into tryptic soy broth and cultured aerobically for 24 h at 37°C with constant agitation to reach its stationary growth phase with a cell density of 10^{9}–10^{10} colony forming units per milliliter (CFU/mL). A fresh culture was then used to make the model water for the disinfection experiments. Culturable counts for *E. coli* were performed using the standard plate count method (*Lazarova et al.* 1998), viable cell counts were determined under 15 MPa to form a GDE. The image of the fabricated GDE is shown in **Figure 1**.

The prepared GDE lay soaked in fresh acetone for 24 h, then was flushed repeatedly with distilled water and dried. The GDEs with the pore-forming agent lay soaked in the boiling deionized water for 30 min afterwards.

**Figure 1 | SEM photographs of the surface of a typical GDE (magnification 30,000 x).**
visually as the number of colonies per plate in serial 10-fold dilutions after incubation at 37°C for 24 h.

**Preparation of SEM specimen for water samples**

The scanning electron microscopy technique was employed to examine the surface structure and morphology of *E. coli* after the EC disinfection. To prepare a SEM specimen for a disinfected water sample, *E. coli* cells in 50 mL of the water was concentrated by centrifugation (Sigma 2-16, Germany) at 10,000 rpm for 20 min. After discarding the supernatant, the *E. coli* cells were re-suspended into 5 ml of 0.85% NaCl solution. The SEM slide sample was then prepared from the cell suspension following the method described by Gu et al. (2001). The samples were coated with gold–palladium and viewed under a SEM (Quanta 200 FEG, FEI Company).

**Exposure of *E. coli* to electric current**

**Batch experiments, electrolysis in a single-chamber cell**

Model wastewater contaminated by *E. coli* culture was used for the experimental investigation. The test water was prepared by diluting a pure culture of *E. coli* to a density of $10^7$ CFU/mL in sterilized deionised water, containing a certain amount of electrolyte Na2SO4. The experimental setup consisted of an oxygen cylinder, an oxygen flow meter, an electrolytic cell and a constant current DC power supply. The homemade active carbon/PTFE GDE sized 5 £ 6 cm² was used as the cathode to reduce oxygen to H2O2, and the graphite plate electrode sized 5 £ 6 cm² was used as the anode. The rectangular anode, thick 5 mm, and the rectangular cathode was located with a spacing of 10 mm in the middle part of the electrolytic cell. The effective area of both the anode and cathode immerged in the model wastewater was 5 £ 4 cm². One oxygenation header was equipped at both sides to ensure equal distribution of oxygen in the electrolytic cell.

The working current between two electrode plates was adjusted by regulating the DC power supply to 0.3 A. 900 mL of the model wastewater was added to the electrolytic cell and adjusted to the appropriate pH with 0.1 mol/L H2SO4 or 0.1 mol/L NaOH before the electrolysis. 1 mL of solution was sampled with a sterilized pipette after placing for 50 min. Subsequently, oxygen flow rate QO2 was regulated with the gas flow meter, and the solution was electrolyzed for 12 min. At timed intervals of 2, 4, 6, 8, 10 and 12 min after the electrolysis, 1 mL of the bacterial suspension was withdrawn and immediately diluted 10-fold in series and plated on sterilized culture plates. Culturable counts for *E. coli* were performed using the plate count method, respectively. Three replicate 1 mL samples of the diluted solutions were plated to determine viable cell counts visually as the number of colonies per plate after incubation at 37°C for 24 h and good reproducibility was shown with a 10% standard deviation. All materials used in the experiments were autoclaved at 121°C for 20 min to ensure sterility.

The bactericidal activities against *E. coli* of the samples were evaluated by the inactivation of *E. coli*, assessed on the basis of the decrease in the number of colonies of *E. coli* formed on an agar plate. The calculating formula of germicidal efficacy $\eta$ was shown below:

$$\eta = \frac{N(t_0) - N}{N(t_0)} \times 100\%$$

where $N_0$ and $N$ are the number of viable *E. coli* cells in colony-forming units before and after the disinfection.

**Electrolysis in a two-chamber cell**

Electrolysis was conducted in an electrolytic cell separated into 2 compartments by a permeable cellulose acetate membrane (Figure 2) to investigate the substances or reactions having a leading function in the bactericidal activity against *E. coli*. Each compartment is of size $5 \times 5 \times 10$ cm³. A graphite plate sized $6 \times 4.8$ cm² was used as the anode, and a fabricated GDE with different compositions sized $6 \times 4.8$ cm² was used as the cathode. The working current between two electrode plates was adjusted by regulating the DC power supply to 0.1 A.

Electrolyzed the model wastewater with *E. coli* contamination having the current under control at 0.1 A. Sampling and colony count was performed according to the procedures specified in the previous section both for the anolyte and catholyte, respectively.
Analytical methods

At timed intervals of 2, 4, 6, 8, 10 and 12 min after electrolysis of the solution with 4% Na$_2$SO$_4$, having the current under control at 0.1 A or 0.3 A. 25 mL of the electrolyzed solution was withdrawn and the peroxide concentration in the electrolytic solutions was determined by titrating an acidified 25 mL of aliquot with 2 mmol/L potassium permanganate solution (Oloman & Watkinson 1979). Three replicate H$_2$O$_2$ solutions were titrated, and good reproducibility was shown with a 0.3 standard deviation.

RESULTS AND DISCUSSION

Description of the theoretical model for mass transfer

When a submerged diffuser is operated, there are two main interfaces over which oxygen transfer occurs. Oxygen transfer occurs across the bubble interfaces as the bubbles rise through the water column. Oxygen transfer also occurs across the water surface at the air–water interface. The latter is negligible, since GDE is fed with pure oxygen. The equilibrium oxygen concentration inside a bubble also depends on gas flow rate and the changing bubble-water transfer coefficient over depth.

In order to describe bubble-transfer behavior, the difference in the liquid-phase equilibrium oxygen concentration of the bubble and the actual DO concentration in the water produces bubble-water oxygen transfer, $J_b$, according to (DeMoyer et al. 2003):

$$J_b = \frac{K_L \alpha_b (C_0 - C)}{V} \int dz$$

where $K_L \alpha_b$ (T$^{-1}$) is the volumetric bulk mass transfer coefficient for the bubble surfaces. The bubble oxygen flux is integrated over the water volume. A constant bubble $K_L$ value over depth is assumed, which should be reasonable if plume turbulence does not vary greatly with depth. In addition, the water concentration is assumed to be constant over space in the tank. With these assumptions, Equation (5) simplifies to,

$$\frac{dc}{dt} = \frac{K_L \alpha_b}{h_d} \int (C_0 - C) dz$$

where $h_d$ is the depth from diffuser to water surface, $z$ is the distance from diffuser. In this work, the oxygenation headers are comparatively big in size ($\Phi = 3$ cm, $h = 5$ cm) compared with the electrolytic cell with the volume of 1 L ($12 \times 7 \times 12$ cm$^3$). Therefore, DO was nearly equal in the cell.

Effects of GDE structure on the disinfection

Effects of Pt load $W_{Pt}$

The effect of $W_{Pt}$ on the disinfection is shown in Figure 3a. It can be seen that the disinfection improved with increasing Pt load $W_{Pt}$, but the germicidal efficacy at $W_{Pt}$ of 3‰ was almost equivalent to that at $W_{Pt}$ of 4‰. Pt increased the current efficiency of H$_2$O$_2$ generation (Figure 3b). But the reaction occurred at catalyst/reaction gas/aqueous solution interface, only the Pt located on the surface may become the active site of the electrode reaction. Therefore, further increase of $W_{Pt}$ in GDE did not accelerate the electrode reactions. Some of the following disinfecting experiments were performed using the GDE with $W_{Pt}$ of 3‰ as the cathode, the Pt surface mass load of which $\rho_{Pt}$ was calculated to be 0.16 mg/cm$^2$. 

Figure 2 | Electrolytic cell for investigating the substances or reactions having a leading function in the bactericidal activity against E. coli (1) Stabilized current supply; (2) diaphragm; (3) graphite plate; (4) GDE.
Effect of pore-forming agent content $W_{\text{NH}_4\text{HCO}_3}$

The major functions of gas diffusion layers were distribution of reactants to the active sites of electrode (pore size as well as porosity distribution), management of water supplied or generated. Hence the necessary properties of gas diffusion layers are good gas diffusion properties with suitable water and air permeability, good electronic conductivity, high mechanical integrity and enhanced oxidative stability (Kannan et al. 2006). On one hand, with increasing pore-forming agent content, gas diffusion layers (GDLs) become porous to allow for distribution of the gases to unexposed areas of the flow channel and for complete utilization of the electrode area; on the other hand, poor electrical conductivity of these GDLs could affect the transport of electrons to the current collector from the electrode. The decrease in the average porosity causes the reduction in oxygen consumption so that less H$_2$O$_2$ is generated during the electrolysis.

The effect of pore-forming agent content on the disinfection is shown in Figure 4a. It can be seen that addition of the pore-forming agent in the appropriate amount improved the disinfection. GDEs with a diffusion layer can provide both a satisfactory yield of hydrogen peroxide and a fast reaction rate of reduction (Figure 4b). The germicidal efficacy was lower than 80% after 12 min electrolysis using the pore-forming agent free GDE as the
cathode. Under the same conditions, the germicidal efficacy reached 94.46% using the GDE with W_{NH4HCO3} of 30% in the disinfection. Further addition of the pore-forming agent shortened the disinfection, thus reducing the equipment investment. The germicidal efficacy reached 91.76% after 10 min of electrolysis using the GDE with W_{NH4HCO3} of 70% as the cathode; under the similar conditions it was 89.14% using the GDE with W_{NH4HCO3} of 40%.

Gas adsorption porosity (BET method) has been tested to determine the pore size distribution and the specific surface area of the diffusion layer. The results showed that GDEs have sufficient hydrophobicity and enough porosity. Additionally, the average pore size in the membrane electrode first decreased significantly with the increasing amount of pore-forming agent, and then increased moderately (see Table 1). This is because incorporating a small amount of pore-forming agent caused a formation of some micropores. However, with the increase in number, a portion of the formed micropores merged into pores with larger apertures. This helped the mass transfer of oxygen at the GDE and accordingly increased current efficiency.

**Effect of operating conditions on the disinfection**

**Effect of pH**

The effect of pH on the disinfection is shown in Figure 5a. The drop of pH value resulted in the rapid rise of the germicidal efficacy. It was primarily because the oxygen reduction equilibrium shifted to the positive direction, thus accelerating the formation of H$_2$O$_2$.

The electrochemical and chemical processes occurring at the cathode in the course of the electrolysis can be expressed by Equation (1) and Equation (7) (Tomat 1984):

\[
H_2O_2 + e^- \rightarrow OH^- + OH
\]

H$_2$O$_2$ reduction equilibrium shifted to the positive direction at acidic pH. Additionally, H$_2$O$_2$ was easier to decompose to highly oxidative hydroxyl at acidic pH. The titration results confirmed that the amount of produced H$_2$O$_2$ at weakly acidic pH was smaller than that at slightly alkaline pH (Figure 5b). However, the oxidizing ability of H$_2$O$_2$ was weak under the alkaline conditions, the germicidal efficacy, therefore, was lower than under acidic and neutral conditions.

**Effect of oxygen flow rate $Q_{O_2}$**

The effect of oxygen flow rate on the disinfection is shown in Figure 6a. It can be seen that continuous oxygenation improved the electrochemical disinfection. The dissolved oxygen tended gradually toward saturation after 10 min

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Results of BET superficial area analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDE sample</td>
<td>BET superficial area (m$^2$/g)</td>
</tr>
<tr>
<td>W_{NH4HCO3} = 0</td>
<td>7.9364</td>
</tr>
<tr>
<td>W_{NH4HCO3} = 30%</td>
<td>9.2340</td>
</tr>
<tr>
<td>W_{NH4HCO3} = 40%</td>
<td>11.2275</td>
</tr>
<tr>
<td>W_{NH4HCO3} = 70%</td>
<td>18.3471</td>
</tr>
</tbody>
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![Figure 5](https://iwaponline.com/wst/article-pdf/61/1/107/447801/107.pdf)

**Figure 5** | Effect of pH on (a) germicidal efficacy and (b) H$_2$O$_2$ production, reaction conditions: GDE with W$_{Pt}$ of 3‰ was used as cathode, W$_{Na_2SO_4}$ in the solution: 4%, $Q_{O_2} = 0.5$ L/min, $J = 10$ mA/cm$^2$. |
oxygenation, which might hinder further generation of H₂O₂. The disinfection shortened with increasing oxygen flow rate: the germicidal efficacy reached 62.7% after 8 min electrolysis at oxygen flow rate of 0.25 L/min using the GDE with WPt of 3‰; in the same time the germicidal efficacy was 67% at oxygen flow rate of 0.5 L/min, over 70% at oxygen flow rate of 0.75 L/min, over 75% at oxygen flow rate of 1 L/min and over 80% at oxygen flow rate of 1.25 L/min. The germicidal efficacy at oxygen flow rate of 0.75 L/min was compared with that at oxygen flow rate of 1 L/min and 1.25 L/min, the results showed that the increasing margin remained unchangeable after 4 min electrolysis, indicating that the increase of oxygen flow rate within limits had little influence on the generation of H₂O₂ and the succeeding disinfection (Figure 6). This was the same as reported in the literature (Qiang et al. 2002). When \( Q_{O_2} = 1.25 \text{ L/min} \), the germicidal efficacy moved up rapidly at the beginning of the electrolysis and reached 80.47% after 8 min electrolysis. Oxygen dissolved rapidly in the water, and a large number of oxygen molecules were adsorbed to the surface of GDE and reduced with the formation of H₂O₂, thus accelerating the disinfection. On one hand, high oxygen flow rate caused an increase in solution’s resistance and energy consumption on the disinfection, which gave rise to an increase in the operating cost of the disinfection with the system; on the other hand, treatment time could be reduced reasonably at high oxygen flow rate, which leads to reduction of equipment investment.

**Effect of salt content**

The effect of salt content on the disinfection is shown in Figure 7. It can be seen that the germicidal efficacy showed relatively smooth change along with the increasing salt content and reached >95% after 12 min electrolysis using the GDE with WPt of 3‰ as the cathode. The results accorded with Faraday’s law of electrolysis, the reduced oxygen amount was proportionate to electric quantity flowing past the solution during the electrolysis. An equal amount of H₂O₂ should be generated after the same electricity passed through the solution, the germicidal efficacy, therefore, was approximately the same. However,
the energy consumption reduced significantly with increasing salt content in the solution. The experimental results showed that the energy consumption on the disinfection reduced by ~45%, when salt content increased from 2% to 10%. This system, therefore, is more suitable for disinfecting salt water.

**Effect of current density**

The effect of current density on the disinfection is shown in Figure 8a. It can be seen that the germicidal efficacy increased with current. However, the accelerating rate was different: it first increased with the current, then decreased, and reached a maximum when $J = 6.7 \sim 8.3$ mA/cm$^2$. The reduction of O$_2$ to H$_2$O$_2$ is a two-electron transfer process. However, electrode polarization increases with current density, subsequently, the generated H$_2$O$_2$ is oxidized to H$_2$O, thus reducing H$_2$O$_2$ in amount and current efficiency (Figure 8b). It is feasible to have current controlled at $8.3 \sim 10$ mA/cm$^2$.

**Investigation of the substances or pathways having a leading function in the bactericidal activity against E. coli**

Electrolysis of the model wastewater with *Escherichia coli* contamination was conducted in the diaphragm electrolytic cell to investigate the substances or pathways having a leading function in the bactericidal activity against *E. coli*. As shown in Figure 9, the germicidal efficacy increased both in the anode and cathode compartments. Additionally, the germicidal efficacy in the cathode compartment was about the same as in the anode compartment indicating the contribution of direct oxidation and indirect treatment of *E. coli* by the hydroxyl radical was similar to the oxidative indirect effect of the generated H$_2$O$_2$. Increase of pore-forming agent in amount improved the disinfection appreciably in the cathode compartment; this was because more H$_2$O$_2$ was generated in the solution (Figure 10). This also evidenced the importance of H$_2$O$_2$ in the bactericidal activity against *E. coli*. The analytical results showed that 12 min after electrolysis 7.5301 mg H$_2$O$_2$ was generated in 1 L 4% Na$_2$SO$_4$ solution at the current of 0.1 A using the GDE with W$_{\text{NH}_4\text{HCO}_3}$ of 40% as the cathode; under the similar conditions 8.0620 mg H$_2$O$_2$ was generated using the...
GDE with $W_{\text{NH}_4\text{HCO}_3}$ of 70% as the cathode. 7.1096 mg H$_2$O$_2$ was generated in the same solution when the GDE with WPt of 3‰ was used as the cathode.

Figure 11 shows electron microscopic images obtained after the treatment, embedding and cutting the microorganisms. Fresh *E. coli* cells receiving no disinfection were full with smooth surface appearance. In cell samples after EC disinfection, the cell membranes or walls remain to a certain degree and are only partially disrupted. As discussed above, this dual electrode process could produce intermediate chemical species as free radicals which have high oxidizing potentials. These strong oxidants could attack the cell membrane and wall, bringing in massive cell kills and lyses, as illustrated by the SEM micrographs.

### Comparison of the disinfection methods

The figures above demonstrate that the electrolysis time of 12 min is a proper operation course, so the energy cost for each process at 12 min was calculated according to the following equation:

$$C_e(Kw\cdot h/t) = \frac{U(V) \times I(A) \times t(\text{min})}{60 \times V(\text{mL})}$$

where $U$ is the average voltage until 12 min and somewhere around 4 V, $I$ is the current of 0.3 A, volume $V$ is 900 mL.

Owing to the high price of pure oxygen, to increase the competitiveness of the electrochemical wastewater treatment, GDE could be fed with air. Oxygen consumption was not taken into consideration when calculating the operating cost of the disinfection.

Table 2 shows the comparison of disinfection methods. As shown in the table, this technology is the most expensive in operating cost, further research is required to advance the understanding and reduce the operating cost of this technology. There are factors influencing the operating cost and one of them is the type of powdered active carbon (PAC) used to fabricate the gas diffusion electrode, special conductive carbon may enhance current efficiency significantly. Type of Pt/C catalyst also has an influence on the bactericidal activity, superfine catalyst particle and PAC are not appropriate for fabrication of GDE because addition of ethanol into it may cause spark and disrupt the morphology of the diffusion layer.
CONCLUSIONS

The effects of Pt load WPt and the pore-forming agent content WNH4HCO3 in GDE, operating conditions such as pH value, oxygen flow rate QO2, salt content and current density on the killing of E. coli were investigated, respectively. Additionally, the substances or pathways having a leading function in the bactericidal activity against E. coli were indicated.

The results showed that Escherichia coli can be completely inactivated within 12 min by the system. The disinfection improved with increasing Pt load WPt, but its efficiency at WPt of 3‰ was equivalent to that at WPt of 4‰. Addition of the pore-forming agent in the appropriate amount improved the disinfection while drop of pH value resulted in the rapid rise of the germicidal efficacy. Under the acidic and neutral conditions, the germicidal efficacy was higher than under alkaline conditions.

The disinfection improved and shortened with increasing oxygen flow rate QO2. On one hand, resistance of the solution and energy consumption on the disinfection increased at high oxygen flow rate, which gave rise to an increase in the operating cost of the disinfection with the GDE system. On the other hand, treatment time could be reduced reasonably at high oxygen flow rate, which leads to reduction of equipment investment.

The increase of salt content resulted in reduction of energy consumption on the disinfection, this system, therefore, is more suitable for high salt water. It is feasible to have electric current density controlled at 8.3 ~ 10 mA/cm².

The germicidal efficacy in the anode compartment was about the same as in the cathode compartment indicating that the contribution of direct oxidation and indirect treatment of E. coli by the hydroxyl radical was similar to the oxidative indirect effect of the generated H2O2 at the GDE.

This technology is expensive in operating cost, further research is required to advance the understanding and reduce the operating cost of this technology.

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