Bactericidal Effect of H^+ and OH^- Ions Generated by Water Dissociation Using the Ion Exchange Membrane Electrodialysis System

Makoto KOGURE*, Yasushi KANZAKI and Tatsuo TANAKA

Received June 23, 1997 ; Accepted September 30, 1997

A new electrodialysis system was designed to examine the disinfection effect of H^+ and OH^- ions separately. The electrodialysis system consists of six compartments in which one compartment is partitioned by a pair of same polar membranes (cation-cation or anion-anion ion exchange membranes). The disinfection effects of H^+ and OH^- ions were investigated in detail on *Escherichia coli* and *Saccharomyces cerevisiae*. Bacteria with negative charge were moved by electrophoresis toward the anion exchange membrane with positive charge and adsorbed on the membrane forming a negatively charged layer. A pronounced decrease in the limiting current density of the anion exchange membrane occurred due to the formation of the negatively charged layer. The water dissociation was accelerated by the increase in the true current density due to the adsorption of bacteria which resulted in the reduction in the effective surface area. It was suggested from the finding that the principle factor of the lethal damage to *Escherichia coli* cells was ascribable to the drastic increase in H^+ ions produced by the acceleration of water dissociation.

1 INTRODUCTION

Disinfection of water can be achieved by various chemical and physical processes. Physical processes include heat treatment, ultrasonic wave and electromagnetic radiation such as u. v. and γ -radiation. A typical chemical process available is to inject chlorine directly into water because chlorine has a strong sterilizing power on bacteria. The process is now widely used because it is inexpensive. However, a novel safe disinfection method instead of chlorine has been required after the findings that chlorine reacts with a variety of organic impurities and produces harmful carcinogens such as trihalomethane.¹⁻³⁾

It is well known that the ion exchange membrane electrodialysis system is an excellent industrial process of desalting or concentrating sodium chloride in sea water. It is necessary for the system to operate below the limiting current density over which water molecules dissociate

into H^+ and OH^- ions on the desalting surface of ion exchange membrane.⁴⁻⁶⁾ Authors et al. have proposed to introduce the novel ion exchange membrane electrodialysis system in anticipation of the disinfection effect of H^+ and OH^- ions based on the water dissociation instead of ordinary chlorine process.⁷⁾ Similar disinfection effect has been reported due to the simple electrolysis of sodium chloride containing solution.⁸⁾ It has the potential that the treatment water becomes neutral due to the recombination of H^+ and OH^- ions. The corrosion of electrode material is feared to occur in the ordinary electrolysis. On the other hand, the electrodialysis system is more safe and clean process because of the use of H^+ and OH^- ions generated as the disinfection species instead of chemicals.

In the previous papers,⁹⁻¹³⁾ the electrodialysis system (cation membrane || anion membrane(C||A) system) consisting of five compartments was separated by either anion or cation exchange membrane alternately as shown on the Fig. 1 a-1. The compartments are one for desalting, two for concentrating, and two for electrode. We have reported that the remarkable disinfection effect of the electrodialysis system on *Escherichia coli* cells is attributable to the formation of acidic H^+ and

Laboratory of Analytical Chemistry, Showa College of Pharmaceutical Sciences (3-3165 Higashi-tamagawagakuen, Machida, Tokyo 194, Japan)

Key Words: disinfection of water, *Escherichia coli*, water dissociation, ion exchange membrane

basic OH^- ions and following neutralization. Such H^+ and OH^- ions are produced continuously by the water dissociation on the surface of the anion exchange membrane and the cation exchange membrane, respectively, in the desalting compartment at the current density higher than each limiting current density. Since the neutralization reaction of H^+ ions with OH^- ions is quite rapid, it has been ambiguous so far which of H^+ and/or OH^- ions had the disinfection effect on microorganisms.

In this study, two new electrodialysis systems are designed which can distinguish the disinfection effect of H^+ and OH^- ions on microorganisms separately. The systems consist of six compartments using two cation (C) and three anion (A) exchange membranes or three cation and two anion exchange membranes. The former is referred to as AIIA system and the latter to CIIIC system as shown in Fig. 1(b-1, c-1). The AIIA system consists of two desalting compartments (I, IV), one concentrating compartment (III) and an anion permeable (II) which was separated by a pair of anion exchange membranes on both sides. In this system only H^+ ions generated by the water dissociation remain in the anion permeable compartment after the permeation of OH^- ions (Fig. 1 b-2). Therefore, the disinfection effect of H^+ ions on microorganisms can be examined independently. On the other hand, the CIIIC system constitutes the cation permeable compartment (II) in which two cation exchange membranes are arranged on both sides. The disinfection effect of OH^- ions on microorganisms can be examined in a similar manner to the AIIA system (Fig. 1 c-2). The purpose of this paper is to make it clear the disinfection effect of H^+ and OH^- ions on microorganisms using the AIIA and the CIIIC systems in comparison with the conventional five compartments cell (CIIA system). *Escherichia coli* cells was used to examine the disinfection effect as a model of prokaryotic organisms in which there is no nucleus. On the other hand, it was also examined the disinfection effect for an yeast of *Saccharomyces cerevisiae* which is one of the eukaryotic organisms containing a nucleus and mitochondria and has more rigid and thicker cell wall than that of prokaryotic organisms.

2 EXPERIMENTAL

2.1 Microorganisms

The microorganisms used were *Escherichia coli* K-12 w3110 (*E. coli*), and *Saccharomyces cerevisiae* IFO 0224 (*S. cerevisiae*). *E. coli* cells were cultured aerobically with a rotary shaker at 37 °C for 18 h in a nutrient broth (pH 7.0) containing 0.3 % of meat extract, 1 % of peptone and 0.5 % of sodium chloride. *S. cerevisiae* cells were incubated aerobically without shaking at 28 °C for 30 h in a yeast glucose broth (pH 6.0) containing 0.3% of yeast extract, 0.3 % of maltose extract, 0.5 % of peptone and 1 % of glucose. Each log-phase culture was centrifuged at 5000 rpm for 10 min and washed twice with sterilized water, and resuspended in a sterilized 0.01 M ($M = \text{mol} \cdot \text{dm}^{-3}$) sodium chloride solution. A final cell concentration of each suspension (sample solution) was 10^7 - 10^8 cells $\cdot \text{cm}^{-3}$.

2.2 Ion exchange membranes

The membranes used were commercially available strong base anion exchange membrane (Selenium[®]AMV) with $-\text{N}^+(\text{CH}_3)_3\text{Cl}^-$ ionic groups and strong acid cation exchange membrane (Selenium[®]CMV) with $-\text{SO}_3^-\text{Na}^+$ ionic groups (Asahi Glass Co., Ltd., Jpn.).

2.3 Electrodialysis apparatus

To investigate the disinfection effects of H^+ and OH^- ions separately on microorganisms in detail, two types of electrodialysis cell were employed in this study according to the ideas mentioned above and are shown in Fig. 1(b-1, c-1). In contrast with the conventional five compartments cell (Fig. 1 a-1), significant difference of the six compartments cell is that it constitutes a compartment which is separated by same ion exchange membranes on both sides. In the AIIA system water dissociation to H^+ and OH^- ions takes place on the surface of A_2 in the anion permeable compartment (II) due to the concentration difference. The limiting current density is roughly proportional to the salt concentration. Therefore the water dissociation does not occur on the desalting surface of the C_2 membrane in the desalting compartment (IV) because the salt concentration of the compartment IV was maintained 100-fold higher than that of the compartment II. Excess OH^- ions generated by

the water dissociation are transported into the concentrating compartment(III) by electrophoresis and diffusion and accumulated there, whereas H^+ ions are accumulated in the compartment II which cannot pass through either A_2 or A_1 . It is possible in the AIIA system to examine the disinfection effects of the residual H^+ ions in the compartment II and of OH^- ions passed through A_2 and accumulated in the compartment III. On the other hand, the disinfection effects of the remaining OH^- ions in the compartment II and of the H^+ ions in the compartment III can be examined in the CIIIC system in a similar manner to the AIIA system.

Cation and anion exchange membranes were arranged parallel to each other as the diaphragms between each compartment in the present systems. The effective area of the membranes was 4 cm^2 and the distance between them was 1.7 cm. Aqueous 0.1 M sodium chloride solution was passed through the electrode (Pt) compartments

located at each end of the electro dialysis cell from the lower inlet to the upper outlet at a flow rate of $6\text{ cm}^3\cdot\text{min}^{-1}$. Desalting compartments I and IV were filled with 8 cm^3 of aqueous 1 M sodium chloride solution. *E. coli* or *S. cerevisiae* cells suspended in aqueous 0.01 M sodium chloride solution (sample solution) was put in either compartment II or III as follows. When compartment II was filled with the sample solution (8 cm^3) to examine the disinfection effect of H^+ or OH^- ions, compartment III was filled with 8 cm^3 of the control solution (0.01 M sodium chloride solution in the absence of microorganisms). On the contrary, compartment II was filled with the control solution when the compartment III was filled with 8 cm^3 of the sample solution.

After setting up all apparatus, the electro dialysis was carried out by keeping the current at each desired density for 20 min. The current density used was $0.25 - 8.75\text{ mA}\cdot\text{cm}^{-2}$.

Fig. 1 Schematic diagrams of CIIA system (a-1) with the conventional electro dialysis cell consisting of five compartments, and of AIIA system (b-1) and CIIIC system (c-1) with the electro dialysis cell consisting of six compartments. The effective area of the membrane used was 4 cm^2 . The distance between the membranes was 1.7 cm. Cell concentration of the sample solution was $10^7 - 10^8\text{ cells}\cdot\text{cm}^{-3}$.

C_1 , C_2 and C_3 , Cation exchange membrane; A_1 , A_2 and A_3 , Anion exchange membrane; I, IV, Desalting compartment (1 M NaCl); II, Ions permeable compartment; III, Concentrating compartment; V, VII, Concentrating compartment (0.1 M NaCl); VI, Desalting compartment; E, Electrode compartment (0.1 M NaCl, flow rate of $6\text{ cm}^3\cdot\text{min}^{-1}$); A, Ammeter; V, Volt meter; +, Anode; -, Cathode

The pH values of the solution in compartments II and III were measured to know the effects of microorganisms on true current density (see §3.2) before and after each electrodialysis with a glass pH-electrode (TOA Electronics Co., Ltd., Jpn.). It is necessary to know the current efficiency (η_H or η_{OH}) for H^+ or OH^- production in order to evaluate the effect of water dissociation. η_H or η_{OH} was calculated from the amount of H^+ produced (pH change) in compartment III and the quantity of electricity according to the Faraday's law. All experiments were performed at room temperature unless otherwise stated.

2.4 Method of counting

Serial 10 fold dilution with 0.1 M phosphate buffer (pH=7.4) was used for viable counts for the cell suspension. Different dilutions containing *E. coli* cells (0.05 cm^3) were dropped out to the nutrient broth plus 1.5 % agar. Dilutions containing *S. cerevisiae* cells were dropped out to the yeast glucose broth plus 1.5 % agar. Colonies which appeared on the coagulated agar plates after incubation for 24 h at 37°C (*E. coli*) and for 48 h at 28°C (*S. cerevisiae*) were counted to obtain the viable cell number. The survival ratios (%) in each solution were estimated from the ratio of viable counts in the solution treated with the electrodialysis to that in the untreated solution.

3 RESULTS AND DISCUSSION

3.1 Determination of limiting current density

It is necessary to know the limiting current density of ion exchange membrane for evaluating water dissociation to H^+ and OH^- ions in an electrodialysis system. An example for determining the limiting current densities of cation and anion exchange membranes is shown in Fig. 2 where the limiting current was determined from the inflection point (arrow) of the current vs. the voltage curves¹⁴⁾ for the salt concentration used in the present study. The observed dependence of the limiting current density on the sodium chloride concentration is shown in Fig. 3. The limiting current density of cation exchange and anion exchange membranes was $0.57 \text{ mA}\cdot\text{cm}^{-2}$ and $0.87 \text{ mA}\cdot\text{cm}^{-2}$ in an aqueous 0.01 M sodium chloride solution, respectively.

Fig. 2 Example of current voltage curves of cation and anion exchange membranes in aqueous 0.01 M sodium chloride solution. The effective area of membranes (C_2 , A_2) was reduced to 0.286 cm^2 by a gasket in order to generate more easily water splitting on the membrane rather than the other membranes used in the apparatus as shown in Fig. 1. The limiting current density was given as the current value of the inflection point divided by the effective area of the membrane. ○, cation exchange membrane (Selemion®CMV); △, anion exchange membrane (Selemion®AMV)

Fig. 3 Relationship between limiting current density of ion exchange membranes and concentration of sodium chloride solution. ○, Selemion®CMV; △, Selemion®AMV

Table 1 Survival ratios of *E. coli* cells and pH changes after 20 min electro dialysis at various current densities on the AllA system and the ClIC system (new electro dialysis cell with six compartments).

Current density (mA·cm ⁻²)	AllA system				ClIC system			
	compartment III		compartment II		compartment III		compartment II	
	Survival ratio (%)	pH	Survival ratio (%)	pH	Survival ratio (%)	pH	Survival ratio (%)	pH
0.25	100	6.47	88.1	6.60	100	6.02	95.8	6.46
0.88	100	6.41	70.5	4.10	100	5.26	94.1	6.87
1.75	100	6.62	2.31	2.46	100	4.94	84.8	7.03
3.50	100	6.62	0.02	2.16	91.2	4.69	74.3	6.81
5.25	100	6.57	0	1.72	92.4	4.62	79.3	6.80
8.75	80.8	6.61	0	1.58	89.7	4.29	22.3	9.97

3.2 Effect of the current density on the survival ratio of *Escherichia coli*

Table 1 shows survival ratios of *E. coli* cells in the salt solution for the compartments II and III and pH values at the outlet of the compartments observed at various current densities either in the AllA or the ClIC system. Disinfection effect was scarcely observed in the compartments II and III of the ClIC system even at high current density. In the AllA system, however, the disinfection effect in the compartment II was significantly depending upon the current density. A remarkable disinfection effect on *E. coli* cells occurred at the current density higher than 3.5 mA·cm⁻². The decrease in pH became significant corresponding to the increase in the current density. Complete disinfection was observed at the current density higher than 5.25 mA·cm⁻², which was six times as high as the limiting current density.

It is necessary to know the current efficiency for H⁺ or OH⁻ production for evaluating the effect of water dissociation on the disinfection of *E. coli* cells. Current efficiencies for H⁺ and OH⁻ production in the compartment II of the ClIC and the AllA systems, η_H (ClIC system) and η_{OH} (AllA system), were calculated from the pH change in the compartment III before and after each electro dialysis. Figure 4 shows plots of η_H or η_{OH} vs. current density. The increase in η_{OH} was more significant in the presence of *E. coli* cells than that in the absence of *E. coli* cells. The former was more than 100 times as large as the latter at the current density higher than 1.75 mA·cm⁻². On the other hand, η_H showed no significant difference. These results suggest that the apparent limiting current density of the anion exchange membrane

(A₂) in the compartment II of AllA system decreased in the presence of *E. coli* cells. Grossman et al.¹⁵⁾ showed that a significant decrease of limiting current density could be caused by membrane fouling (poisoning) due to the sorption of impurities with positive or negative charges on the surface of cation or anion exchange membrane, respectively. *E. coli* cells with negative charge were transported by the electrophoresis toward the positive A₂ membrane and adsorbed on the desalting surface forming a negatively charged layer. The formation of the layer resulted the reduction in the effective surface area of the A₂ membrane and a pronounced decrease of the limiting current density. The water dissociation must be accelerated on the surface of

Fig. 4 Current density vs. η_H or η_{OH} plots. ○, η_H ; △, η_{OH} : *E. coli* cells; ●, η_H ; ▲, η_{OH} : Control (without *E. coli*)

the A_2 membrane because of the significant increase in the true current density due to the synergistic effect of the formed layer. The decrease in the survival ratio of *E. coli* cells can be attributed to the increase in the resultant H^+ ions concentration in the compartment II because of the acceleration of water dissociation in which η_{OH} was significantly increased.

It is known that the effect of pH on the survival ratio of *E. coli* cells becomes significant in the region of pH below 2 or above 12.⁹⁾ Thus the simple pH effect was examined on the influence of the survival ratio of *E. coli* cells apart from the electrodialysis. Figure 5 shows survival ratios of *E. coli* cells against the exposure time in various pH solutions. The survival ratio decreased with increasing the exposure time and was zero after 2 and 5 min at pH 1.05 and pH 1.54, respectively. The result obtained by the electrodialysis treatment was consistent well with the present chemical treatment, i.e. the lethal damage to *E. coli* cells was attributable to the increase in the concentration of H^+ ions in either treatment.

For comparison the disinfection experiment on *E. coli* cells was performed in the desalting compartment (VI) of the conventional five compartments cell (CIIA system) as shown in Fig. 1(a-1). Figure 6 shows the result. It was found that the decrease in the survival ratio and the decrease in pH against the current density corresponded well similar to that of the compartment II in the six compartments cell (AIIA system). The disinfection effect of the CIIA system on *E. coli* cells can be attributed to the increase in the concentration of H^+ ions in a similar manner to the AIIA system. The disinfection effect was weak in comparison with that of the AIIA system because the pH values of the compartment VI was in the region of pH 2 - 3 due to the influence of OH^- ions generated on the surface of the C_2 membrane in contrast to below pH 2 of the compartment II. The result is in good agreement with the data shown in Fig. 5.

3.3 Effect of the current density on the survival ratio of *Saccharomyces cerevisiae*

It is interesting that the electrodialysis system may be effective for the disinfection of fungi. *S. cerevisiae* which is one of the typical yeast may

have a considerable resistance to the increase in the concentration of H^+ and OH^- ions because it has more rigid and thicker cell wall than that of *E. coli*. The disinfection effect on *S. cerevisiae* cells was examined in the CIIA and AIIA systems and

Fig. 5 Survival ratios of *E. coli* cells treated during 20 min at various pH values. pH of tested solutions was adjusted by adding 0.1 M HCl aqueous solution to a sterilized 0.01 M NaCl solution. *E. coli* cells was then added to the solution prepared at a desired pH. pH : ○, 1.05; △, 1.54; □, 2.08; ●, 2.58; ▲, 3.13

Fig. 6 Survival ratio of *E. coli* cells and pH change after 20 min electrodialysis at various current densities on the CIIA system (conventional electrodialysis cell with five compartments).

Table 2 Survival ratios of *S. cerevisiae* cells and pH changes after 20 min electro dialysis at various current densities on the AllA, ClIC and ClIA systems.

CD	AllA system				ClIC system				ClIA system	
	compartment III		compartment II		compartment III		compartment II		compartment VI	
	SR	pH	SR	pH	SR	pH	SR	pH	SR	pH
0.25	99.2	5.56	99.0	5.56	100	6.05	98.6	5.90	99.3	6.30
0.88	98.6	5.66	99.7	5.35	100	5.85	99.3	5.94	99.2	3.29
1.75	99.5	5.91	100	3.23	99.4	4.90	99.7	6.36	98.4	3.15
3.50	99.5	6.14	97.1	2.24	99.4	4.42	99.7	6.42	92.5	2.83
5.25	99.5	6.04	86.8	1.97	97.2	4.62	99.4	6.44	92.1	3.14
8.75	99.5	6.32	77.6	1.64	97.9	4.29	96.6	6.89	----	----

CD: Current density / mA·cm⁻² SR: Survival ratio / %

for comparison the ClIA system. Table 2 shows survival ratios of *S. cerevisiae* cells and pH changes of compartments II, III and VI at various current densities. No significant disinfection effect was observed in the ClIA system and the both compartments of the ClIC system over every current densities examined. In the AllA system, the decrease in pH in the compartment II was significant in comparison with that of the ClIA system although only 22 % of the cells was disinfected at 8.75 mA·cm⁻² (pH=1.64), which was ten times as high as the limiting current density. Simple pH effect on the survival ratio of *S. cerevisiae* cells was examined in a similar manner to that of *E. coli* cells. The effect of bulk pH was almost negligible in the pH region 2 to 11. Only 40 % of the cells was disinfected even at pH=1 as shown in Fig. 7.

To examine the effect of water dissociation, the current efficiency of H⁺ (η_H) and OH⁻ (η_{OH}) was examined in advance. Figure 8 shows η_H and η_{OH} against current density in the ClIC and in the AllA systems when *S. cerevisiae* cells were added to the compartment II. η_H change was irrespective of the presence of *S. cerevisiae* cells in a similar manner to that of *E. coli* cells (see Fig. 4). On the other hand, the increase in η_{OH} was more significant in the presence of *S. cerevisiae* cells at the current density of 1.75 mA·cm⁻² though the change was small in comparison with that of *E. coli* cells. These results suggest that the increase in the concentration of H⁺ and OH⁻ ions generated by the water dissociation were independent of the disinfection effect on *S. cerevisiae* cells which had the considerable resistance for the acidic and basic conditions.

Fig. 7 Survival ratio of *S. cerevisiae* cells treated at various pH values for 20 min.

Fig. 8 Current density vs. η_H or η_{OH} plots.
○, η_H ; △, η_{OH} : *S. cerevisiae* cells

4 CONCLUSIONS

The new electrodialysis systems, AllA and ClC systems, were designed in which the disinfection effect of H^+ and OH^- ions generated by water dissociation can be examined separately. The current efficiency of H^+ production, η_H was similar irrespective of the presence of *E. coli* or *S. cerevisiae* cells over every current densities examined in the ClC system. On the other hand, the increase of η_{OH} was significant in the presence of *E. coli* or *S. cerevisiae* cells in the AllA system at the current density higher than the limiting current density. The decrease in the survival ratio of *E. coli* and *S. cerevisiae* cells was scarcely observed in the ClC system. In the AllA system the decrease in the survival ratio of *E. coli* cells was significant and pH values decreased correspondingly. No disinfection effect was observed for *S. cerevisiae* cells even in the ClA or AllA system. The authors propose the following mechanism for the disinfection effect of electrodialysis system: *E. coli* and *S. cerevisiae* cells with negative charge were transferred by electrophoresis toward an anion exchange membrane with positive charge and adsorbed on a desalting surface forming a negatively charged layer. The formation of the layer resulted the reduction of effective surface area of the anion exchange membrane and a pronounced decrease in the apparent limiting current density. The water dissociation was accelerated on such membrane surface by the significant increase in the true current density. The decrease in the survival ratio of *E. coli* cells can be attributed to the increase in the H^+ ions concentration due to the acceleration

of water dissociation. No significant disinfection effect of the H^+ ions was observed for *S. cerevisiae* cells which can form a spore.

References

- 1) J. J. Rook, *Water Treat. Exam.*, **23**, 234 (1974).
- 2) J.T. Ling, *J. Am. Water Works Assoc.*, **68**, 659 (1976).
- 3) M. Schalekamp, *J. Am. Water Works Assoc.*, **71**, 638 (1979).
- 4) A. M. Peers, *Discuss. Faraday Soc.*, **21**, 124 (1956).
- 5) R. Simons, *Electrochim. Acta*, **29**, 151 (1984).
- 6) Y. Tanaka and M. Seno, *J. Chem. Soc., Faraday Trans. 1*, **82**, 2065 (1986).
- 7) T. Tanaka, *Eisei Kagaku*, **16**, 105 (1970).
- 8) Y. Yamagishi, M. Watanabe and T. Katsunuma, *J. Antibact. Antifug. Agents.*, **16**, 323 (1988).
- 9) T. Sato, T. Tanaka and T. Suzuki, *Denki Kagaku*, **52**, 239 (1984).
- 10) T. Sato, T. Tanaka and T. Suzuki, *Denki Kagaku*, **53**, 706 (1985).
- 11) T. Sato, T. Tanaka and T. Suzuki, *Denki Kagaku*, **54**, 696 (1986).
- 12) T. Sato, T. Tanaka and T. Suzuki, *Denki Kagaku*, **56**, 100 (1988).
- 13) T. Sato, T. Tanaka and H. Ohya, *Bioelectrochem. Bioenerg.*, **21**, 47 (1989).
- 14) N. W. Rosenberg and C.E. Tirrell, *Ind. Eng. Chem.*, **49**, 780 (1957).
- 15) G. Grossman and A. A. Sonin, *Desalination*, **12**, 107 (1973).