Inactivation of Chlorine-Resistant Microorganisms by a Pulsed Discharge in Water

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A pulsed discharge in water was applied to inactivate *Cryptosporidium parvum* (*C.parvum*) in filter backwash water from a water purification plant. Effects of water quality and discharge conditions on the degree of inactivation were investigated by batch experiments. A high inactivation ratio (> 99%) was obtained under conditions of low turbidity and high total energy. In 10L backwash water of turbidity 40, 98% of oocysts were inactivated by applying a total energy of 17520 J for 10L (1752 J/L). Experimental results suggest that UV emission is mainly effective for inactivation.

Keywords : pulse, discharge, inactivation, *Cryptosporidium*, UV, water treatment

1. Introduction

A pulsed discharge in water is known to simultaneously cause an intense shockwave, UV emission and the generation of oxidizing species⁽¹⁾⁽²⁾. Therefore, it has been studied for various industrial uses, such as decomposition of chemicals⁽²⁾⁻⁽⁴⁾ and disinfection to eliminate microorganisms⁽¹⁾⁽⁵⁾⁻⁽⁷⁾. In particular, disinfection by pulsed discharges has many advantages over other disinfection procedures because it needs no chemicals, energy requirements is low and disinfection can be completed without raising the water temperature⁽¹⁾.

C.parvum is one of the aquatic infective microorganisms that cause severe diarrhea. In the past decade, large outbreaks of *C.parvum* were reported in Milwaukee (USA) in 1993 and Ogose (Japan) in 1996. More than 400,000 patients, including over 100 dead in Milwaukee and more than 8,000 patients in Ogose were reported. Because *C. parvum* is resistant to chlorine, it cannot be inactivated by chlorine disinfection, which is the standard procedure of water purification.

In water purification plants, it is becoming important to reuse filter backwash in view of effective water use. Since purified water is used for backwashing, it is a waste of a water resource to throw away backwash water without reuse. However, backwash water contains many suspended solids on which *C.parvum* adheres. Therefore, some inactivation method that is effective in high-turbidity water is required before reuse in order to ensure the safety of water purification.

Disinfection by means of a pulsed discharge in water have been studied since 1960's⁽¹⁾⁽⁵⁾⁻⁽⁷⁾, but most of experiments have been conducted in clean water and no studies have been done for inactivation of *C.parvum*. In this study, we applied a pulsed discharge in water to inactivate *C.parvum* in filter backwash water and investigated the influences of water quality and discharge conditions on inactivation.

2. Experiments

2.1 Reagents and Preparation Filter backwash water used in experiments was sampled from a water purification plant and turbidity was adjusted from 2 to 55 by decantation or centrifugation before use. Model suspension was prepared by adding sodium chloride (Kanto Chemical) and kaolin (Kanto Chemical) to distilled water (Wako Chemical) for adjusting the conductivity to 0.2 mS/cm and the turbidity to 55, respectively. Severe Combined Immunodeficiency (SCID) mice (C.B-17/Icr-scid Jcl, 5-week-old females) were purchased from CLEA Japan, Inc. *C.parvum* oocysts (HNJ-1 strain, isolated from a nonimmunosuppressed diarrheal Japanese patient and has been maintained with SCID mice) were supplied from Osaka City University. Other chemicals used were of reagent grade.

2.2 Experimental Apparatus Figure 1 shows a schematic diagram of the experimental apparatus with a 1L vessel. This stainless steel vessel is equipped with one rod electrode (diameter 6 mm, stainless steel) on its lid through a polyethylene insulator and another one (diameter 6 mm, stainless steel) welded on its bottom. The inner space of the vessel is designed to be an ellipsoidal column (long axis 13.9 cm, short axis 12.1 cm, height 9.3 cm and approximate inner volume 1230 cm³). The electrodes

Fig. 1. Schematic diagram of experimental apparatus using 1L vessel

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are positioned at one focus of the ellipsoid, and the gap length between electrodes can be adjusted by shifting the upper electrode. In this work, the gap length was adjusted to 2 mm before each experiment started.

Pulsed power supply was composed of a high voltage supply (CCS08040P1000C, General Atomics Energy Products), capacitors (5 μ F, Model 30594, General Atomics Energy Products), a controller (Kyusyu Keisokki Co., Ltd.) and an original air-gap switch with a spark plug triggered by the controller. Voltage and current were measured on a digital oscilloscope (LC-374A, LeCroy Corporation) using a voltage divider (SK-301, IWATSU ELECTRIC Co., Ltd.) and a Rogowski coil (CM-1-L, Ion Physics Corporation). Voltage was variable, but 5.4 kV was chosen in all experiments on the basis of the results of preliminary discharge tests. Although frequency was also variable, 0.2 Hz was adopted to maintain a sufficient mixing time of suspension between each discharge. The number of capacitors was varied from 1 to 15 to change the pulsed energy. The high voltage line from the power supply was connected to the electrode on the lid, and the ground line was connected to the vessel itself.

Figure 2 shows a schematic diagram of a 10L vessel. This stainless steel vessel is designed to have a cylindrical inner space (diameter 20 cm, height 35.5 cm and inner volume 11150 cm³), resembling a part of a water pipe. Two electrodes (diameter 6mm, steel) are both attached on the sidewall of the vessel through insulators. A stirring rod is also furnished through a hole in the lid. Power supply and other components are the same as shown in Figure 1.

2.3 Analysis Turbidity was measured using TY-4L (Nihon Kouden Kenkyusyo K.K.) and conductivity was measured using D-24 (Horiba, Ltd.). UV transmission of water (200-600 nm) was measured using a UV-visible spectrometer (UV mini 1240, Shimadzu Corporation) with a 1 cm long quartz cell. UV intensity in a range of 200 nm to 400 nm was measured by PMA-11 (Hamamatsu Photonics K.K.) through a quartz basis (OFSQP50C10-10, Sigma Koki Co., Ltd.) using a UV reflection filter of 1% transmission (FNDU20C02-1, Sigma Koki Co., Ltd.). A microscope (E600, Nikon) was used to observe the morphology and to count the number of oocysts. The pressure waveforms in water were observed by using a poly-vinylidene fluoride (PVDF) film sensor (DST1-028K, TOKYO SENSOR Co., Ltd) with its surface covered by self-fusion insulating tape (Scotch 130C, SUMITOMO 3M Limited) to reduce electrical noise on signals caused by a discharge.

Fig. 2. Schematic diagram of 10L vessel

2.4 Experimental Procedure Oocysts were added to the sample water in the vessel to make the oocyst concentration at 5×10^6 /L. Before each experiment started, 0.1L of suspension (containing 5×10^5 oocysts) was sampled as untreated oocysts (control) and then discharges were applied under appropriate conditions.

After each experiment finished, 0.1L of suspension was sampled as treated oocysts. To evaluate the viability of oocysts, each sample was concentrated by centrifugation (1700g, 10min) and resuspended in 0.5mL of sterile water and 10^5 oocysts were administered by gastric gavage to three SCID mice each. As control, two groups of three mice each were administered 10^5 and 10^3 viable oocysts, respectively. After administration, viable oocysts started to proliferate in the intestines of a SCID mouse and the number of oocysts in a SCID mouse increased with housed days. The number of excreted oocysts in feces also increased with housed days and housed days required for 10^5 oocysts excretion were correlative with the number of viable oocysts administered. Therefore, we counted the number of excreted oocysts in feces everyday for 1 month using the qualitative sugar flotation method and a microscope, and calculated the number of viable oocysts administered to each mouse. The inactivation ratio of treated oocysts was calculated by Eq. (1). N_T is the total number of oocysts administered ($=10^5$ oocysts for each sample) and N_V is the number of viable oocysts calculated from infection tests. For estimation or discussion on each experiment, the average value of three mice was used.

$$\text{Inactivation ratio} = 100 (N_T - N_V) / N_T \quad (\%) \quad (1)$$

The influence of suspension composition was evaluated from the results for backwash water compared with those for model suspension. Pulsed energy was varied from 73 to 1094 J/pulse by changing the number of capacitors. The number of discharges was also varied to investigate the effect of total energy per unit volume. The influence of the treated volume was evaluated by comparing the results for the 1L vessel with those for the 10L vessel.

3. Results

Figure 3 shows typical voltage and current waveforms of a discharge. Although the conductivity of backwash water ranged from 0.23 to 0.42 mS/cm depending on the season or the weather,

Fig. 3. Typical V-I waveforms of a discharge (5.4 kV, 292 J/pulse, turbidity 40)

waveforms were almost the same in each experiment.

The effects of total energy on the inactivation ratio were investigated using a 1L vessel by changing the number of discharges of 292 J/pulse. Figure 4 shows the inactivation ratios at various total energies. The inactivation ratio is about 75% at 3 discharges (876 J/L, equal to 0.24 kWh/m³), but increases as total energy increases and exceeds 99% at 6 discharges (1752 J/L, 0.49 kWh/m³). Although the inactivation ratio by 24 discharges (7008 J/L, 1.95 kWh/m³) was supposed to be much higher than 99%, we

Fig. 4. Effects of total energy on inactivation ratio (5.4 kV, 292 J/pulse, turbidity 40)

Fig. 5. Effects of turbidity on inactivation ratio (5.4 kV, 292 J/pulse, 3 discharges for 1L)

Fig. 6. Effects of suspension composition on inactivation ratio (5.4 kV, 73 J/pulse, 12 discharges for 1L)

could not estimate the accurate value because of the limitation of infection tests.

Figure 5 shows effects of turbidity on inactivation at the same total energy of 867 J/L. Inactivation ratio exceeds 99% for both turbidity 2 and 10. Although it is supposed to be higher for turbidity 2 because two of three mice did not become sick for turbidity 2 though all three mice became sick for turbidity 10 in 1 month infection tests, we also could not estimate because of the limitation of the tests. For turbidity 40, the inactivation ratio decreases to 76%, indicating that the inactivation ratio decreases as turbidity increase.

Figure 6 shows the effects of the suspension composition on the inactivation ratio. The result for model suspension of turbidity 55 is almost the same (>99%) as that for model suspension of turbidity 0, but is higher than that for backwash water (93%) of same turbidity. These results indicate that inactivation is affected by the suspension composition as well as turbidity.

Figure 7 shows the inactivation ratios for the 10L vessel and the 1L vessel at the same total energy per unit volume (1752 J/L). 6 discharges for 1L and 60 discharges for 10L were conducted at the same pulsed energy condition. The inactivation ratio for the 10L vessel reaches 98%, slightly lower than that for the 1L vessel (>99%), indicating that increasing treated volume reduces the energy efficiency slightly.

Figure 8 shows the inactivation ratio for the 10L vessel under various pulsed energy conditions but the same total energy per unit volume (438 J/L). 4 discharges of 1094 J/pulse result in an

Fig. 7. Effects of treated volume on inactivation ratio (5.4 kV, 292 J/pulse, 1752 J/L, turbidity 40)

Fig. 8. Effects of pulsed energy on inactivation ratio (5.4 kV, 438 J/L, turbidity 40)

extremely lower inactivation ratio than 15 discharges of 292 J/pulse.

4. Discussion

Disinfection to eliminate microorganisms in water by means of a pulsed discharge has been studied in many papers⁽¹⁾⁽⁵⁾⁻⁽⁷⁾. In previous papers, different factors, such as high electric fields, shockwaves, UV emission and oxidizing species, have been reported to be effective for disinfection, depending on the discharge conditions or apparatuses used.

In our experiments, arc discharges occur in the 2mm gap between two electrodes, and almost all currents, except leakage current before the discharge, flow only through the gap. This means that the electric field is highest (about 25 kV/cm) within the gap and is very low (<2 kV/cm) in a large area of treated water, even in a 1L vessel. Therefore, the effect of electric fields, which causes cell wall destruction, is very small. Considering lifetimes and diffusion velocity, the effect of oxidizing species, generated directly by current, is also small.

As shown in Figure 9, no morphological changes of oocysts were observed before and after discharges. This fact indicates that inactivation of oocysts is not caused by external damage but internal damage.

Since both of shockwaves and UV emission were able to cause internal damage, we observed pressure waveforms in water by a pressure sensor to estimate the damping of shockwaves. Figure 10(A) shows the waveform of voltage and 10(B) shows typical pressure waveforms in water of turbidity 10 and 40 at a distance of 10cm from the discharge point. Since the propagation velocity of pressure waves in water is about 1500 m/sec, equal to that of sound waves, it is expected to take 0.073 msec for the first pressure wave to arrive at the sensor after a discharge occurs. In Figure 10(B), some electrical noise by a discharge is observed from 0 to 0.073 msec, but much larger sensor output, caused by pressure waves, is observed after 0.073 msec. Figure 10(B) also shows that pressure waveforms are almost same even in water of different turbidity. Considering about the large effects of turbidity on inactivation ratio in Figure 5 and that shockwaves are kinds of pressure waves, we suppose that shockwaves are not mainly effective for inactivation in our experiments. However, further experiments will be needed to confirm this supposition because the damping characteristic varies with a frequency.

As discussed above, factors other than UV emission are unlikely to be mainly effective for inactivation in our experiments. Figure 11 shows intensity curve of UV emitted by a discharge in tap water measured by PMA-11 at a distance of about 11cm from discharge point, through 10 cm water and a 1 cm thick quartz window of 10 L vessel. Wavelengths of emitted UV are ranged widely from 230 nm to 400 nm. Intensity increases gradually from 230 nm to 260 nm, but keeps almost constant over 260 nm. To estimate the reduction of UV in water, we also measured UV transmission of water by UV-visible spectrometer with a 1 cm long quartz cell. Figure 12 shows UV transmission of backwash water at various turbidities. It shows that UV transmission decreases with turbidity increases, and transmission of 260 nm at turbidity 40 is only about 60% of that at turbidity 10. Figure 13 shows UV transmission curves in suspensions of various compositions at the same turbidity. It is obvious that UV transmission is different from each other. Since turbidity is an index of visible ray transmission, the relationship between

turbidity and UV transmission differs with suspension composition, specifically with the qualities of suspended solids and dissolved substances. In dilute solutions, the reduction of the monochromatic light caused by absorption obeys the Lambert-Beer law (Eq. (2)). Here I_0 is the intensity of incident

$$-\log(I/I_0) = acI \quad (2)$$

light, I is the intensity of transmitted light, a is constant of proportion, c is the concentration of solute and I is transmitting

Fig. 9. Morphology of oocysts before and after discharges (5.4 kV, 292 J/pulse, 15 discharges; before (left); after (right))

Fig. 10. Effects of turbidity on pressure waveform in water (A: Voltage waveform; B: PVDF output at a distance of 10cm from the discharge point. Numbers in a graph are turbidity. A broken line indicates 0.073 msec after a discharge occurred.)

Fig. 11. Intensity curve of UV emitted by a discharge (5.4 kV, 292 J/pulse, 1 pulse, in tap water)

distance. However, a more complex equation is needed to express the reduction of UV in backwash water because the effects of reflection and scattering by suspended solids should be concerned.

Assuming that UV emission is mainly effective for inactivation, the effects of turbidity and suspension composition on inactivation ratio can be explained as follows. In Figure 5, a close relationship between turbidity and inactivation ratio is observed because turbidity has a close relationship with UV transmission as shown in Figure 12. In Figure 6, since the relationship between turbidity and UV transmission differs with suspension composition as shown in Figure 13, the inactivation ratios at the same turbidity are different from each other.

We then discuss about the effects of treated volume, the number of discharges and pulsed energy on energy efficiency for inactivation. The energy efficiency is determined by the ratio of the amount of UV irradiated to each oocyst to the induced energy. The amount of UV irradiated to each oocyst is determined by various factors, UV intensity emitted per 1 discharge and water quality, for example. At the same discharge condition and water quality, a volume irradiated by UV is considered to be same because the intensity of UV and the reduction of UV in water are almost same. Therefore, the ratio of irradiated volume to treated volume decreases with treated volume increases. This suggests that the reduction of energy efficiency may occur by the shortage of mixing even at the same total energy condition. The number of discharges is also considered to affect on the energy efficiency because it has a close relation to the electrode erosion. The

electrode erosion may cause not only the increase of dissolved substances, causing the reduction of UV transmission, but also the variation of UV intensity by varying the discharge condition, specifically a gap length. In addition, the variation of discharge condition may be fed back to the erosion rate of the electrode. As for the pulsed energy, it is supposed to affect on both of the amount of UV emitted per 1 discharge and the electrode erosion.

Considering about the discussion above, the slight reduction of inactivation ratio in 10 L vessel in Figure 7 might be caused by the shortage of mixing or by the variation of the discharge condition related to the number of discharges. Similarly, the extreme reduction of inactivation ratio in Figure 8 might be caused by the variation of the discharge condition caused by the immediate electrode erosion or by the shortage of the number of discharges.

Although inactivation of *C. parvum* has been reported to occur with relatively low UV doses⁽⁸⁾, further experiments are needed to confirm the mechanism of inactivation, including the effect of shockwaves and the secondary generation of oxidizing species by intense UV. To investigate the effects of discharge condition is also important for the energy efficiency of this treatment.

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References

- (1) M. Allen and K. Soike : "Sterilization by Electrohydraulic Treatment", *Science*, Vol.154, pp.155-157 (1966-10)
- (2) A. C. Elmore and W. A. Lee : "Pilot Study Evaluation of Pulsed Plasma Arc Ground-Water Treatment", *Practice Periodical of Hazardous Toxic and Radioactive Waste Management*, Vol.3, No.4, pp.178-182 (1999)
- (3) P. Sunka, V. Babickey, M. Clupek, P. Lukes, M. Simek, J. Schmidt, and M. Cernak : "Generation of chemically active species by electrical discharges in water", *Plasma Sources Science Technology*, Vol.8, pp.258-265 (1999)
- (4) A. K. Sharma, B. R. Locke, P. Arce, and W. C. Finney : "A Preliminary study of Pulsed Streamer Corona Discharge of Phenol in Aqueous Solutions", *Hazardous Waste & Hazardous Materials*, Vol.10, No.2, pp.209-219 (1993)
- (5) S. E. Gilliland, and M. L. Speck : "Inactivation of Microorganisms by Electrohydraulic Shock", *Appl. Microbiology*, Vol.15, No.5, pp.1031-1037 (1967)
- (6) E. J. M. van Heesch, A. J. M. Pemen, P. A. H. J. Huijbrechts, P. C. T. van der Laan, K. J. Ptasinski, G. J. Zanstra, and P. de Jong : "A Fast Pulsed Power Source Applied to Treatment of Conducting Liquids and Air", *IEEE Trans. Plasma Sci.*, Vol.28, No.1, pp.137-143 (2000)
- (7) A. M. Anpilov, E. M. Barkhudarov, N. Christofi, V. A. Kop'ev, I. A. Kossyi, M. I. Taktakishvili, and Y. Zadiraka : "Pulsed high voltage electric discharge disinfection of microbially contaminated liquids", *Appl. Microbiology Lett.*, Vol.35, pp.90-94 (2002)
- (8) T. Hirata, S. Morita, and A. Hashimoto : "Water Treatment and Disinfection Technologies against Cryptosporidium Pollution", *J. Water*, Vol.44, No.4, pp.32-40 (2002)

Fig. 12. UV transmission curves in backwash water of various turbidities (numbers in a graph are turbidity)

Fig. 13. UV transmission curves in model suspension and in backwash water at turbidity 40

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