KKU Res. J. 2014; 19(Supplement Issue): 81-88
http : //resjournal.kku.ac.th

Potential application of pressurized carbon dioxide for agricultural irrigation water disinfection

Huy Thanh Vo[1], *Tsuyoshi Imai*[1,*], *Tuan Van Le*[1], *Kanthima Phummala*[1], *Takaya Higuchi*[1], *Ariyo Kanno*[2], *Koichi Yamamoto*[1] *and Masahiko Sekine*[2]

[1] *Division of Environmental Science and Engineering, Yamaguchi University, 2-16-1 Tokiwadai, Yamaguchi 755-8611, Japan*

[2] *Division of Civil and Environmental Engineering, Yamaguchi University, 2-16-1 Tokiwadai, Yamaguchi 755-8611, Japan*

**Correspondent author: imai@yamaguchi-u.ac.jp*

Abstract

Irrigation water and recycled water used for farm gardens can be a potential source of contamination of microbial pathogens that cause harmful illness. This study investigated the use of pressurized carbon dioxide to inhibit pathogens in water sources. An apparatus producing microbubbles was operated with pressure up to 0.7 MPa, room temperature and a common period for disinfection, 25 minutes. Target environmental water samples, including distilled water, artificial ground water and effluent wastewater, were subjected to microbial contamination with desired concentrations of *Escherichia coli* (ATCC 11303, ATCC 23631 and ATCC 13706) and bacteriophages. Under identical conditions, approxi-mately 4.0 – 5.0 log of *E. coli* were inactivated in water samples, whereas the reduction ratio of bacteriophages are nearly 3.0 – 4.0 log. The chemical nature of CO_2 molecule (acidification, diffusivity and solubility in water) was indicated to be the main factors causing the microorganism deaths. Besides that, high pressure, depressurization rate, characteristics of microbubbles and pumping cycle contributed to microorganism inhibition. These findings in this investigation may be considered to use carbon dioxide as a novel disinfectant to water treatment in agricultural irrigation. Moreover, carbon dioxide treatment produces no disinfection by-products and excessive pressure after disinfection can be an advantage to enhance irrigating to plants.

Keywords: irrigation water, carbon dioxide, inactivation effect, water disinfection, microbubbles.

1. Introduction

Water resources used for various targets in agricultural irrigation require preliminary treatment to be safe to use. Water disinfection is an important treatment to control the microorganism growth in the irrigation water system and minimize the diseases related to the waterborne pathogens, e.g., bacteria, viruses, fungi, cysts…. Agricultural reuse of wastewater becomes the potential irrigation water in the big cities and urban. The water resources from secondary treatment contain the residual viruses and pathogens, which can persist to varying degrees after release to the environment (11). Hence, the effluent waste-water is required special care before irrigating the crops for direct human consumption (14). Especially, agricultural food crops, such as vegetable fields (barley, avocado,

cabbage, lettuce, strawberry…), orchards and vineyards, nurseries (flowers)…are required secondary treatment and disinfection for irrigated water (1). Irrigation water can be disinfected using non-chemical methods (heat, Ultraviolet radiation and filtration), or chemical methods (chlorine, chlorobromide, ozone, chlorine dioxide…). UV disinfection is effective and environmentally friendly treatment. However, this requires the water to be free of suspended particles and UV-absorbing substances which exist abundantly in agricultural irrigation water. Chlorination is the most widely used disinfectant in water treatment. Recently, many potential problems have arising due to the reaction of residual chlorine with natural organic matter (NOM) in water causing health effect in humans. Whereas ozone, chlorine dioxide and hydrogen peroxide react with water contaminants are transferred to a series of free-radicals to oxygen as the end reaction product. These reactions cause harmful to the plant and reduce its growth rate. Using high pressure carbon dioxide (CO_2) to inhibit the microorganism growth is considered as a novel disinfectant for water treatment without forming disinfection by-products (DBPs).

The sterilizing technique by high pressure CO_2 has been successfully implemented in the preservation of food and concluded to be effective for inactivation of variety pathogens (2, 3, 5, 6, 8, 10, 13). A recent study by Kobayashi et al. (2009) involved to apply high pressure carbon dioxide for water disinfection. His group found that Escherichia coli (*E. coli*) were inactivated up to 6 log at the pressure of 2 MPa around 40°C after 60 minutes. Our preliminary investigation indicated that CO_2 microbubbles at 0.7 MPa significantly inhibited *E. coli* cells in distilled water to approximately 5.0 log reduction (12). However, no attempt was investigated to inactivate various microorganisms and environmental waters by high pressure CO_2.

In order to assess inactivation effect of CO_2 in many different microorganisms and in environmental water resource, the experiments in this study were run using three kinds of *E. coli* (ATCC 11303, ATCC 23631, ATCC 13706) and three kinds of bacteriophages (T4, Qβ, ΦX174) and tested water samples includes effluent wastewater, artificial ground water and distilled water. By using different water samples, this study aims to apply pressurized carbon dioxide for garden irrigation water disinfection with small scale.

2. Materials and Methods

2.1. Preparation of microorganisms

Three kinds of *E. coli* cells and bacteriophages were used as target pathogens for disinfection. Escherichia coli ATCC 11303, ATCC 23631 and ATCC 13706 from stock cultures (American Type Culture Collection, Manassas, VA, USA) were respectively propagated in flasks containing 100 ml Luria-Bertani (LB) broth media (Wako Chemical Co. Ltd., Osaka, Japan) and incubated at 37 °C with continuous shaking for 16-18 h at 150 rpm. Whereas, bacteriophage suspensions were prepared from T4 (ATCC 11303-B4™), Qβ (ATCC 23631-B1™) and ΦX 174 (ATCC 13706-B1™) and grown to high titers by overnight incubation at 37 °C in *E. coli* hosts ATCC 11303, 23631 & 13706, respectively. The remaining cells and cell debris were eliminated by centrifugation at 2,000 × g for 10 min. The supernatant, including the phage, was then filtered through a membrane filter with a pore size of 0.20 mm (Millipore, Carrigtwohill, County Cork, Ireland). Cells and virus suspensions with initial concentrations of 10^7–10^9 PFU/mL were stored in 20% glycerol. For storage, samples were initially refrigerated at -20 °C for 24 h, and then reduced to -80ฐC to prevent temperature shock.

2.2. Microbial enumerated tests

2.2.1. Bacteria enumeration

The cell concentration was determined by spreading aliquots on LB agar plates (Wako), incubating the samples overnight at 37 °C, and then determining the number of colony-forming units (CFU) from plates containing 25–300

colonies. The initial concentration was estimated to be approximately 10^7-10^9 CFU/mL. For each experiment, 100 mL of *E. coli* stock inoculated LB was incubated at 37°C and 150 rpm for 12–18 h.

2.2.2. Bacteriophage titer

Surviving infectious T4, Qβ andΦX174 were enumerated by forming lawns of sensitive strains of *E. coli* hosts and then conducting plaque phage assays using double layers of agar on the plates. Initially, 0.1 ml phage suspension was mixed with 0.2 ml *E. coli* host culture and incubated at 37°C (50 rpm) for 30 min. This mixture was then blended directly in a test tube containing 5 ml of top layer of liquefied Tryptic Soy Agar (TSA) 0.7% [wt/vol] (Wako) and poured rapidly onto a Petri dish containing TSA at 1.5% [wt/vol]. Plaque-Forming Units (PFU) was determined after overnight incubation at 37 °C based on plates containing 30–300 PFU.

2.3. Preparation of water samples

Microorganism suspensions and distilled water, artificial ground water and effluent wastewater before disinfection (Ube wastewater treatment plant, Yamaguchi, Japan) were intermingled to attain the desired concentration at room temperature as the wastewater samples. The artificial groundwater was made from $CaCl_2$ 0.125mM; $MgCl_2$ 0.05mM; KCl 0.103 mM; $NaHCO_3$ 1.5 mM (Wako) and autoclaved in 15 min at 121°C before using (15). Whereas, the components of effluent wastewater were pH (7.1), COD (9.6 mg/L), BOD (6.2 mg/L), SS (4.0 mg/L), N (17.9 mg/L), P (1.24 mg/L) (Ube city environment department).

2.4. Apparatus and procedure for disinfection

The disinfection device was tested based on the high contacted efficacy between CO_2 and water (Fig. 1). Highly dissolved CO_2 in water was distributed thoroughly inside due to high pressure and pump cycle. Influent water pumped with high speed (13-15 l/min) knocks to the shield inside strongly and suddenly. A lot of microbubbles are formed and dissolved with carbon dioxide. Initial temperature was remained unchanged from 20-25 °C.

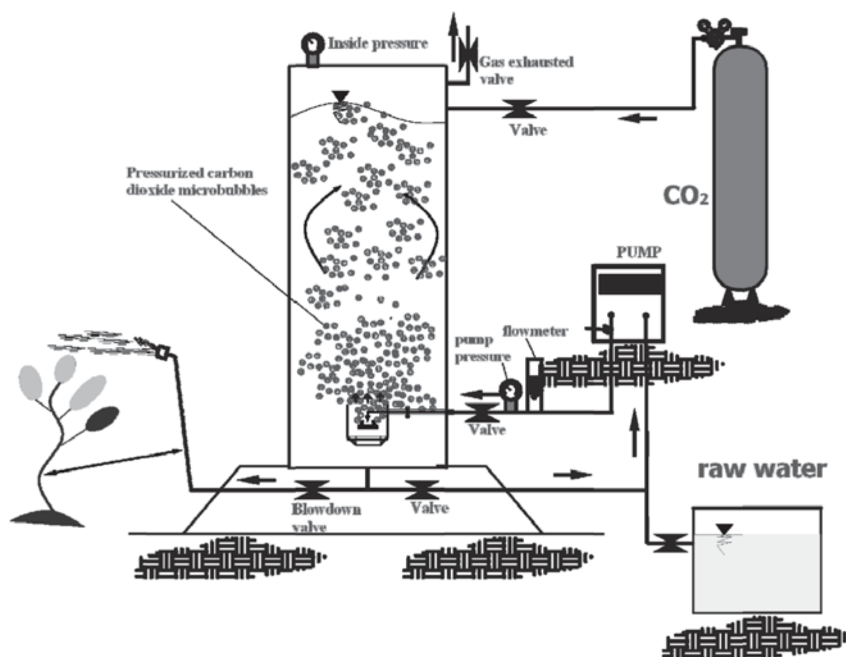

Figure 1. Schematic of experimental apparatus.

At the beginning, 7000 mL of wastewater contaminated microorganisms was pumped into and operated during treatment time, 25 minutes at flow rate of 13-15 l/min. The working pressure indicated from optimum condition from previous study (12) was 0.7 MPa. Blowdown valve was used to take the samples.

Inactivation effect was assessed via the inactivation results at various microorganisms and environmental water resources.

2.5. Inactivation rate

The calculation of inactivation rate was based on slope of the linear relationship between log (N/N_0) and time t, where N and N_0 are the final and initial plate count numbers per milliliter (PFU/mL) and t represents time in minute.

3. Results and Discussion

3.1. Inactivation effect to different bacteria.

The first set of analysis examined the impact of carbon dioxide disinfection to variety bacteria. Reduction ratios of all three kinds of *E. coli* over the time change similarly. After 20 minutes of inactivation, *E. coli* ATCC 11303 was inhibited a nearly 4.2 log reduction, while *E. coli* ATCC 23631 and 13706 were inactivated for 3.9 log and 3.8 log, respectively (Fig. 2). Interestingly, inactivation effect reached the same reduction ratio for all bacteria, approximately 4.5 log after 25 min.

As showed on Fig. 2, inactivation rates increased slightly in the first 15 minute (2.0-2.5 log/15 min), but then grew significantly after that. This agrees with the earlier result by Vo et al. (2013) that CO_2 treatment with 20 min at 25°C, *E. coli* was completely inactivated with the initial concentration of around 10^5-10^6 CFU/mL. In general, inactivation rate of pressurized carbon dioxide against *E. coli* follows the first-order kinetics and was indicated to be 0.18 log /min (R^2>0.945). This finding has important implications for predicting the inactivation process of *E. coli* by CO_{-2} in water. CO_2 microbubbles under high pressure were considered to be effective to diffuse and disintegrate *E. coli* cells. They permeate through cell wall membranes, disorder cell components and exceed intracellular pH change (5, 6, 8).

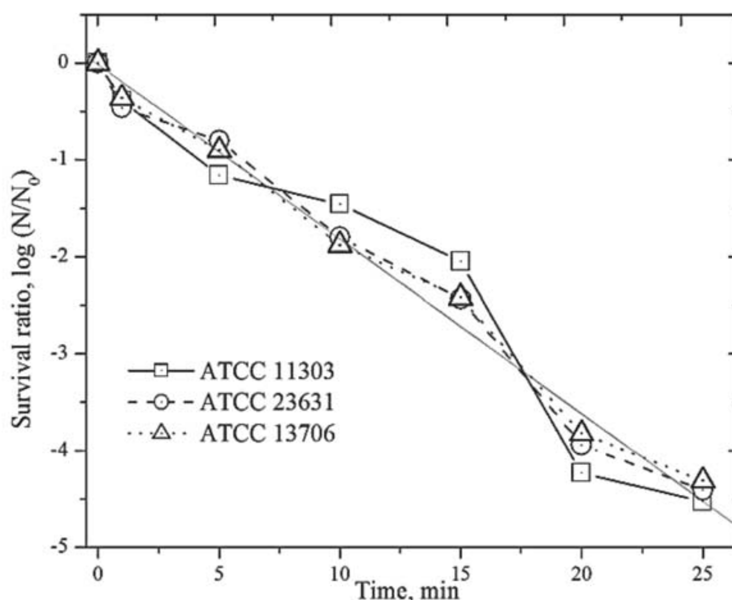

Figure 2. Inactivation effect of pressurized CO_2 (0.7 MPa) against different bacteria. Environmental waters were distilled water. Initial concentration: 10^7-10^9 CFU/mL. Room temperature (22 °C).

3.2. Inactivation effect to different bacteriophages.

In another experiment, bacteriophages were used as virus indicators. Phage T4 (double stranded DNA) and ΦX174 (single stranded DNA) representative for DNA viruses, phage Qβ (single stranded RNA) is as RNA virus. Under the same conditions of *E. coli* inactivation tests, approximately 4.0 log of phage T4 was inhibited by CO_2 treatment, whereas the reduction ratios of phage Qβ and ΦX174 were nearly 3.4 log and 2.9 log, respectively. During the treatment time by CO_2, pH decreased to approximately 4.0 from the first minute for all experiment. Demonstrated on Fig. 3, during the first 15 minutes the inactivation rates to all phages are familiar. However, after that the inactivation rates are different. The reduction rate of phage T4 increased significantly, 0.16 log/min (R^2>0.999), while the inactivation rate of phage Qβ increased slightly, 0.13 log/min (R^2>0.995) and the survival ratio of phage ΦX174 was highest with the only decrease of 0.11 log/min (R^2>0.96).

The high inhibition of phage T4 was indicated to be sensitive to pressurized CO_2 microbubbles. One possibility is that the large size of phage T4, 90 nm wide and 200 nm long, linked by a long tail and head is easy to be broken under pressurized CO_2 molecules (9). Phage Qβ and ΦX174 has much smaller sizes, only 25–30 nm that will be difficult for CO_2 microbubbles to diffuse effectively as phage T4 shapes. Phage Qβ was found that it survived better in an alkaline environment than in the water containing a lot of hydrogen ions. In this study, phage Qβ was inhibited more effectively than phage ΦX174, this agrees with the previous investigation (4). Inactivation mechanism of pressurized CO_2 against bacteriophages is similar to one of *E. coli* cells. Molecular CO_2 with high pressure can also penetrate through protein coat of coliphage. Once accumulated excessively, they will change the order loss of the lipid chains and destruct the domains. In addition, a strongly decrease intracellular pH denaturing DNA and RNA characteristics leads to the inhibition of coliphage.

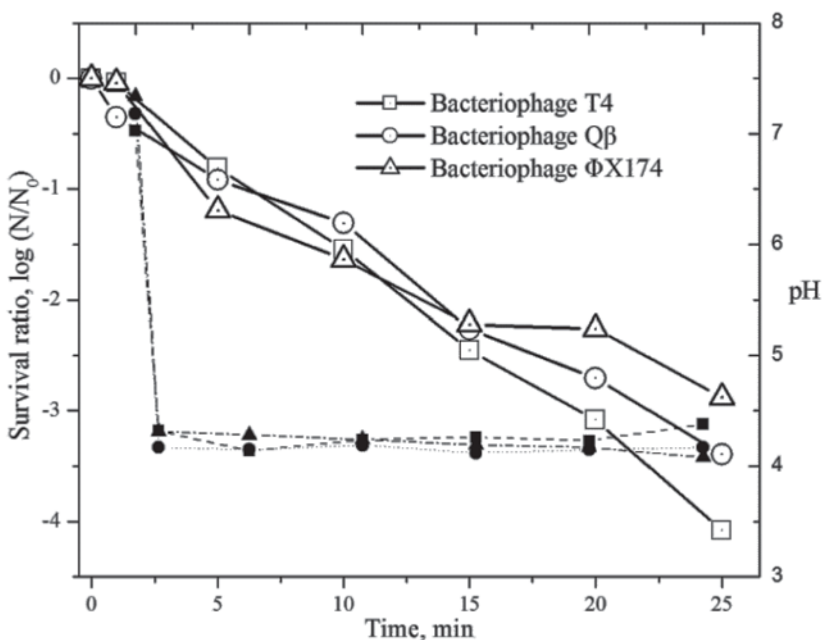

Figure 3. Inactivation effect of pressurized CO_2 (0.7 MPa) against different bacteriophages. Wastewater were distilled water contaminated by bacteriophages. Initial concentration: 10^7-10^9 PFU/mL. Room temperature (22 °C). Dotted lines illustrate pH change over the time: (■) T4, (●) Qβ, (▲) ΦX174.

3.3. The influence of environmental to inactivation effect.

The environmental water samples contaminated by *E. coli* were compared in order to assess inactivation effect of CO-$_2$. The results obtained from the preliminary disinfection of the wastewater made by distilled water, the artificial groundwater and the real effluent wastewater are presented in Fig 4. The reduction ratio of *E. coli* in the effluent wastewater only 3.5 log. And this reduction is also lower over the time than others. Whereas, both distilled water and the artificial groundwater had the similarly high inactivation ratios, approximately 4.5 log (Fig. 4). Compared to pH change in water on Fig. 3, the pH change of three samples in this case had a slight difference. One possibility is that buffering capacity of chemical components in the artificial groundwater and the effluent wastewater are higher. pH after the first-minute treatment reached nearly 5.0, while pH of the distilled water attained around 4.0.

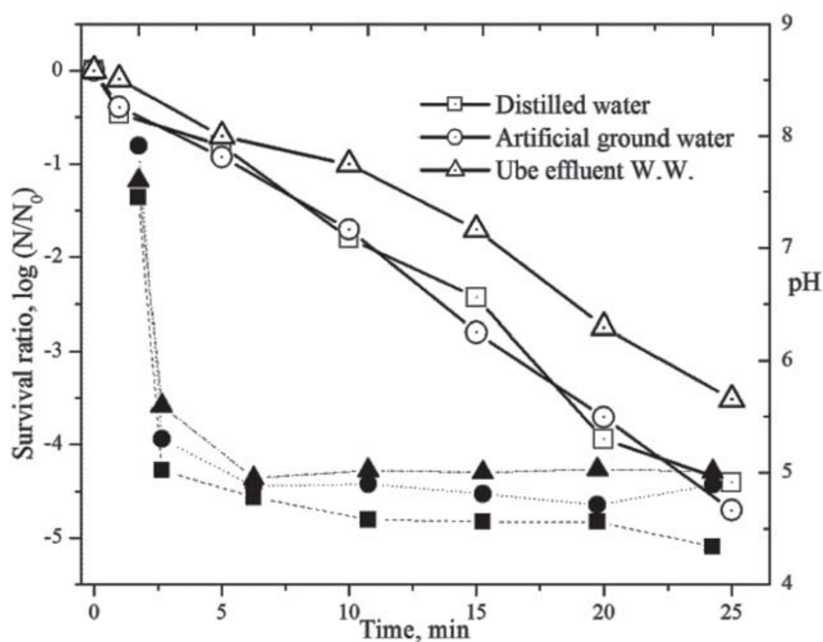

Figure 4. Inactivation effect of pressurized CO_2 (0.7 MPa) against *E. coli* ATCC 11303 in different environment waters. Initial concentration: 10^7-10^9 CFU/mL. Room temperature (22°C). Dotted lines illustrate pH change over the time: (■) Distilled water, (●) Artificial ground water, (▲) Ube effluent wastewater.

The suspended solids (SS=4.0 mg/L) in the effluent wastewater as the particles of turbidity provide shelter for *E. coli* cells and reduce their exposure to CO_2 microbubbles. For this reason, the inactivation rate against *E. coli* in the effluent wastewater is 0.14 log reduction/min (R^2>0.990), slower than in the distilled water (0.18 log/min, R^2>0.992) and the artificial groundwater (0.184 log/min, R^2>0.998). SS factor may explain the relative good correlation between the effectiveness of disinfection process and water quality. However, the environmental samples in this study were artificially contaminated to desired high microbial concentration. In the real conditions it may be more effective to inactivate complete pathogens. This finding, while preliminary, suggests that the inactivation effect of pressurized CO_2 reaches the higher rate in the raw water with lower turbidity.

Depressurization rate after discharging treated water also concerns to cell deaths (3). The change of

pressure as shear force makes physiological characteristics adapt suddenly and breaks cell walls and viral coat proteins. Moreover, long exposure time with continuous pumping cycle (25 min) causes to microorganism inhibition.

4. Conclusion

The present study was designed to determine the inactivation effect of pressurized CO_2 microbubbles against pathogen indicators. Under identical pressure condition (0.7 MPa) and around room temperature (22°C), approximately 4.5 log of *E. coli* cells and nearly 3.0–4.0 log of bacteriophages (T4, Qβ,ΦX174) were inhibited by CO_2 microbubbles. The evidence from this study suggests that the irrigating water quality with low turbidity has higher inactivation effect. Moreover, the excessive pressure after treatment remains high and a good condition to utilize for irrigating to plants at far distance. This research will serve as a base for future studies and potential application of pressurized CO_2 for the agricultural irrigating water and wastewater disinfection. However, with a small scale and the batch model, caution must be applied, as the further inactivation effect has not deeply investigated to continuous model.

5. References

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