

Enhancement of sunlight irradiation for wastewater disinfection by mixing with seawater

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ABSTRACT

There is a need for developing a simple and easy-to-maintain disinfection technique for sewage treatment for use in developing countries and disaster-affected areas. We propose a novel disinfection technology that inactivates bacteria in wastewater via sunlight irradiation under high salt concentration by mixing with seawater. The disinfection efficiency of the proposed method was quantitatively evaluated and examined using fecal indicator bacteria. When the salinity in wastewater was adjusted to 30 practical salinity units by mixing with seawater, the constant of inactivation irradiation energy K_s (m^2/MJ) was 1.6–2.2-fold greater than that without seawater for total coliforms and *Escherichia coli*. By contrast, although enterococci were inactivated by sunlight irradiation, an increase in salinity did not enhance disinfection. On setting the irradiation energy of sunlight to $5.5 MJ/m^2$, >99% of the fecal indicator bacteria were inactivated. Finally, we examined the relationship between the attenuation of irradiance and water depth and accordingly proposed a design of a treatment system wherein wastewater and seawater were adequately mixed and passed via a disinfection tank under the natural flow with sunlight irradiation.

Key words: disinfection tank, enterococci, *Escherichia coli*, inactivation irradiation energy, salinity

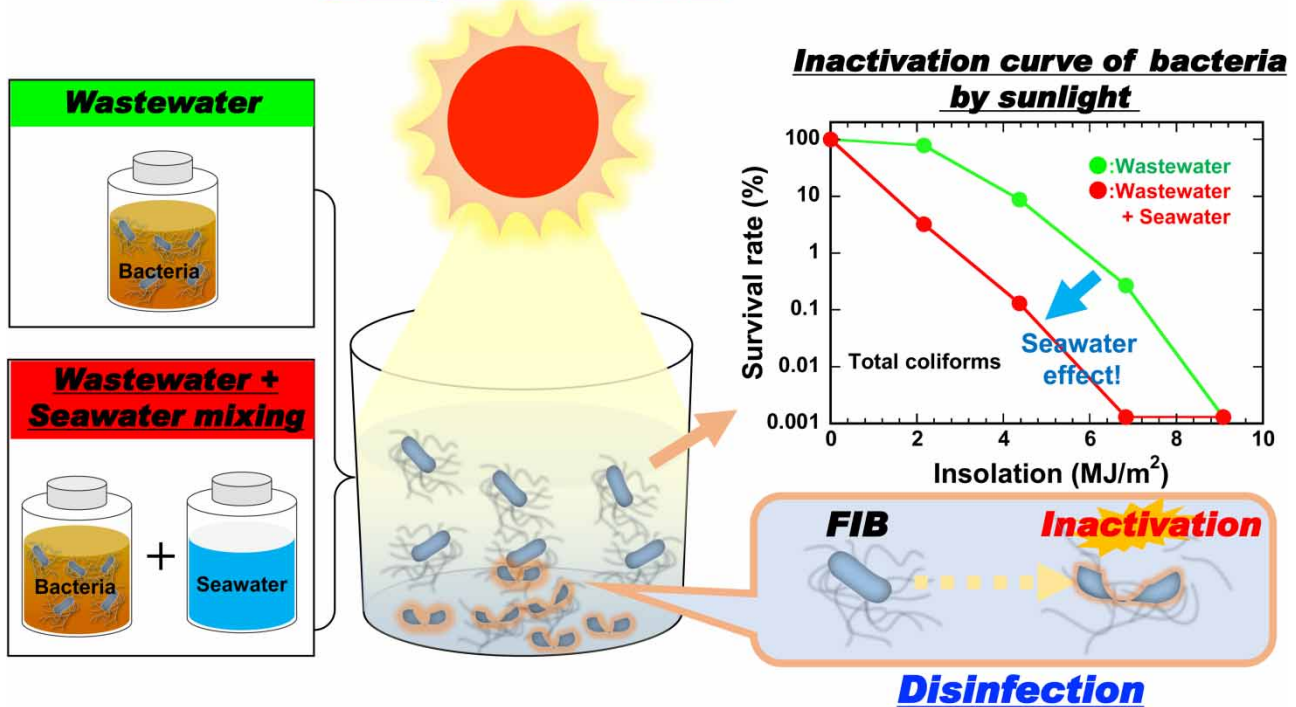
HIGHLIGHTS

- Quantitative evaluation of the appropriate salinity in wastewater mixed with seawater.
- Inactivation of fecal indicator bacteria by sunlight with seawater mixing.
- Disinfection process based on irradiance and the water depth.
- Proposed disinfection is extremely simple and environmentally friendly.

GRAPHICAL ABSTRACT

Disinfection Process through Sunlight Irradiation with Seawater Mixing

Sunlight Irradiation



INTRODUCTION

The wastewater treatment system is an important urban infrastructure that improves public health and protects water environment by preventing the discharge of pollutants from domestic and industrial sewage into it. Wastewater treatment systems are becoming common in developed countries, with approximately 70% of the sewage being treated in high-income countries (1). By contrast, in developing countries, because of budget and power supply limitations, the sewer system is merely installed and not fully operational. In low-income countries in particular, only 8% of the sewage is treated, i.e., >90% is discharged without treatment (Sato *et al.* 2013). In middle- and low-income countries, 842,000 people die annually because of contaminated drinking water and inadequate sanitation (World Health Organization 2014).

Even in developed countries that have sophisticated wastewater treatment systems, the loss of treatment function in the event of a disaster poses substantial environmental and human health risks. For example, after the Great East Japan Earthquake that occurred on 11 March 2011, 120 sewage treatment facilities along the Pacific Ocean were damaged and 48 of these were completely shut down (Study Committee on Countermeasures in Sewerage against Earthquake and Tsunami 2012; Satoh 2017). Thereafter, a significant amount of untreated domestic sewage was released into the environment. The largest wastewater treatment plant damaged by the earthquake and tsunami, the Minami-Gamo Wastewater Treatment Plant, needed 5 years for reconstruction (Kato *et al.* 2017). As an emergency measure immediately following the earthquake, domestic wastewater was treated by primary sedimentation and disinfection with a high concentration of chlorine to meet the national discharge standards of biochemical oxygen demand of <160 mg/L (<120 mg/L on average) and total coliform count of <3,000/mL (Study Committee on Countermeasures in Sewerage against Earthquake and Tsunami 2012; Satoh 2017).

While chlorine disinfection can reduce microbial load in wastewater relatively easily, it could not be performed in some areas because chlorine supply was interrupted immediately after the earthquake.

Therefore, there is a considerable need to develop a simple and easy-to-maintain disinfection method that can be applied in developing countries and as an alternative to chlorination in the event of a disaster. Such disinfection methods should meet the following requirements: (1) have a high inactivation rate for the target microorganisms; (2) are easy and economical to use; and (3) require no chemicals or electric power. However, presently, such a wastewater disinfection technology is still under investigation.

As an alternative disinfection method that meets the above-mentioned requirements, we evaluated sunlight irradiation involving contact with seawater. Sunlight irradiation can inactivate microorganisms in several wastewater types (Davies & Evison 1991; Davies-Colley *et al.* 1999; Sinton *et al.* 1999; Rozen & Belkin 2001; Sinton *et al.* 2002; Noble *et al.* 2004; Kadir & Nelson 2014; Dahl *et al.* 2017). Furthermore, the high salt concentration in seawater leads to pronounced osmotic stress for bacteria. The number of bacteria in coastal waters has been reported to decrease with an increase in salt concentration (Munro *et al.* 1989; Troussellier *et al.* 1998; Sinton *et al.* 2002). Moreover, it has been documented that sunlight affects the survival of fecal bacteria in seawater (Fujioka *et al.* 1981). Based on these findings, it is expected that the inactivation of microbes would be enhanced when seawater is mixed with the wastewater and irradiated with sunlight (Davies-Colley *et al.* 1994; Sinton *et al.* 1994, 1999, 2002).

The appropriate salinity of wastewater mixed with seawater under different levels of sunlight irradiation, however, has not been quantitatively evaluated, which is necessary to design the disinfection process. Hence, in the present study, we assessed the inactivation efficiency of sunlight for the fecal indicator bacteria, such as total coliforms, *Escherichia coli*, and enterococci, in the secondary effluent collected from municipal wastewater treatment plant in Japan under various sunlight irradiation conditions and mixing ratios of treated wastewater and seawater. Using the subsequent results, we designed a disinfection process on the basis of the relationship between the attenuation of irradiance and water depth.

MATERIALS AND METHODS

Sampling of treated wastewater and quality water analyses

Samples collected from an actual wastewater treatment plant were used for disinfection experiments. This plant has a separate treatment system that supports an area with a population of 10,000 (average treated water volume: 4,200 m³/day), and the plant employs an oxidation ditch as a biological treatment process. In general, the treated water is chlorinated after solid-liquid separation in the final settling tank and subsequently discharged into the river. For the study, overflow water was collected from the final settling tank in the latter phase of the biological treatment process, and this undisinfected secondary treated wastewater was used for the disinfection experiments. This secondary treated wastewater was sent to the laboratory immediately after sampling, and experiments were initiated within 2 h of receiving the samples. The quality parameters of raw water, including pH and electrical conductivity (EC), were determined by a pH/water quality analyzer (LAQUA F-74, 9615-10D; HORIBA, Ltd). Moreover, turbidity (kaolin standard unit) was measured using a turbidity meter (SEP-PT-706D; Mitsubishi Chemical Co.).

Artificial seawater

For disinfection, artificial seawater was used for bioassay (United States Environmental Protection Agency 2002a), which was set to have a salinity of 60 practical salinity units (PSUs, PSU is regarded as ‰ parts per thousand) assuming dilution with freshwater. The prepared artificial seawater was filtered via a membrane filter (a 47-mm diameter, 0.45- μ m pore, mixed cellulose ester membrane filter; Advantec Co.) and subsequently treated by high-pressure steam sterilization (121 °C, 15 min).

Sunlight irradiation experiment

An ultra-small memory illuminometer (MDS-MKV/L; diameter: 18 mm; total length: 93 mm; light-receiving part diameter: 22 mm; JFE Advantech Co.) was fixed vertically at the bottom of a circular transparent polystyrene container (inner diameter: 238 mm; depth: 117 mm). After filling 5 L of the water sample in the container (water depth: 112 mm), it was exposed to sunlight. The sunlight-exposed water sample in the container was mixed every hour, after which the water was sampled. The sunlight irradiated on the sample was measured as photosynthetic photon flux density (μ mol/m²/s) by using the memory illuminometer in water for continuous monitoring at an interval of 1 s during the experimental period. As a control test to compare with the disinfection effect of sunlight irradiation, a similar experiment was performed under the dark condition.

The entire water container was covered with an aluminum foil box to block the sunlight, and the experiment was performed in a clear weather.

The exposure time of the sunlight irradiation experiments without seawater mixing was set to 5 h. The temperature of the test water during the experiments was measured every hour of sampling, and the average value was considered. Temperature during the experiments was obtained from the database of the Japan Meteorological Agency (<https://www.data.jma.go.jp/obd/stats/etrn/index.php>) (accessed date: 2 February 2016). Sunlight irradiation experiments without seawater mixing were repeated thrice using each treated wastewater collected on different days.

For sunlight irradiation experiments with seawater mixing, 2.5 L of sterilized artificial seawater (0, 20, and 60 PSUs) was adjusted with sterilized distilled water mixed with 2.5 L of treated wastewater so as to make the total amount to 5 L. The final salinity of each water sample was adjusted to 0, 10, and 30 PSUs, respectively. The exposure time of the solar irradiation experiment by mixing seawater was set to 3–4 h, and for the control test, the same experiment was performed under the dark condition. Sunlight irradiation experiments with seawater mixing were also repeated thrice using each treated wastewater collected on different days.

Enumeration of bacteria

The total coliform and *E. coli* counts in the water samples were measured using the Colilert-18 kit (IDEXX Laboratories) as per manufacturer's instructions. The Colilert system calculates the most probable number (MPN) based on the number of fluorescing wells (*E. coli*-positive wells) on Quanti-Tray. The upper and lower detection limits in the Colilert-18 kit were set to 2,419.6 and 1 MPN/100 mL, respectively.

Enterococci in the water samples were enumerated using the membrane filtration method (United States Environmental Protection Agency 2002b) on mEI agar. In brief, water samples (100 mL) were filtered via a membrane filter (Advantec Co.) and placed on mEI agar. After incubation at 41 °C for 24 h, colonies showing blue halos were counted as enterococci; the counts were reported as the mean colony-forming units (CFUs) of three plates. The detection limit of enterococci in the water was set to 0.3 CFUs/100 mL.

Evaluation of the disinfection effect via integrated irradiance

The continuously monitored photosynthetic photon flux density was converted to irradiance ($W/m^2 = J/s/m^2$) using the conversion coefficient given by Thimijan & Heins (1983) and the integrated irradiance was obtained with the irradiation time, i.e., the irradiation energy S (MJ/m^2). The disinfection effect by sunlight irradiation was then evaluated from the irradiation energy and the percent survival rate of each indicator bacterium ($P = 100 N/N_0$). Here, N_0 is the number of bacteria before sunlight irradiation (MPN or CFUs/100 mL), and N is the number of bacteria after time (h) (MPN or CFUs/100 mL). Irradiation energy S (MJ/m^2) and the plot of the survival rate log of each fecal index bacterium were fitted in a regression model, and the absolute value of the slope was used as the inactivation rate coefficient K_s (m^2/MJ) of each fecal indicator bacterium under sunlight irradiation (Davies-Colley *et al.* 1994). Moreover, the irradiation energy S_{99} (MJ/m^2) required for inactivating 99% of each fecal indicator bacterium was obtained from the regression model.

Vertical distribution of irradiance in the water column

When the treated wastewater was irradiated with sunlight, the irradiance of the water column decreased with increasing water depth. Hence, the vertical distribution of irradiance in the water column with sunlight irradiation was estimated. The treated wastewater was stored in a light-shielded cylindrical container (diameter: 0.1 m; length: 1.0 m) wrapped in aluminum foil, with only the water surface exposed to sunlight. The change in the photosynthetic photon flux density at an interval of 0.2 m from just below the water surface to the bottom of the water was determined using the illuminance meter. Then, the obtained photosynthetic photon flux density was converted to irradiance. The measurement of irradiance in the water column was repeated five times with each of the treated wastewater collected on different days.

RESULTS AND DISCUSSION

Disinfection by sunlight irradiation with and without seawater mixing

For the sunlight irradiation experiment without seawater, the inactivated irradiation energy constants K_s and S_{99} of each fecal indicator bacterium were calculated based on the results of Figure 1 by fitting the regression model. The absolute values of the slope are listed in Table 1. The turbidity of the treated wastewater was $<2^\circ$ as the kaolin standard unit in all samples (samples

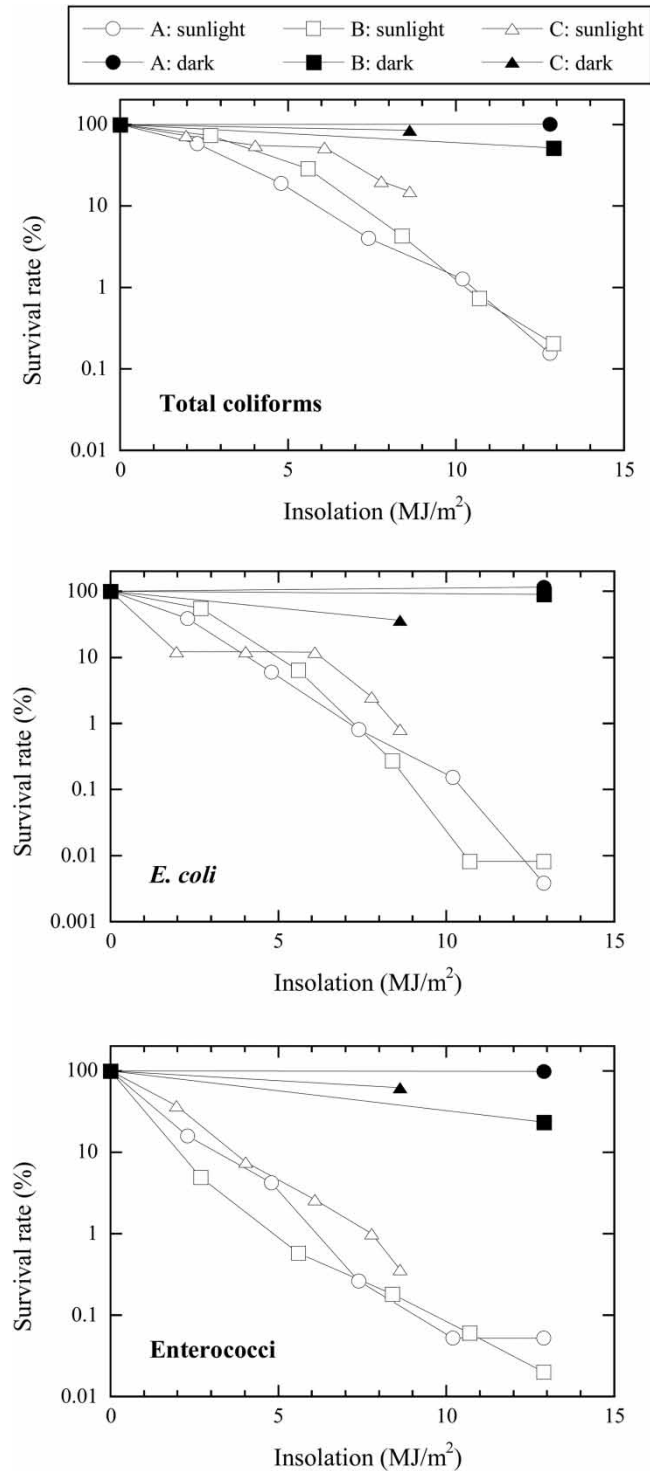


Figure 1 | Change in the survival rate of fecal indicator bacteria (total coliforms, *E. coli*, and enterococci) with the sunlight irradiation time. A, B, and C mean each sample of treated wastewater shown in Supplementary Material, Table S1.

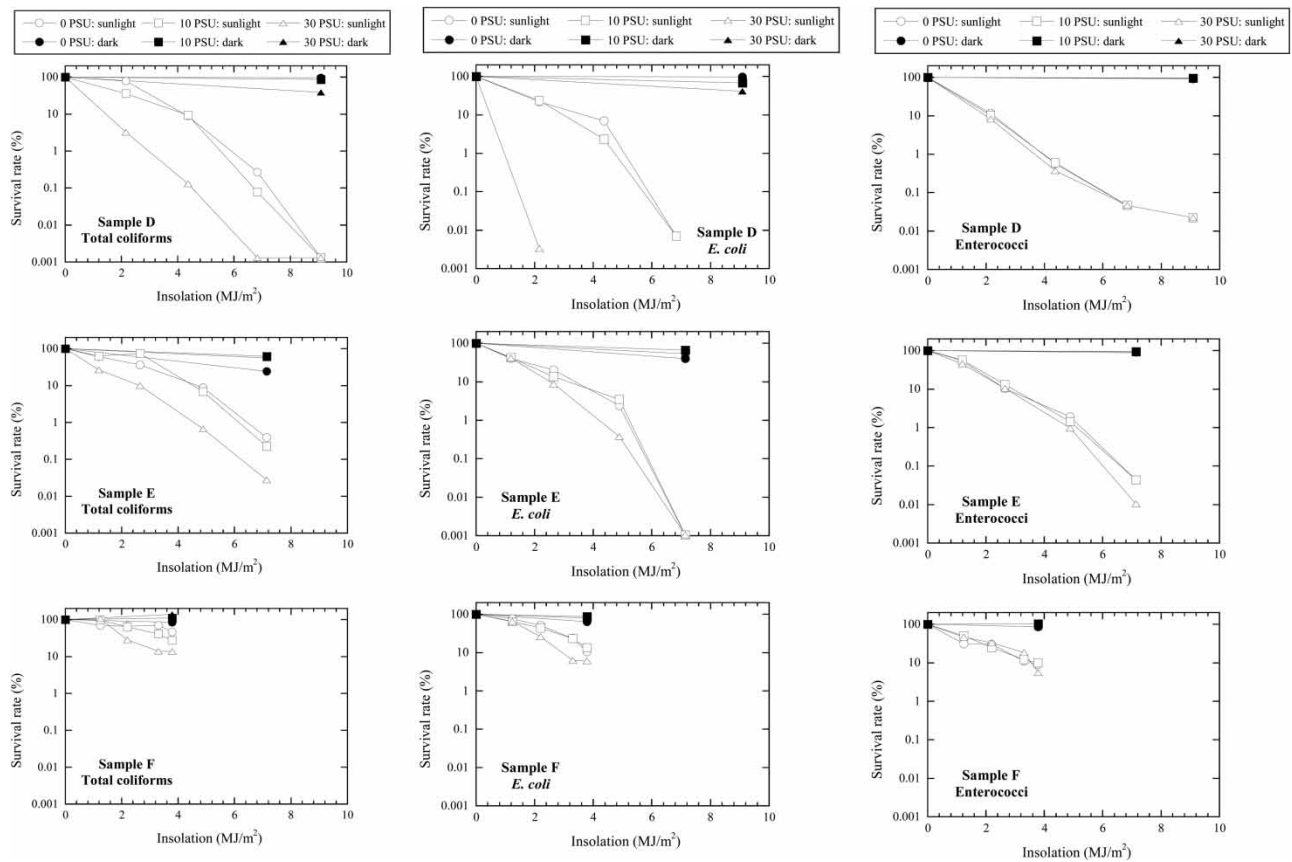
A, B, and C), and there were negligible suspended particles (Supplementary Material, Table S1). The K_s values of total coliforms, *E. coli*, and enterococci were 0.406, 0.684, and 0.631 m^2/MJ , respectively. The irradiation energy S_{99} (MW/m^2) required for inactivating 99% of each fecal indicator bacterium was estimated from the average of the results of the three experiments. The S_{99} values of total coliforms, *E. coli*, and enterococci were 14.5, 7.84, and 6.70 MJ/m^2 , respectively.

Table 1 | Sunlight inactivation kinetic parameters for total coliforms, *E. coli*, and enterococci in the experiment of sunlight irradiation without seawater mixing

Sample	K_s (m ² /MJ)			S_{99} (MJ/m ²)		
	Total coliforms	<i>E. coli</i>	Enterococci	Total coliforms	<i>E. coli</i>	Enterococci
A	0.50	0.77	0.63	10.0	7.4	6.7
B	0.51	0.84	0.63	10.6	6.6	6.0
C	0.21	0.45	0.63	22.9	9.5	7.4
Mean ± SD	0.41 ± 0.14	0.68 ± 0.17	0.63 ± 0.00	14.5 ± 6.0	7.8 ± 1.2	6.7 ± 0.6

SD, standard deviation.

For all samples (samples D, E, and F) of the sunlight irradiation experiment with seawater mixing, similar to those of the sunlight irradiation experiment without seawater mixing, the turbidity of the treated wastewater was $<2^\circ$, and there were few suspended solids that inhibited the penetration of the sunlight (Supplementary Material, Table S2). The EC of the treated wastewater samples with salinity adjusted to 30 PSUs was in the range of 37.2–38.9 mS/cm, and the pH was increased from 7.4 to 7.9. Figure 2 illustrates the relationship between the irradiation energy of the treated wastewater under different salinity conditions and the survival rate of each indicator bacterium. Similar to the results observed in Figure 1, the three kinds of fecal indicator bacteria were slightly inactivated even under dark conditions. During sunlight irradiation, the survival rate of each bacterium decreased significantly with an increase in the irradiation energy. It was clear that total coliforms and *E. coli* were more susceptible to inactivation, as inferred from a marked decrease in the survival rate at 30 PSUs. However,

**Figure 2** | Sunlight inactivation curves of fecal indicator bacteria (total coliforms, *E. coli*, and Enterococci) for three separate experiments with seawater mixing under different conditions of salinity. The conditions of samples D, E, and F are shown in Supplementary Material, Table S2.

the survival rates of total coliforms and *E. coli* in sample F were higher, and the inactivation effect of sunlight irradiation and salinity was lower than that in the other two samples. The temperature of sample F was 17 °C, which was the lowest among the three samples. It appears that the low water temperature limited the inactivating effect of irradiation on total coliforms and *E. coli* (10). Table 2 shows the calculations of the inactivated irradiation energy constants K_s and S_{99} for each indicator bacterium at each salinity based on the results of Figure 2 by fitting the regression model and using the absolute value of the slope. The average K_s of total coliforms was 0.71, 0.82, and 1.13 m^2/MJ in the samples adjusted to 0, 10, and 30 PSUs, respectively. At a salinity of 30 PSUs, K_s was 1.6-fold higher than that of 0 PSUs. The behavior of *E. coli* was similar to that of total coliforms, and the K_s of *E. coli* at a salinity of 30 PSUs was 2.39 m^2/MJ , which was 2.2-fold higher than that of 0 PSUs. The disinfecting effect of irradiation toward total coliforms and *E. coli* was improved when the salinity of the sample was set to 30 PSUs. At a salinity of 10 PSUs, which is near the salt content of physiological saline (0.85% NaCl), the disinfection effect was not improved by adding seawater. By contrast, the K_s values of enterococci in 0, 10, and 30 PSUs were 0.88, 0.90, and 0.97 m^2/MJ , respectively. The K_s values of enterococci remained unchanged in the salinity range of 0–30 PSUs. Therefore, enterococci cannot be expected to be inactivated by irradiation with an increase in salinity due to seawater mixing.

The average S_{99} values of total coliforms were 14.0, 8.6, and 5.1 MJ/m^2 in the samples adjusted to 0, 10, and 30 PSUs of salinity, respectively. For total coliforms, S_{99} decreased with increasing salinity, and at 30 PSUs, the S_{99} value decreased to $\leq 40\%$ of that at 0 PSU. The inactivated percent 90 (S_{90}) of total coliforms in the seawater reportedly ranges from 2.2 to 10 MJ/m^2 (Davies-Colley *et al.* 1994; Sinton *et al.* 1994). Hence, the S_{99} obtained in the present study is within the range of the previously reported S_{90} values. For *E. coli*, the S_{99} at 30 PSUs decreased by 2–3.6 MJ/m^2 compared with 0 and 10 PSUs. The inactivating effects on total coliforms and *E. coli* were significantly higher under the salinity condition of 30 PSUs. By contrast, the S_{99} of enterococci was within a narrow range of 5.1–5.5 MJ/m^2 at 0–30 PSUs, and high salinity did not have an inactivation-promoting effect. Although salinity and irradiance were not precisely controlled as in the present study, Davies-Colley *et al.* (1994) and Sinton *et al.* (1994) previously stated that enterococci are more resistant to sunlight in seawater compared with coliforms. It is assumed to be enterococci are halophilic bacteria.

Sunlight irradiation for disinfecting wastewater by mixing it with seawater: A proposal

Figure 3 illustrates the relationship between irradiance (W/m^2) and water depth (m). In treated wastewater, irradiance decreases exponentially with the increase in water depth. As per this relationship, we derived a simple regression formula to explain water depth as a function of irradiance: $y = 3.03 - 1.16 \log(x)$ ($r = 0.994$), where x is the irradiance and y is the water depth. The average irradiance of the surface layer was 431 W/m^2 . Because the average irradiance of sunlight in summer, which is typical in Japan, is 1,000 $W/m^2 = 1,000 J/s/cm^2$, we estimated that irradiance is attenuated by 60% just

Table 2 | Sunlight inactivation kinetic parameters for total coliforms, *E. coli*, and enterococci in the experiment of sunlight irradiation with seawater mixing

Salinity (PSU)	Sample	K_s (m^2/MJ)			S_{99} (MJ/m^2)		
		Total coliforms	<i>E. coli</i>	Enterococci	Total coliforms	<i>E. coli</i>	Enterococci
0	D	1.24	1.20	0.98	5.0	4.4	4.4
	E	0.75	1.50	1.06	6.8	3.9	4.8
	F	0.15	0.56	0.61	30.2	8.6	7.4
	Mean \pm SD	0.71 \pm 0.45	1.09 \pm 0.39	0.88 \pm 0.20	14.0 \pm 11.5	5.7 \pm 2.1	5.5 \pm 1.3
10	D	1.26	1.20	0.97	4.7	4.3	4.4
	E	0.84	1.47	1.09	7.2	3.9	4.7
	F	0.35	0.51	0.63	13.9	9.2	7.4
	Mean \pm SD	0.82 \pm 0.37	1.06 \pm 0.40	0.90 \pm 0.20	8.6 \pm 3.9	5.8 \pm 2.4	5.5 \pm 1.3
30	D	1.63	4.77	0.96	3.0	1.2	4.3
	E	1.13	1.58	1.26	4.3	3.5	4.2
	F	0.62	0.82	0.67	7.9	5.9	7.0
	Mean \pm SD	1.13 \pm 0.41	2.39 \pm 1.71	0.97 \pm 0.24	5.1 \pm 2.0	3.6 \pm 1.9	5.1 \pm 1.3

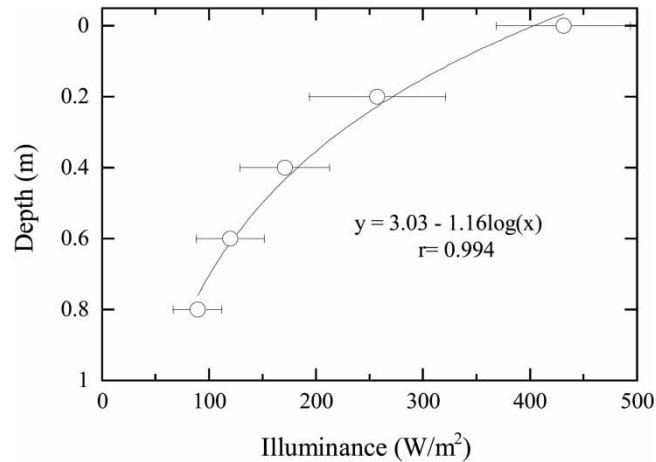


Figure 3 | Relationship between sunlight irradiance and water depth in the treated wastewater.

below the water surface. Assuming that the attenuation of irradiance because of water depth is permitted up to 216 W/m², which is 50% of the surface layer, the depth is 0.32 m. Under a water depth of 0.32 m, the irradiation time to obtain a value of 5.5 MJ/m² (= 5,500,000 J/m²), which nearly covers S₉₉ of the three types of fecal indicator bacteria, is 7.1 h (5,500,000 J/m² ÷ 216 J/s/cm² = 25,500 s = 7.1 h). Hence, if the average hydraulic retention time of the disinfection tank is set to 7.1 h, the surface area of the disinfection tank with a depth of 0.32 m can be calculated from the water volume for planning the treatment and the disinfection tank can be appropriately designed. However, sunlight is not available at night, and irradiance would fluctuate significantly depending on the weather conditions. Hence, a storage tank for the secondary treated water to adjust the flow rate is crucial before disinfection by sunlight irradiation. For this purpose, we propose the use of small- and medium-sized batch-type treatment plants. Without seawater application, the S₉₉ for total coliforms and *E. coli* was 2–3-fold higher in only wastewater than that in using seawater. It is necessary to set the irradiation time of the disinfection process to 14–21 h. Since the hours of sunlight are limited, disinfection cannot be completed in a day by sunlight irradiation only treated wastewater without seawater.

A challenge involved in the sunlight irradiation of wastewater after mixing with seawater as proposed in this study is to adjust the salinity to 30 PSUs after the mixing of wastewater and seawater. Because salinity in the normal coastal waters is approximately 32–33 PSUs, it is necessary to set the mixing ratio of wastewater and seawater to 10:90 for adjusting the salinity to 30 PSUs. However, as the amount of the treated water increases and the size of the disinfection tank irradiated with sunlight increases, locating a site near the coast becomes a key constraint. Assuming that the mixture ratio of wastewater and seawater is 50:50, it is necessary to acquire high salinity seawater of 60 PSUs, similar to that used in this study. We propose the utilization of concentrated seawater generated by seawater desalination via reverse osmosis or sun drying. Indeed, in desalination facilities, the treatment and disposal of concentrated seawater has become a problem as high saline waste, and some facilities dilute it with treated sewage and discharge it to the sea. Because salt production by sun drying is a simple technology, it is easy to obtain salt-rich seawater. Therefore, the problem of salinity adjustment can be resolved by using concentrated seawater.

Treated wastewater, which is freshwater, is valuable as a water resource. Therefore, in the hydrological cycle, it is inappropriate to use seawater as a disinfection process for wastewater treatment plants located in the upstream and middle basins, because it causes salinization of freshwater. The disinfection process proposed in this study is intended to be supposed into sewage treatment plants located near the coastal areas.

CONCLUSIONS

Based on our analyses, it appears necessary to develop a simple and easy-to-maintain disinfection method for use in developing countries and in the event of a disaster. Therefore, assuming a simple disinfection process without needing chemicals and electricity for treating wastewater, we quantitatively evaluated and examined the disinfection effects of fecal indicator bacteria via sunlight irradiation with the mixing of seawater. For total coliforms and *E. coli*, the inactivation

irradiation energy constant K_s was 1.6–2.2-fold higher in the wastewater by adjusting the salinity to 30 PSUs by mixing seawater than that in only wastewater. By contrast, although enterococci were confirmed to be disinfected by irradiation with sunlight, the disinfecting effect was not enhanced by mixing with seawater. Indeed, >99% of all fecal indicator bacteria can be inactivated by setting the irradiation energy of sunlight to 5.5 MJ/m². In addition, the relationship between the attenuation of irradiance and water depth was evaluated, and we could design the specification of a sunlight disinfection tank. The proposed disinfection process, which could be achieved by simply mixing wastewater and seawater and which can pass through a disinfection tank under the natural flow after sunlight irradiation, is an extremely simple, environmentally friendly, and attractive disinfection technique.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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