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Disinfection and Reactivation of Microorganisms after UV Irradiation for Agricultural Water Reuse of Biofilter Effluent

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Abstract

A pilot study was performed to examine the feasibility of UV disinfection system and the reactivation of indicator microorganisms (TC, FC, *E. coli*) after UV irradiation for agricultural reuse of reclaimed water. Photoreactivation and dark repair enable UV-inactivated microorganisms to recover and may reduce the efficacy of UV inactivation, which might be drawbacks of the UV disinfection method. The effluent of biofilter for 16-unit apartment house was used as input to the UV disinfection system, and average SS and BOD concentration were 3.8 and 5.7 mg/L, respectively, and the mean level of total coliform was in the range of 1.0×10^4 MPN/100mL. UV disinfection was found to be effective and it reduced mean concentration of indicator microorganisms (total coliform, fecal coliform, and *E. coli*) to less than 100 MPN/100mL within 60s exposure using 17, 25, and 40W lamps. Two UV doses of 6 and 16 mW · s/cm^2 were applied and microorganisms reactivation was monitored under the dark, photoreactivating light, and solar irradiation. Microorganisms reactivation was observed in the UV dose of 6 mW · s/cm^2, and numbers increased up to 5% at the photoreactivating light and 1% at the dark. However, microorganisms were inactivated rather than reactivated at the solar radiation and numbers decreased to non-detectible level about below 2 MPN/100mL in 4 hours. In the case of 16 mW · s/cm^2, microorganism reactivation was not observed indicating that UV dose might affect the reactivation process such as photoreactivation and dark repair. Therefore, concerns associated with microorganism reactivation could be controlled by sufficient UV dose application. Agricultural reuse of reclaimed water might be even less concerned due to exposure to the solar irradiation that could further inactivate microorganisms. The pilot study result is encouraging, however, sanitary concern in water reuse is so critical that more comprehensive investigation is recommended.

Keywords : Photoreactivation, Dark repair, UV disinfection, UV dose, Total coliform, Fecal coliform, E. coli, Water reuse, Guidelines for agricultural reuse

I. Introduction

There has been growing concern that the world is moving toward a water crisis, in 2025 two thirds of world's population will be suffering moderate to high water stress and about half of

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the population will face real constraints in their water supply (Lazarova et al., 2001). Water reuse is one of main options being considered as a new source of water in regions where water is scarce. Korea is a densely populated country and classified as a country of water shortage. Several regions suffer water stress and also increasing sewage discharges associated with the growing population threaten water quality of 5.5 billion m^3/yr, and about 200 million m^3/yr of it has been reused in urban areas and additionally about 450 million m^3/yr is estimated to be feasible for agricultural irrigation. Agricultural reuse of reclaimed water recently receives growing attention in Korea, and UV disinfection of secondary level effluent is being as a practical alternative for agricultural irrigation.

A major disadvantage of UV-disinfection lies in the ability of microorganism to repair damages caused by to their DNA. The principal type of cell damage from UV-irradiation is the formation of pyrimidine dimmers in the DNA modules, and these DNA-lesions are repairable by the process of photoreactivation which is attributed to the ability of light in the wavelength range between 330~480 nm (Liltved et al., 2000; USEPA, 1986). Disinfection of wastewater by chlorination produces residuals that are toxic to aquatic organisms and may cause the formation of carcinogenic compounds (George and burton, 1991). UV disinfection systems have been increasing in the past decades because such a system is easy to maintain, needs no chemical input, and produces no hazardous by-products (Crites and Tchobanoglous, 1998; WERF, 1995). Inactivation of microorganisms by UV irradiation is effected through the formation in the genomic DNA of the organisms (Abdennaceur et al., 2000). The major lesions induced by the germicidal UV light (253.7 nm) are pyrimidine dimmers. The presence of UV-induced lesions would inhibit the normal replication of DNA and therefore result in inactivation of the microorganisms.

Some organisms, however, are known to possess the ability to repair their DNA by mechanisms such as photoreactivation and dark repair (Friedderg et al., 1995). Photoreactivaion is the phenomenon by which UV-inactivated microorganisms recover activity through the repair of primidine dimmers in the DNA under near-UV light (310 to 480nm) with the enzyme. DNA repair mechanisms other than photo repair, such as excision repair, are named dark repair because they can repair the damaged DNA without light. Photoreactivation and dark repair enable UV-inactivated microorganisms to recover and may reduce the effect of UV inactivation. Therefore, they disadvantage the UV disinfection methods (Kumiko et al., 2001).

Korea is a densely populated country with about 47 million people live in approximately 100,000 km^2, and is classified as a country of water shortage. Several regions suffer water stress even with an average annual precipitation of 1,285 mm nationwide. Increasing sewage discharges associated with the growing population threatens water quality of receiving water bodies, and many areas experience water quality problems. Domestic wastewater is known to be one of the major pollutant sources, and legislation on the effluent water quality is forcing wastewater treatment plants to meet stringent standards. The largest water demands in national water use is associated with agricultural irri-

gation (15.8 billion m^3/yr) representing approximately 48% of total water use (33.1 billion m^3/yr) in Korea. However, planned agricultural reuse has not been practiced yet because of water quality concerns even though about 450 million m^3/yr of treated wastewater is estimated to be feasible for agricultural irrigation. Korea had not existed as wastewater reuse guidelines for agricultural irrigation. Therefore, Water quality of effluent from wastewater treatment plants (WWTPs) was reviewed to examine the feasibility of agricultural reuse using USEPA and WHO guidelines. It might meet the guidelines for BOD and SS, however, the most critical microbiological concentration was too high and further treatment is required.

The purpose of this study was to find feasibility of UV-disinfection system and compare a photoreativation and dark repair that their degree of interaction throughout a time course, for three indicator microorganisms, total colifom, fecal coliform, and *E. coli* when exposed to solar irradiation, visible light, and kept in darkness after UV irradiation. An adequate knowledge of the extent and rate of photoreactivation and dark repair of indicator microorganisms is important to predict the fate of these organisms in agricultural reuse of reclaimed water for water reuse guidelines.

Ⅱ. Materials and Methods

1. Study sites, experimental water condition, and water reuse guidelines

The study took place in a small sewage treatment plant (absorbent biofilter) for 16-unit apartment houses in Yangpyeong, Korea. Effluent from the biofilter was used as input to the UV-disinfection system. Average TSS and BOD concentration of biofilter effluent was 3.8 and 5.7 mg/L, respectively, and the mean level of total coliform was in the range of 1.1 x 10^4 MPN/100mL. The biofilter effluent was collected in a storage tank and pumped into the UV-disinfection system at predetermined flow rate. Table 1 summarizes the characteristics of water quality of biofilter in comparison with conventional wastewater treatment plants (WWTP) in Korea and suggested guidelines for water reuse in agriculture. The concentration of biofilter effluent was slightly higher than the WWTP effluent, partly because the former included only septic tank and absorbent biofilter while the latter included primary, secondary, and in many plants advanced treatments with disinfection. WWTP and biofilter might meet the USEPA guidelines for BOD and SS, however, the most critical microbiological concentration was too high and

Table 1 Water quality characteristics of conventional wastewater treatment plants and biofilter / Suggested guidelines for water reuse in agriculture

Parameter	WWTP[a]	Biofilter[b]	WHO[c]	USEPA[d]
Coliform	4,029 TC/mL	11,684 TC/100mL	10^3 - 10^5 FC/100mL	ND - 200 FC/100mL
BOD_5 (mg/L)	6.5	5.7	.	10 - 30
SS(mg/L)	5.3	3.8	.	30

a : Notional mean concentration of WWTPS from January to October in 2002. (MOE, 2002).
b : Mean concentration from March to December in 2002.
c : Recommended revised microbiological guidelines for treated wastewater use in agriculture (WHO, 1989).
d : Suggested guidelines for water reuse in agriculture (USEPA, 1992).

further treatment is required. The WHO and USEPA guidelines were made based on data from upland field agricultural system and may not be directly applicable to the paddy field agricultural system in Korea.

2. UV irradiance experiment

The flow-through type UV pilot equipment, with a useful volume of about 6L, was constituted with a stainless cylindrical container that involved a low-pressure mercury vapor discharge lamp inserted into a quartz sleeve used to mechanically protect and seal the lamp. The system had two cylindrical UV chambers in series with one lamp each as shown in Fig. 1. Only one chamber was actually used to examine the efficiency in the main experiment, because preliminary study demonstrated complete removal when two chambers were used. The pilot system has been running continuously over one year and the quartz sleeve being cleaned mechanically in every two hours to prevent filthiness.

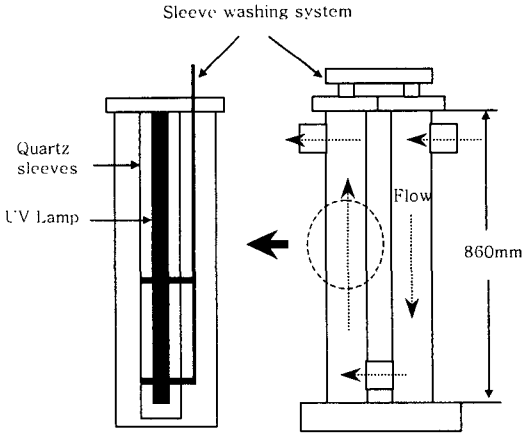

Fig. 1 Schematic of experimental UV disinfection system

The incident UV intensity (mW/cm^2) at 254 nm was measured with a calibrated dosimeter (VLX-3W CE, Vilber Lourmat, France) and the average intensity was calculated by the point-source summation method (USEPA, 1986; AWWA, 2000).

Three lamp intensities (17, 25, and 40 W) as shown in Table 2 and four flow rates (10, 20, 30, and 40 m^3/day) were used to evaluate the disinfection efficiency for the biofilter effluent with respect to lamp intensity and exposure time. The dose of UV light was calculated using equation 1 (Karl and Jeannue, 1994).

$$D = I \times t \quad \cdots\cdots (1)$$

where

D = UV dose (mW · s/cm^2)

I = intensity of UV light (mW/cm^2)

t = exposure time (second)

The disinfection study concerned agricultural reuse of the effluent from sewage treatment plant, and consisted in monitoring water quality before and after UV irradiation. For each experiment, samples were collected before and after pass through the UV-disinfection system, and analysis was done immediately on receipt of

Table 2 Characteristics of UV lamps used for experiment

Wattage (W)	Length (mm)	Diameter (mm)	UV Output (μW/cm^2 @1m)	MFG/MODEL
17	357	13	57	Lighttech/G10T5L (Hungary)
25	610	13	82	Atlantic/G24T5L (USA)
40	842	13	140	Philips/TUV36WT5 (Hollands)

samples in the laboratory usually within 2 hours of sampling. Total coliform (TC), fecal coliform (FC), and Escherichia *coli* (*E. coli*) were analyzed as indicator microorganisms by Standard Methods (APHA, 1998) as well as physical and chemical analyses.

3. DNA repair experiments

Two low-pressure UV lamps (17W, 40W) were used for the UV irradiation procedures. The incident intensity of the UV at a wavelength of 253.7 nm was 0.08 and 0.11 mW/cm^{-2} with 30 m^3/day respectively (Table 2). For each experiment, samples were collected after UV irradiation, and DNA repair experiment was done immediately on the receipt of samples in the laboratory usually within 2 hours of sampling.

A fluorescent lamp (DULEX L 36W, OSRAM, Germany) was used to study the effects of visible light to study indicator microorganism photoreactivation. A Fluorescent lamp was about 40cm above the liquid surface of sterile petri dishes (D 90 mm × 15 mm) to 25 and 27 ℃ incubator for 12 h. Light in the wavelength range of 350 – 790 nm was measured to 1,500 lx (Fig. 2). Photoreactivation is the phenomenon by which

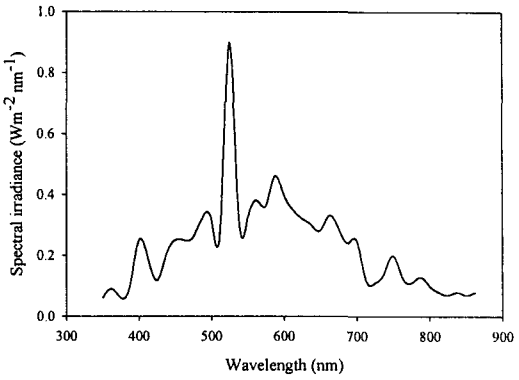

Fig. 2 Spectral irradiance of the fluorescent lamp used

UV inactivated microorganisms regain their activity by mean of photo repair of UV induced lesions in the DNA by utilizing the energy of near UV light (310 to 480 nm) (Kumiko et al. 2002, Friedberg et al. 1995). Sample collection was intermittently performed during the exposure to fluorescent light for 12h. Sampling time were 1, 2, 3, 4, 8, and 12 h after exposure each treatment, and analysis of indicator microorganisms was done immediately. For the investigation of dark repair, samples were kept in darkness to 22 and 25 ℃ incubator for 12 h after the UV irradiance (Fig. 3).

Sterile petri dishes were placed in the college house top for various exposure times to study

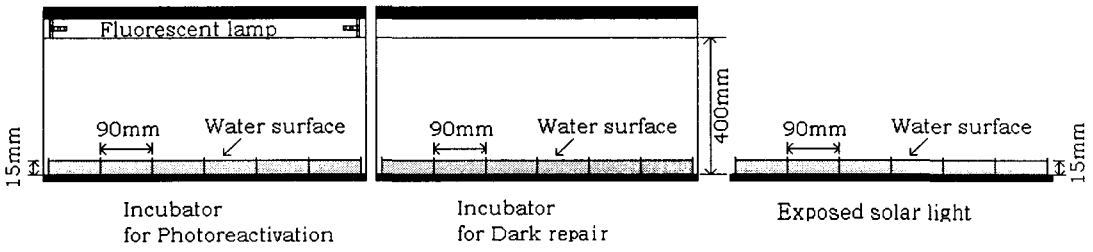

Fig. 3 Schematic of experimental UV disinfection and repair systems

reactivation and inactivation effects by solar irradiance as shown Fig. 3. Experiments were investigated on sunny days morning, from August to November 2002 in Seoul, Korea. The incident UV intensity (mW/cm^2) at 365 nm was measured with a calibrated radiometer (VLX-3W CE, Vilber Lourmat, France). The sunlight intensity during the trials was measured in the sensor with a digital lux meter (INS DX-100, Tiwan). Control experiment was done in the same way of fluorescent light experiment.

Ⅲ. Results and Discussion

1. Inactivation of UV irradiation in each UV dose

The microorganism concentrations before and after UV-disinfection with different lamp intensities and flows rate are shown in Fig. 4, and the survival ratio is summarized in Table 3. All the indicator microorganisms reduced to generally below 100 MPN/100mL in less than 60 seconds irradiation. The difference between 17 and 25 W lamps was not apparent in removal rates, but 40W lamp demonstrated complete removal in all the flow rates tested. ANOVA tests revealed that the differences in survival ratio with flow rates

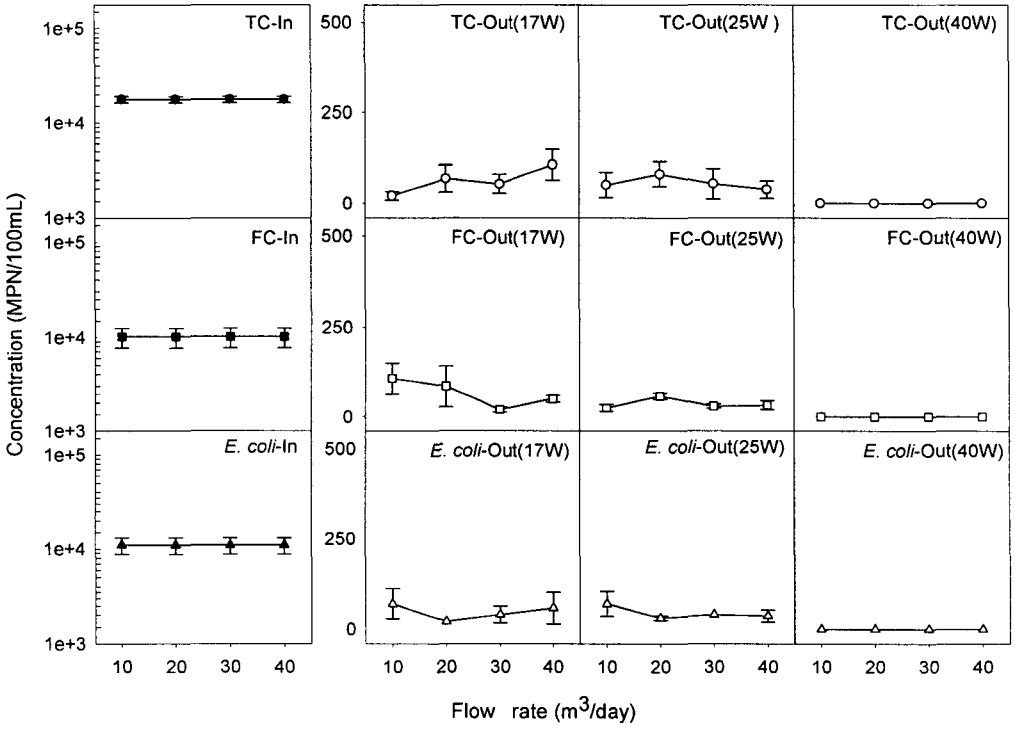

Fig. 4 Microorganism concentrations before and after UV disinfection with different lamp intensities and flow rates (mean ± S.E., n = 5)

Table 3 Survival ratio with lamp type and dose in the UV disinfection system

Lamp	Flow., rate (m^3/day)	Exp. time (s)	UV dose (mW·s/cm^2)	Survival ratio (% mean ± S.E.*)		
				TC	FC	E. coli
17W	10	20.4	18.2	2.2±1.46	1.4±1.13	0.7±0.33
	20	10.2	9.1	0.4±0.21	0.3±0.13	0.2±0.08
	30	6.8	6.1	0.3±0.14	0.8±0.22	0.4±0.31
	40	5.1	4.6	0.6±0.26	0.7±0.41	0.6±0.26
25W	10	34.8	26.2	0.3±0.11	0.4±0.18	0.7±0.50
	20	17.4	13.1	0.7±0.19	0.9±0.16	0.3±0.16
	30	11.6	8.1	1.5±0.44	0.5±0.08	0.4±0.16
	40	8.7	6.6	2.0±1.35	0.5±0.13	0.4±0.16
40W	10	48.0	48.0	0.0±0.00	0.0±0.00	0.0±0.00
	20	24.0	24.0	0.0±0.00	0.0±0.00	0.0±0.00
	30	16.0	16.0	0.0±0.00	0.0±0.00	0.0±0.00
	40	12.0	12.0	0.0±0.00	0.0±0.00	0.0±0.00

* Standard error

for each lamp were not significant. Incomplete removal by 17 and 25W lamps could be partly explained by lamp length in the chamber and UV dose. Notice that 17 and 25W lamp lengths filled about 40 and 70% of chamber height, respectively, while 40W lamp filled the whole height and showed a substantially higher UV output. Comparing survival ratio of 40W and the other two lamps, there was an indication that high lamp intensity with low exposure time could be more effective than the low lamp intensity with high exposure time for the similar UV dose. Flow-through type system used in the study allows close exposure of microorganisms to the UV lamp with distance less than 5 cm. UV intensity decreases as distance from the source increases, and it is inversely proportional to the square of distance (AWWA, 2000). The effect of flow rate was not apparent under the experimental condition, and it might be less significant for the thin flow in normal range of operation.

2. Photoreactivation and dark repair against UV dose

In 1951, Kelner recommended that photoreactivation be quantified as follows :

$$\text{Degree of photoreactivation} = (N_{pr} - N)/(N_0 - N) \quad \cdots\cdots (2)$$

$$\text{Percent photoreactivation} = 100((N_{pr} - N)/(N_0 - N) \quad \cdots\cdots (3)$$

$$\text{Log survival} = \log (N_{pr}/N_0) - \log(N/N_0) = \log (N_{pr}/N) \quad \cdots\cdots (4)$$

Where

N_0 = number of organisms prior to UV irradiation

N = number of organisms surviving UV disinfection

N_{pr} = number of organisms after photoreactivation

The degree of photoreactivation represents the fraction of inactivated cells that has been photoreactivated as shown in equation 2. Most studies involving photoreactivation of wastewater have employed a log increase methodology to quantify photoreactivation as shown in Equation 3. In this study, percent phothoreactivation was used for represents the photoreactivation and dirk repair (Equation 3) because of Equation 4 had not contained concentrations of microorganisms prior to UV irradiation.

Fig. 5 shows percent photoreactivation results of photoreactivaion and dark repair after UV irradiation about 6 and 16 mW · s/cm^2 of UV dose. Concentrations of TC, FC, and *E. coli* in the water of UV irradiated by UV dose of 6 mW · s/cm^2 showed some growth in viability during exposed visible light and kept in darkness was 0.2 ~ 5.0% and 0.2 ~ 1.1% respectively. Under the darkness their initial concentration of 10 ~ 30 MPN/100mL increased to the level of 100 MPN/100mL after 12 hours, which implied that part of damaged microorganisms by UV-irradiation might be repairable with time. Under the fluorescent lamp, photoreactivation was more apparent that their concentration increased up to 1,000 MPN/100 mL that might significantly repair. Against 6 mW · s/cm^2, photoreacivation and dark repair a little observed in UV irradiance by UV dose of 16 mW · s/cm^2. In this study, photoreactivation more observed than dark repair about five times. Most repairs of microorganism caused a great deal of recovery with in 3 to 4 hour. Result of early report on photoreacitvation pure cultures of *E. coli* by Novick and Szilard (1949) and Kelner (1951) indicated that as the UV dose increased, the number of cells able to be recover decreased.

The UV dose required for 90% inactivation of *E. coli* photoreactivation and dark repair were 7.5 and 2.5 mWs/cm^2, respectively (Harris et al., 1987). From the results of Kashimada et al. (1996) the UV dose required for 90% inactivation of fecal coliforms, photoreactivation and dark repair, is computed to be 24 and 5.2 mW · s/cm^2, respectively. In this study, the UV irradiated by

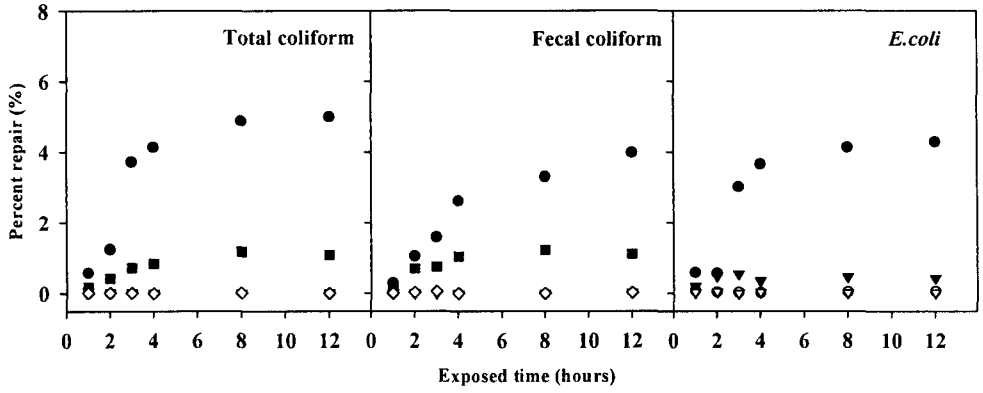

Fig. 5 Percent photoractivation as a function of exposure to photoreactivating light and dark for low and high UV dose (● : 6 mW · s/cm^2 and with photoreactivation ■ : 6 mW · s/cm^2 with dark repair ◇ : 16 mW · s/cm^2 with photoreactivation ▽ : 16 mW · s/cm^2 with dark repair)

6 and 16 mW · s/cm^2 UV dose microorganisms photoreactivation and dark repair was with 95 to 99.8 and 99 to 99.8 % inactivation, respectively. Therefore, It is possible to remove photoreactivation and dark repair problem by UV irradiation of sufficient UV dose.

The apparent photoreactivation with visible light from a fluorescent lamp was observed in indicator microorganisms. Therefore, Photoreactivation enable UV-inactivated microorganisms to recover and may reduce the efficacy of UV-disinfection. They disadvantage the UV disinfection methods.

3. Photoreactivaion and more inactivation with solar radiation

Fig. 6 shows results of photoreactivaion and more inactivation with solar radiation after UV irradiation about 6 mW · s/cm^2 of UV dose. TC, FC, and *E. coli* in UV irradiance water showed apparent decrease within 4 hours. The initial concentration of TC, FC, and *E. coli* were 11, 28, and 16 MPN/100mL after UV irradiation, respectively. Nevertheless, microorganisms decreased to the level of below 2 MPN/100mL within 4 hours. On a sunny day during the fall in Seoul, the solar irradiation intensity at 365 nm is about 1~4 mW · s/cm^2. The sunlight intensity during the trials was measured in the range of 10^2~10^6 lx perpendicular to the sensor with a digital lux meter.

UV irradiation decreased down to below 2 MPN/100 mL under the solar radiation primarily due to additional inactivation by solar radiation rather than photoreactivation. Samples not disinfected by UV irradiation also demonstrated substantial decrease of their concentration under solar radiation from about 5,000 MPN/100 mL to less than 30 MPN/100 mL in 24 hours as shown Fig. 7. Nevertheless, photoreactivation may occur in microorganisms, especially in UV-treated wastewater after its discharge to watersheds, because UV-inactivated microorganisms would normally be exposed to solar radiation, including visible light. Moreover direct reuse of effluent without disinfection is not recommended because natural decay by solar radiation may

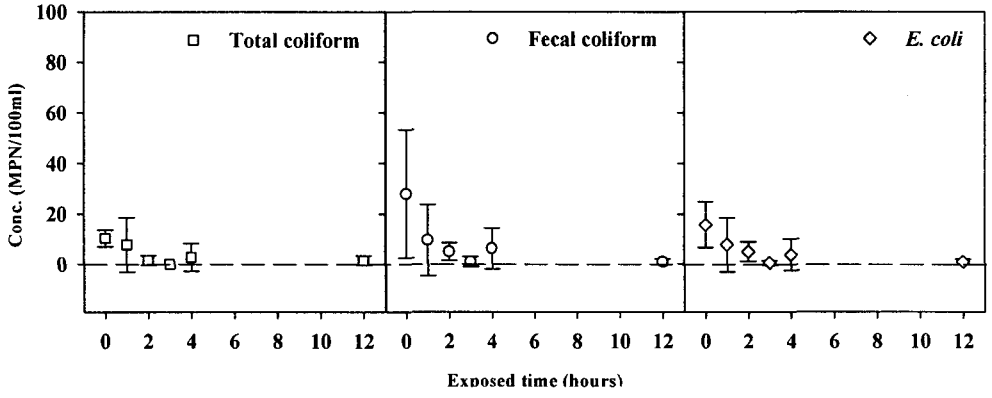

Fig. 6 Change of indicator microorganisms concentrations after UV disinfection water exposed to solar radiation

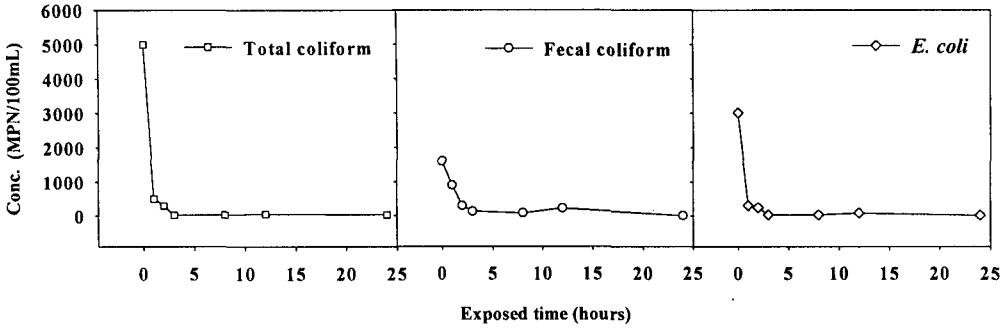

Fig. 7 Change of concentration before UV disinfection water exposed to solar radiation

take time and be affected by climatic conditions.

4. Photoreactivation and dark repair for agricultural water reuse

Fig. 8 shows results of photoreactivaion and more inactivation after UV irradiation about 6 and 16 mW · s/cm^2 of UV dose considering of waste-water reuse guidelines for agriculture. Microorganism concentration level of 1,000 MPN/100 mL was guideline suggested by WHO to unrestricted irrigation for eaten uncooked such as vegetable and salad crops. Regarding to photoreactivation in low UV dose (6 mW · s/cm^2), the indicator microorganisms concentrations to meet the WHO guidelines, but hardly meet the USEPA guidelines (Table 1).

The developed countries have tended to adopt

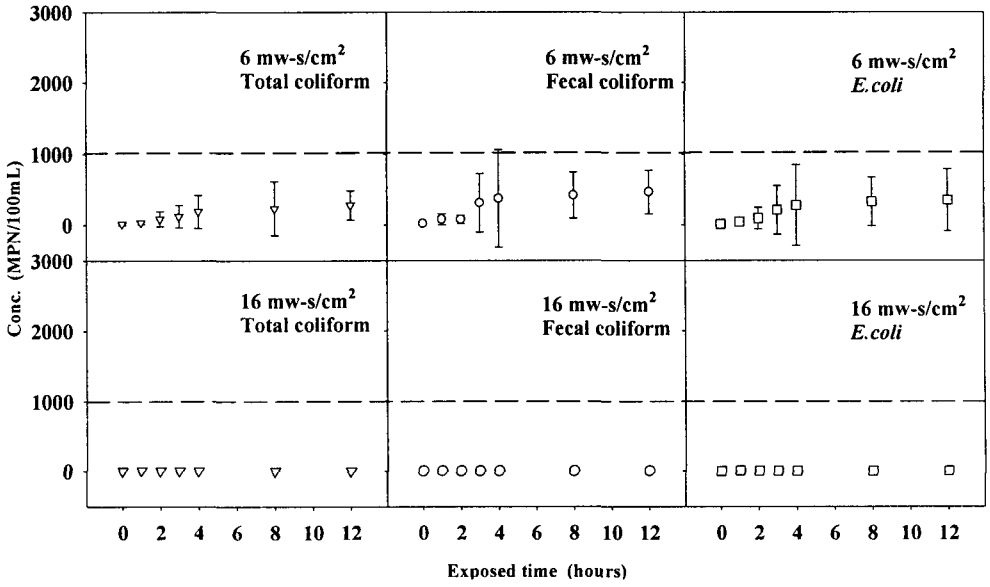

Fig. 8 Microorganisms concentrations before and after exposure to photoreactivating light and dark for low and high UV dose

an quantitative risk assessment approach which lead to conservative high technology/high cost/low risk guidelines or regulations of which USEPA are the best known examples. Some countries have endeavored low risk in practice because of insufficient money, experience or regulatory controls. Limits of affordability have led some developing countries to follow the low technology/low cost/controlled risk path of the risk approach that is embodied in the WHO guidelines. The WHO approach aims to provide guidance that can be adapted to national conditions and constraints, and allows the introduction of threshold criteria devised from balancing risk and affordability (Anderson et al, 2001).

1,000 TC/mL is the microorganism's standards for WWTP effluent effective from 2003 in Korea, and currently 100 MPN FC/100mL is being considered as microorganism's standards for agricultural reuse of reclaimed water in Korea (Seoul National Univ., 2002). This study demonstrated the similar result with other studies about UV inactivation and photoreactivation, and about 30 mW · s/cm^2 might be adequate for the disinfection of secondary effluent to keep the mean concentration of indicator microorganisms under 100 MPN/100mL for agricultural reuse.

Ⅳ. Conclusions

The result of this study showed that the UV system was found to be effective and reliable in reducing microorganisms and a feasible disinfection measure for agricultural reuse of effluent from wastewater treatment plants.

Three lamp intensities of 17, 25, and 25 W were all adequate to produce mean concentration of indicator microorganisms (TC, FC, and *E. coli*) less than 100 MPN/100mL within 60 s, but they often exceeded 100 MPN/100 mL for 17 and 25 W lamps when one-chamber was used. The indicator microorganisms were completely removed and after passing two chambers in series for all the tested lamps and also one-chamber with 40 W lamp, where flow thickness was less than 5 cm. About 30 mW · s/cm^2 was thought to be adequate to disinfection of secondary effluent for agricultural reuse, which was within the range of other studies.

High efficiency of UV disinfection to the biofilter effluent implies that it could be applicable to the conventional WWTP in Korea whose effluent concentration was lower than the biofilter. The flow-through type UV disinfection system might be more favorable than the open-channel system for small scale sewage treatment plants in rural area due to close exposure and more effective disinfection.

Microorganisms repair was observed in the UV dose of 6 mW · s/cm^2, and numbers increased up to 5% at the photoreactivating light and 1% at the dark. However, microorganisms were inactivated rather than reactivated at the solar radiation and numbers decreased to non-detectible level about below 2 MPN/100mL in 4 hours. In the case of 16 mW · s/cm^2, microorganism repair was not observed indicating that UV dose might affect the repair process such as photoreactivation and dark repair. Therefore, concerns associated with microorganism repair could be controlled by sufficient UV dose application. Photoreactivation problem after UV disinfection might be less concerned in agricultural reuse due to exposure

to the solar radiation and resulting inactivation. Overall, UV disinfection of effluent from wastewater treatment plants was thought to be an effective and feasible alternative for agricultural reuse, and thus strongly recommended.

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