33

f

An Bord Pleanála Oral Hearing

Irish Water

Greater Dublin Drainage

UV Disinfection Response to Inspector

– Dara White (Irish Water)

1.5 As UV treatment requires the use of energy, the best practice approach for UV disinfection of wastewater is to use dynamic dosing which adapts depending on the characteristics of the effluent such as total suspended solids (including metals) and turbidity, thereby continuously providing a sufficient dose while minimising energy requirements. This is controlled by an energy management system.

1.6 The UV treatment system proposed will be designed and operated to achieve 20,000 E Coli/100ml or less, with an average concentration in the order of 5,000- 6,000 E coli/100ml in the final effluent.. At this concentration, there will be no impact on the designated shellfish water. The inclusion of the proposed UV treatment system at the wastewater treatment plant will provide a combined 99.9% E Coli reduction across the entire plant.

1.7 These UV systems are designed specifically for each plant to achieve a reduction in the E Coli levels which is appropriate to

- the designation of the waters e.g. bathing / shellfish
- the distance of the discharge from the designated waters,
- the local current and tidal system
- the flow discharged from the WWTP.

1.8 Some concerns have been raised about the appropriateness of UV treatment, which I will now address.

1.9 Photoreactivation is the process whereby bacteria recover after being inactivated by UV light in the presence of daylight. The use of medium pressure UV treatment reduces the ability of bacteria to photoreactivate compared to low pressure systems. Appendix 3 provides an evaluation of low pressure and medium pressure UV systems and confirms that the use of medium pressure UV systems is more effective at reducing the ability of bacteria to photoreactivate.

1.10 Irish Water will install a medium pressure UV system to control photoreactivation. Furthermore photoreactivation requires the final effluent to be exposed to daylight. At the proposed wastewater treatment plant, the final effluent will not be exposed to daylight for about 4 hours after the UV treatment due to the length of the proposed outfall pipe. Therefore further preventing the photoreactivation process.

1.11 Preventative Maintenance: The UV system will include automatic cleaning as well as additional stand-by units to facilitate continued operation during maintenance. Instruments will be installed to continuously monitor the UV dose being applied in accordance with performance requirements. This will facilitate additional cleaning or bulb replacement as required. In addition, regular inspections of the UV system will be completed.

1.12 Total Suspended Solids

The UV treatment system will achieve the required performance reduction in E Coli at the design emission level value (ELV) for total suspended solids (TSS). The TSS ELV is anticipated to be 35mg/l as set out in the Urban Wastewater Treatment Directive or lower as directed by the Environmental Protection Agency in the wastewater discharge licence.

Appendix 1: WWTP's with UV treatment systems installed

Agglomeration Code	Agglomeration Name
D0014-01	Sligo
D0021-01	Malahide
D0023-01	Balbriggan
D0024-01	Swords
D0030-01	Wexford town
D0034-01	Ringsend
D0040-01	Tralee
D0055-01	Westport
D0056-01	Midleton
D0113-01	Carndonagh/Malin
D0114-01	Portrane/Donabate
D0132-01	Kinsale
D0168-01	Bantry
D0186-01	Ballyheigue
D0198-01	Clifden
D0285-01	Sneem
D0287-01	Waterville
D0296-01	Baltimore
D0444-01	Churchtown and Environs
D0459-01	Ballylongford
D0511-01	Achill Sound
D0541-01	Belgooly
D0024-01	Swords
D0074-1	Belmullet
D0170-01	Dunmore East
D0130-01	Bundoran
D0139-01	Youghal

Osberstown WWTP E Coli Performance 2017			
Month	Day	Flow m3/day	E Coli cfu/100ml
Contractual ELV for E Coli			N/A
Jan'17	02	25191	12540
	04	25965	20640
	16	25327	13590
	22	24033	36540
	25	24468	2560
Feb'17	01	29647	155310
	06	29034	100
	20	26036	310
Mar'17	01	35043	30760
	14	30046	1350
	20	29744	51720
	29	29194	36540
Apr'17	03	27782	198630
May'17	01	23008	3550
	03	23317	13330
	08	23304	1750
	17	26392	32230
	24	24740	29240
June'17	05	32579	240000
	07	30163	240000
	12	31040	14140
July'17	04	25720	8820
	13	23726	11870
	20	28654	3930
	28	23828	6020
Aug'17	02	23288	3890
	16	29957	129970
	23	26276	3790
Sept'17	06	24890	200
	13	33562	5830
	20	24700	9880
	27	25853	25950
Oct'17	04	24434	3360
	11	29964	4650
	18	26610	48840
Nov'17	01	26636	5120
	19	27465	15000
	22	47892	10540
	29	33921	10950
Dec'17	06	27602	9340
	13	37318	4960
	20	30133	520

Portrane WWTP E Coli performance 2018			
Month	Day	Flow m3/day	E Coli cfu/100ml
Contractual Elv for E Coli /100ml			2000
March	1	6549	1860
	7	8524	1800
	8	7804	1000
	9	7978	760
	10	11120	860
	11	9539	820
	12	9635	680
	13	8396	180
	14	11030	460
	15	16629	240
	16	13719	700
	17	11256	940
	18	10086	680
	19	9367	1060
	20	8993	1880
	21	8551	820
	22	7831	87
	23	8534	1500
	24	7845	1140
	25	7084	740
	26	7486	860
	27	7927	1420
	28	7152	1120
	29	7093	1260
April	1	7381	1580
	2	15168	1600
	3	14284	930
	5	13220	1
	6	10695	1
	7	10092	1
	8	9377	3
	9	9646	1
	10	8096	1
	11	8280	1020
	12	7841	1000
	13	7891	1800
	15	7929	1750
	16	7234	1280
	17	7077	1300
	20	6725	1800
	21	6708	1850
	24	8524	4
	26	7341	31
	28	6249	1080
	29	6778	980
	30	6666	480
May	1	6727	850
	3	6925	1400
	5	6340	740
	6	6472	440
	7	6088	900
	8	6400	660
	9	6625	1040
	10	6438	550
	11	4168	20
	12	6759	1
	13	6561	620
	14	6015	80
	20	5928	500
	21	6071	1200
	22	5989	450
	23	5768	900
	24	6205	500
	25	5647	1850
	26	6144	500
	27	6153	1720
	28	5782	740
	31	6102	1250

Portrane WWTP E Coli performance 2018			
Month	Day	Flow m3/day	E Coli cfu/100ml
Contractual Elv for E Coli /100ml			2000
October	1	5380	33
	2	5125	36
	3	5051	260
	4	5344	71
	5	8287	330
	6	5878	46
	7	5724	39
	8	5298	52
	9	5385	820
	10	5597	850
	11	5915	900
	12	8309	480
	13	12675	66
	14	7862	57
	15	6676	98
	16	5855	30
	17	5971	850
	18	5638	490
	19	5753	1580
	20	5745	35
	21	5658	52
	22	5597	55
	23	5475	1400
	25	5455	980
	26	5445	1860
	27	5678	500
	28	5376	300
	29	5749	320
	30	5170	500
	31	6090	1500
November	1	5627	1300
	2	5677	950
	3	5703	35
	4	5592	101
	5	9665	66
	6	6923	1450
	7	15905	980
	8	8357	650
	10	14013	800
	11	9218	420
	12	8347	520
	13	7517	134
	14	7076	78
	15	6731	51
	16	6325	43
	17	6770	7
	18	6552	9
	19	6190	21
	20	7749	9
	21	17313	52
	22	10889	27
	23	8382	15
	29	16943	1750
	30	11234	1350
December	1	10738	1460
	2	11461	700
	3	10555	580
	5	17903	1850
	6	12553	1680
	7	12156	700
	8	10204	260
	9	9605	108
	10	8629	320
	29	7587	61
	30	6905	77
	31	7652	66

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2002, p. 6029–6035 Vol. 68, No. 12
0099-2240/02/$04.00+0 DOI: 10.1128/AEM.68.12.6029–6035.2002

Photoreactivation of *Escherichia coli* after Low- or Medium-Pressure UV Disinfection Determined by an Endonuclease Sensitive Site Assay

Kumiko Oguma,* Hiroyuki Katayama, and Shinichiro Ohgaki

Department of Urban Engineering, University of Tokyo, Bunkyo-ku, Tokyo, Japan

Received 14 March 2002/Accepted 23 September 2002

Photoreactivation of *Escherichia coli* after inactivation by a low-pressure (LP) UV lamp (254 nm), by a medium-pressure (MP) UV lamp (220 to 580 nm), or by a filtered medium-pressure (MPF) UV lamp (300 to 580 nm) was investigated. An endonuclease sensitive site (ESS) assay was used to determine the number of UV-induced pyrimidine dimers in the genomic DNA of *E. coli*, while a conventional cultivation assay was used to investigate the colony-forming ability (CFA) of *E. coli*. In photoreactivation experiments, more than 80% of the pyrimidine dimers induced by LP or MPF UV irradiation were repaired, while almost no repair of dimers was observed after MP UV exposure. The CFA ratios of *E. coli* recovered so that they were equivalent to 0.9-, 2.3-, and 1.7-log inactivation after 3-log inactivation by LP, MP, and MPF UV irradiation, respectively. Photorepair treatment of DNA in vitro suggested that among the MP UV emissions, wavelengths of 220 to 300 nm reduced the subsequent photorepair of ESS, possibly by causing a disorder in endogenous photolyase, an enzyme specific for photoreactivation. On the other hand, the MP UV irradiation at wavelengths between 300 and 580 nm was observed to play an important role in reducing the subsequent recovery of CFA by inducing damage other than damage to pyrimidine dimers. Therefore, it was found that inactivating light at a broad range of wavelengths effectively reduced subsequent photoreactivation, which could be an advantage that MP UV irradiation has over conventional LP UV irradiation.

UV irradiation is one of the effective treatments used for disinfection. The numbers of water and wastewater treatment plants equipped with UV disinfection systems have been increasing in the past few decades in many countries, because such a system is easy to maintain, needs no chemical input, and produces no hazardous by-products (21). The ability of UV light to inactivate microorganisms (in other words, the sensitivity of microorganisms to UV light) is known to differ from organism to organism (1, 14, 25). Many researchers have pointed out that parasites such as *Cryptosporidium* and *Giardia*, the most problematic waterborne pathogens, can be inactivated effectively by UV irradiation (1, 2, 5, 6, 7, 15). This should be a great advantage of UV disinfection systems, because such parasites are known to be highly resistant to conventional chemical disinfectants, such as chlorine.

The mechanisms by which UV light inactivates microorganisms are different at different wavelengths (14). The germicidal effect of short-wavelength UV light (UV-C and UV-B; 220 to 320 nm) is mainly due to the formation of *cis-syn* cyclobutane pyrimidine dimers in the genome DNA of the organisms, while (6-4) photoproducts and other photoproducts are also produced at lower ratios (4, 14). The lesions inhibit the normal replication of the genome and result in inactivation of the microorganisms. Besides genomes, proteins and enzymes with unsaturated bonds are known to absorb UV-C and UV-B, which may also result in significant damage to the organisms (17). On the other hand, long-wavelength UV light (UV-A; 320 to 400 nm) is known to damage organisms mainly by exciting photosensitive molecules inside the cell to produce active species such as $O_2^{\cdot -}$, H_2O_2, and $^{\cdot}OH$, which damage the genome and other intracellular molecules and cause lethal and sublethal effects, such as mutations and growth delay (8, 16, 22, 23, 24).

Some organisms are known to possess mechanisms to repair UV-damaged DNA. Photoreactivation is one DNA repair mechanism, while other mechanisms are commonly referred to as dark repair in contrast to photoreactivation (11). Special attention has been paid to photoreactivation because it may greatly impair the efficacy of UV disinfection within a few hours after treatment. Photoreactivation is the phenomenon by which UV-inactivated organisms regain their activity via photorepair of UV-induced lesions in the DNA by utilizing the energy of near-UV light (310 to 480 nm) and an enzyme, photolyase (11, 14). Therefore, UV-A is essential for photoreactivation, although it also has lethal and sublethal effects on organisms, as mentioned above. Jagger called this phenomenon concomitant photoreactivation because the inactivating light itself has the potential to photorepair the dimers (16). The ability to perform photoreactivation differs from species to species, and most strains of *Escherichia coli*, the indicator bacterium used in water quality control, are known to be capable of photoreactivation. The photolyase of *E. coli* is basically specific for repair of pyrimidine dimers, while some organisms were recently found to have a photoreactivating enzyme specific for (6-4) photoproducts (19, 27, 28). The diversity and distribution of photolyase are still controversial issues, and it is therefore important to investigate the photoreactivation ability of key microorganisms, such as indicator bacteria. Moreover, quantitative determination of photoreactivation is essential in

* Corresponding author. Mailing address: Department of Urban Engineering, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8656, Japan. Phone: 81-3-5841-6242. Fax: 81-3-5841-8533. E-mail: oguma@env.t.u-tokyo.ac.jp.

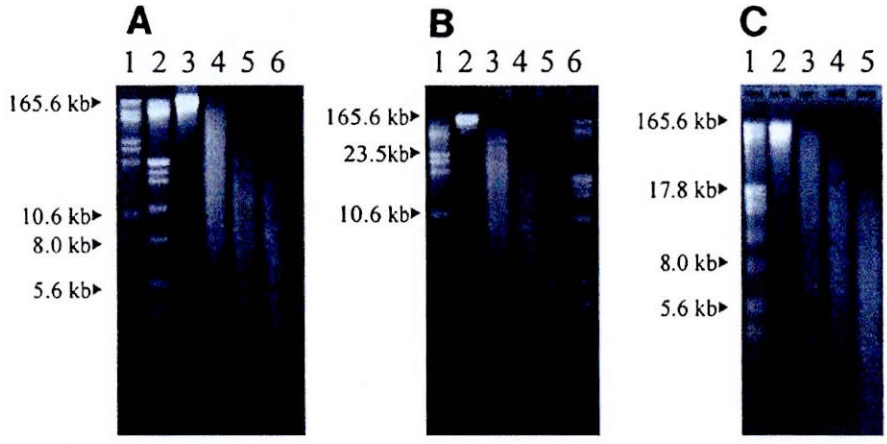

FIG. 1. Gel images for ESS assays of *E. coli* during exposure to LP, MP, or MPF UV lamps. (A) Exposure to LP UV. Lanes 1 and 2, standard markers; lane 3, no UV; lanes 4 to 6, UV doses of 1.9, 3.8, and 5.7 mJ · cm^{-2}, respectively. (B) Exposure to MP UV. Lanes 1 and 6, standard marker; lane 2, no UV; lanes 3 to 5, UV doses of 2.1, 4.2, and 6.3 mJ · cm^{-2}, respectively. (C) Exposure to MPF UV. Lane 1, standard marker; lane 2, no UV; lanes 3 to 5, UV doses of 1.8, 3.6, and 5.4 mJ · cm^{-2}, respectively.

resulted in fragmentation of DNA into shorter molecules. Figure 1 was analyzed to obtain Fig. 2, which shows profiles of the numbers of ESS in *E. coli* during exposure to LP, MP, or MPF UV. As shown in this figure, the number of ESS induced by UV irradiation increased along with the increase in UV doses from each lamp. Figure 3 shows the ratio of CFA during exposure of *E. coli* to LP, MP, or MPF UV. The CFA ratio decreased log linearly with increasing UV doses for all lamps. There was no clear difference among LP UV irradiation, MP UV irradiation, and MPF UV irradiation in terms of the ESS and CFA profiles for UV doses during inactivation procedures, as shown in Fig. 2 and 3.

Figure 4 shows the relationships between the number of ESS and the CFA ratio during exposure to LP, MP, or MPF UV. The CFA ratio showed a log-linear relationship with the number of ESS for each type of lamp, while the ESS-CFA relationships did not differ significantly among LP UV exposure, MP UV exposure, and MPF UV exposure.

Photoreactivation after LP, MP, or MPF UV inactivation. Figure 5 shows typical gel images of ESS assay mixtures for *E. coli* during fluorescent light exposure after LP, MP, or MPF UV exposure, which were analyzed to determine the ESS remaining ratio, as shown in Fig. 6. The ESS induced by LP and MPF UV irradiation were gradually repaired during fluorescent light exposure; on average, 84 and 83% of the total ESS were repaired in 3 h, respectively. On the other hand, almost no ESS were repaired by fluorescent light exposure after MP UV irradiation.

Figure 7 shows the results of photorepair treatment in vivo, in vitro with intact photolyase, and in vitro with MP UV-exposed photolyase. This figure shows that MP UV-induced ESS in *E. coli* were photorepaired in vitro with either intact or MP UV-exposed photolyase, suggesting that no repair of ESS in vivo was caused by a disorder with the endogenous photolyase of MP UV-irradiated *E. coli*.

Figure 8 shows the profiles of the CFA ratio of *E. coli* during fluorescent light exposure after LP, MP, or MPF UV inactivation. After 3-log inactivation by exposure to LP and MPF UV,

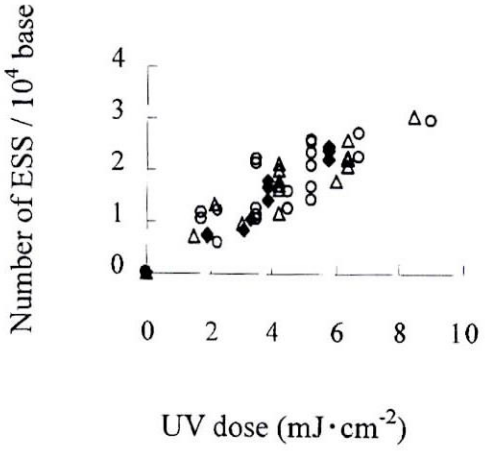

FIG. 2. Numbers of ESS in *E. coli* after exposure to an LP UV lamp (♦), an MP UV lamp (△), or an MPF UV lamp (○). The data are the results of five independent exposures to each type of lamp.

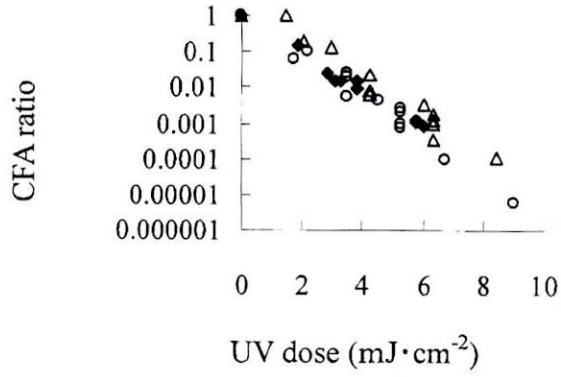

FIG. 3. CFA ratios for *E. coli* after exposure to an LP UV lamp (♦), an MP UV lamp (△), or an MPF UV lamp (○). The data are the results of five independent exposures to each type of lamp.

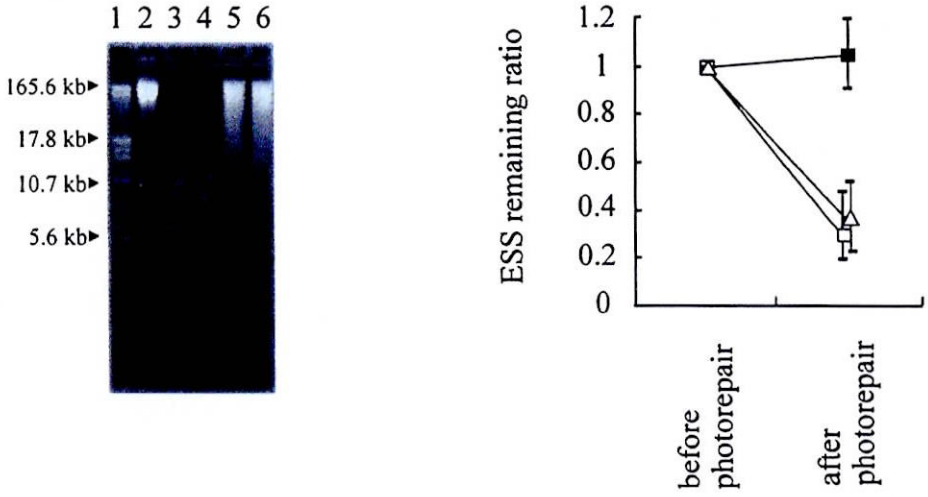

FIG. 7. Photorepair of ESS in vivo (■), in vitro with intact photolyase (□), or in vitro with MP-exposed photolyase (△) after MP inactivation. Lane 1, standard marker; lane 2, no UV; lane 3, MP UV dose of 6.3 mJ · cm^{-2}; lane 4, MP UV dose of 6.3 mJ · cm^{-2}, followed by photorepair in vivo; lanes 5 and 6, MP UV dose of 6.3 mJ · cm^{-2}, followed by photorepair in vitro with intact photolyase (lane 5) or with MP-exposed photolyase (lane 6). For photorepair in vivo, MP UV-irradiated *E. coli* was subsequently exposed to fluorescent light. For photorepair in vitro, DNA of MP UV-irradiated *E. coli* was exposed to fluorescent light in vitro with intact or MP UV-exposed photolyase. The symbols indicate the means from two or three independent experiments, and the bars indicate the maximum and minimum values.

The ratio of CFA showed log-linear relationships with the number of ESS during exposure to LP, MP, or MPF UV, while the ESS-CFA relationships were not clearly different for the three types of lamps. This suggests that the numbers of ESS necessary to decrease the CFA of *E. coli* are not significantly different for inactivation with the different wavelengths (254, 220 to 580, and 300 to 580 nm). This may imply that the culturability of *E. coli* is regulated mostly by pyrimidine dimers and is not greatly affected by other damage during inactivation.

Figure 7 shows that even ESS in MP UV-irradiated *E. coli*, which were not repaired by exposure to fluorescent light in vivo, were photorepaired in vitro with either intact or MP UV-exposed photolyase. This suggests that the MP UV-induced pyrimidine dimers were not structurally different from other photorepairable dimers and that the failure to repair MP UV-induced ESS in vivo was caused by a disorder with the endogenous photolyase in *E. coli*. Moreover, even MP UV-exposed photolyase could repair ESS in vitro, indicating that the photolyase itself was not inactivated by MP UV irradiation. It was therefore assumed that MP UV irradiation did not affect the activity of endogenous photolyase but reduced the amount of photolyase in *E. coli*, possibly by affecting regulation of the photolyase gene to lower expression. The failure in ESS repair was not observed after MPF UV treatment; it was observed only after MP UV treatment. This suggests that the disorder of photolyase was caused by wavelengths between 220 and 300 nm, although it is possible that the difference in irradiance between MP UV and MPF UV affected this phenomenon. The detailed mechanisms of exposure to MP UV that reduce the repair of ESS may be an interesting subject for further investigation. The results of photorepair treatment in vitro suggested that the MP UV lamp was effective at reducing the subsequent photorepair of pyrimidine dimers at the enzyme level.

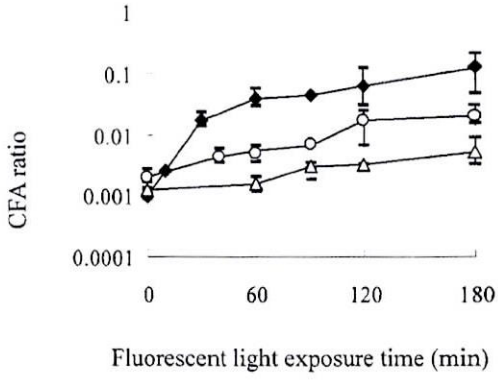

FIG. 8. CFA ratios after exposure to fluorescent light after LP UV (◆), MP UV (△), or MPF UV (○) inactivation. The symbols indicate the means from two or three independent experiments, and the bars indicate the maximum and minimum values.

Table 1 and Fig. 9 show that both the repair of ESS and the recovery of CFA were observed after exposure to LP or MPF UV, while neither was apparently observed after exposure to MP UV irradiation. Table 1 and Fig. 9 also indicate that MPF

TABLE 1. Photoreactivation characteristics of *E. coli* after LP, MP, or MPF UV inactivation

Irradiation	Repaired ESS (%)	Repaired CFA[a] (log_{10})	Final inactivation of CFA[b] (log_{10})
LP UV	84.2 (72.6–94.8)[c]	2.09 (2.00–2.18)	0.92 (0.83–1.02)
MP UV	<0 (<0–3.2)	0.61 (0.53–0.83)	2.29 (2.07–2.51)
MPF UV	83.1 (75.1–93.1)	1.02 (0.95–1.38)	1.70 (1.67–1.73)

[a] Log (CFA ratio after photoreactivation) − log (CFA ratio before photoreactivation).
[b] −Log (CFA ratio after photoreactivation).
[c] Mean based on two or three independent experiments. The values in parentheses are minimum and maximum values.

Hydrogen peroxide is responsible for UVA-induced DNA damage measured by alkaline comet assay in HaCaT keratinocytes. J. Photochem. Photobiol. B Biol. **59**:123–131.

24. **Ramabhadran, T. V., and J. Jagger.** 1976. Mechanism of growth delay induced in *Escherichia coli* by near ultraviolet radiation. Proc. Natl. Acad. Sci. USA **73**:59–63.
25. **Sommer, R., T. Haider, A. Cabaj, W. Pribil, and M. Lhotsky.** 1998. Time dose reciprocity in UV disinfection of water. Water Sci. Technol. **38**:145–150.
26. **Sutherland, B. M., and A. G. Shi.** 1983. Quantitation of pyrimidine dimer contents of nonradioactive deoxyribonucleic acid by electrophoresis in alkaline agarose gels. Biochemistry **22**:745–749.
27. **Todo, T., H. Takemori, H. Ryo, M. Ihara, T. Matsunaga, O. Nikaido, K. Sato, and T. Nomura.** 1993. A new photoreactivating enzyme that specifically repairs ultraviolet light-induced (6–4) photoproducts. Nature **361**:372–374.
28. **Uchida, N., H. Mitani, T. Todo, M. Ikenaga, and A. Shima.** 1997. Photoreactivating enzyme for (6–4) photoproducts in cultured goldfish cells. Photochem. Photobiol. **65**:964–968.
29. **Veatch, W., and S. Okada.** 1969. Radiation-induced breaks of DNA in cultured mammalian cells. Biophys. J. **9**:330–346.
30. **Water Works Association.** 1993. Standard methods for the examination of water. Japan Water Works Association, Tokyo, Japan.