22 March 2019

Dara White

(Wastewater Treatment Specialist, Irish Water)

Witness Statement for Greater Dublin Drainage Oral Hearing

1 ADDITIONAL INFORMATION ON PROPOSED UV TREATMENT OF EFFLUENT

1.1 UV light technology has been developed over the last 30 years to provide UV disinfection for both water and wastewater. It is a mature technology that is used worldwide (with more than 10,000 installations) to meet water quality objectives in receiving waters. In wastewater treatment plants, banks of UV emitting bulbs are provided in modules within concrete channels. These channels are designed to provide hydraulic residence time for the effluent to be irradiated by UV light of the particular wavelengths specified and to achieve a design reduction in bacteria numbers.

1.2 Irish Water has installed and operated UV disinfection systems at 27 no. WWTP's around the country for both designated shellfish water and designated bathing water protection. For shellfish water the systems are operated all year round. A list of Irish WWTP's with UV treatment systems installed, is provided in Appendix 1.

1.3 Irish Water is proposing to provide medium pressure (MP) UV treatment of the final effluent to provide a further reduction in the E Coli concentrations to further protect the designated shellfish waters. With secondary treatment the discharge concentrations of coliforms in the effluent are variable and are dependent on: the combined or otherwise nature of the sewerage network; the organic load to the treatment plant; the flow on any given day; the temperature; and the design residence time in the treatment plant.

1.4 Therefore, E coli concentrations vary from below 10,000 E Coli/100ml up to about 300,000 E Coli/100ml in an unpredictable manner from day to day. UV treatment reduces, and controls the spikes and variability of the concentrations of E Coli discharged from a Wastewater Treatment Plant (WWTP) and provides for an upper level of E Coli concentration in the final effluent E Coli. In this way, UV treatment protects designated waters from the variability associated with secondary treatment and thus provides greater assurance in meeting water quality standards. Attached in Appendix 2 are two tables;

- Table 1 show the final effluent coliform concentrations from Osberstown WWTP (130,000pe) which has no UV treatment installed
- Table 2 shows the final effluent coliform concentrations from Portrane WWTP (65,000pe) which has UV treatment.

These tables provide a comparison of the E Coli levels in final effluent from similar WWTP with and without UV treatment and clearly demonstrates the effectiveness of the UV treatment particularly in relation to the reduction in the variability of E Coli.

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, This will further inhibit 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.

1.13 Impact on operational capacity

The proposed UV treatment system will be designed for the flows at the plant and will be installed on the final effluent line. Accordingly, the proposed UV treatment will have no impact on the operational capacity of the wastewater treatment plant.

1.14 In conclusion, the proposed UV treatment system is appropriate and adequate for the requirements of further protecting the designated shellfish waters.

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

Appendix 2

- Table 1 show the final effluent coliform concentrations from Osberstown WWTP (130,000pe) which has no UV treatment installed

- Table 2 shows the final effluent coliform concentrations from Portrane WWTP(65,000pe) which has UV treatment.

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**
January	4	12975	87
	5	10920	73
	6	8366	140
	7	8146	200
	8	7821	230
	9	8129	87
	10	7539	810
	11	7605	920
	12	6643	990
	13	7612	62
	14	7338	64
	15	7729	63
	16	7488	54
	17	8225	820
	18	8322	870
	19	7282	710
	20	11499	63
	21	19340	70
	22	18023	82
	23	10688	43
	24	16565	12
	25	11946	6
	26	9273	1
	27	10427	1
	28	8798	1
	29	9886	1
	31	7668	690
February	2	8117	370
	3	8410	860
	4	8111	700
	5	7493	760
	6	7881	57
	7	7601	110
	8	7425	830
	9	7470	1000
	10	8936	1500
	11	8099	1960
	12	7871	1660
	13	8198	1400
	14	7588	730
	15	7061	68
	16	5783	29
	19	8505	4
	24	6606	820
	25	6409	740
	26	6266	560
	28	6558	1220

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**
June	5	6090	1400
	14	5395	1000
	24	5172	21
	26	5459	11
July	4	4907	1750
	5	4892	1900
	6	4994	950
	7	4842	1200
	8	4814	400
	11	4890	700
	16	4916	400
	17	4865	2000
	18	4608	800
	19	5071	54
	20	9549	101
	21	6173	50
	22	5335	300
	23	5473	850
	24	4998	1350
	25	5204	800
	26	5410	128
	27	5090	400
	28	10733	55
	29	10508	3
	30	6669	6
	31	6070	1450
August	1	7173	440
	2	6011	74
	3	5759	460
	4	5870	2
	5	5457	1
	6	5918	2
	7	5354	3
	8	5298	83
	9	5884	37
	10	5564	5
	11	5819	1
	12	5890	1
	13	5851	1
	14	5807	1
	15	5707	1510
	16	5924	1050
	17	5903	460
	18	5648	32
	19	5608	44
	20	5323	39
	21	5644	550
	22	5081	150
	23	5471	45
	24	5903	990
	25	5327	1
	26	8428	1
	27	5794	1
	30	5333	1200
	31	5422	200
September	1	5180	27
	2	5865	39
	3	5435	1
	4	5199	23
	6	6235	1700
	8	6934	60
	9	5948	40
	10	5338	50
	11	5719	330
	12	5267	340
	13	5655	540
	14	5573	650
	15	5586	32
	16	5544	38
	17	5013	39
	18	5852	24
	19	4970	890
	20	7790	290
	21	7641	190
	22	6102	38
	23	5566	55
	24	5501	40
	27	5386	760
	29	5386	100
	30	5414	30

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

Appendix 3

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

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.

order to be able to calculate the UV doses necessary to compensate for the potential repair in advance.

The most conventional UV lamps used for disinfection are low-pressure (LP) UV lamps, while medium-pressure (MP) UV lamps have also been used. LP UV lamps have monochromatic emission at a wavelength of 254 nm, which is most efficiently absorbed by DNA bases and therefore has some of the greatest germicidal effects among UV wavelengths (14). On the other hand, MP UV lamps emit polychromatic light at a broad range of wavelengths, from around 200 to 600 nm. MP UV lamps can emit light at a high intensity, which allows MP UV systems to be operated at higher flow rates than LP UV systems (12, 17). MP UV lamps are known to be as effective as conventional LP UV lamps at inactivating microorganisms or more effective (6, 7, 13), and the photoreactivation that occurs after MP UV disinfection results in a requirement for further inactivation because of its importance.

The purpose of this study was to compare a polychromatic MP UV lamp (220 to 580 nm) with a monochromatic LP UV lamp (254 nm) in terms of photoreactivation of *E. coli*. In addition, photoreactivation of *E. coli* after exposure to a filtered MP (MPF) UV lamp (300 to 580 nm) was also investigated in order to clarify the effects of inactivating light wavelengths on the subsequent photoreactivation. An endonuclease sensitive site (ESS) assay, which previously proved to be useful for determining the number of pyrimidine dimers in the genomic DNA of *E. coli* (20), was used along with a conventional cultivation assay in order to investigate UV inactivation and subsequent photoreactivation of *E. coli* both at the genomic level and at the colony-forming-ability (CFA) level.

MATERIALS AND METHODS

Microorganism. A pure culture of *E. coli* K-12 strain IFO 3301 was used as the test microorganism. A few discrete colonies of *E. coli* were selected from growth formed on Luria-Bertani (LB) agar (Merck) and were incubated in LB broth (Difco) at 37°C overnight until the stationary phase was reached. The growth was collected by centrifugation (7,000 × g, 10 min), washed twice with a sterilized phosphate buffer solution (pH 7.6), and subsequently suspended in the phosphate buffer at an initial concentration of 2.5×10^7 to 4.0×10^7 CFU · ml^{-1}. Forty milliliters of the suspension of *E. coli* was placed into a sterilized petri dish (diameter, 100 mm) and subjected to the light exposure procedures.

Light exposure. Two LP UV lamps (20 W; Stanley GL6; Toshiba) or an MP UV lamp (330 W; B410MW; Ebara) was used for inactivation. In order to investigate the effect of long wavelengths, the MP UV lamp emission was filtered through a Pyrex glass plate (thickness, 1 mm). A multichannel photodetector (MCPD-2000; Otsuka) showed that the emissions of the LP, MP, and MPF UV lamps were at wavelengths of 254, 220 to 580, and 300 to 580 nm, respectively. The germicidal intensity of the light emitted from each lamp was standardized by determining the irradiance of light at 254 nm with a biodosimeter by using F-specific RNA coliphage Qβ (18). Briefly, a pure-culture suspension of phage Qβ at an initial concentration of 2.0×10^6 PFU · ml^{-1} was exposed to the LP, MP, and MPF UV lamps to determine the inactivation curves by a double-agar-layer method with LB agar (Merck) by using *E. coli* K-12 strain F^+ A/λ as the host organism. The rate of inactivation of phage Qβ for each lamp was compared with the inactivation rate constant for phage Qβ at 254 nm to determine the irradiance values for the LP, MP, and MPF UV lamps (0.24, 3.0, and 0.25 mW · cm^{-2}, respectively). The irradiance values were fixed throughout the experiment, and UV doses were controlled by changing the exposure time.

Each 99.9% (3-log) inactivation of the CFA ratio (see below) was followed by exposure to fluorescent lamps (18 W; Hitachi) for 3 h to allow photoreactivation. The irradiance of the photoreactivating light at 360 nm was 0.1 mW · cm^{-2}, as measured with a UV radiometer (UVR-2 UD-36; Topcon). All preparations of *E. coli* were constantly stirred magnetically throughout the experiment and kept in the dark except during exposure to UV and fluorescent light. The sample temperature was kept at 20°C by circulating cooling water around the petri dishes.

Cultivation assay. The CFA of *E. coli* was investigated by using a deoxycholate acid agar medium (Eiken) in a dark room and the standard methods for examination of water (30). The number of CFU after incubation at 37°C for 18 h was determined, and the ratio of the CFA of *E. coli* was calculated as follows: $CFA_t = N_t/N_0$, where CFA_t is the ratio of CFA at irradiation time t, N_t is the number of CFU at irradiation time t, and N_0 is the number of CFU before UV irradiation.

ESS assay. An ESS assay allows recognition of pyrimidine dimers in DNA at ESS by treatment of DNA with a UV endonuclease, which incises a phosphodiester bond specifically at the site of a pyrimidine dimer. The molecular lengths of fragmented DNA are determined by alkaline agarose gel electrophoresis, followed by a theoretical calculation to obtain the number of ESS (26).

The conditions for the ESS assay used in this study were basically the same as those described previously (20). After the irradiation procedures, the *E. coli* suspensions were centrifuged (5,000 × *g*, 10 min), and the pellets were subjected to DNA extraction procedures (Genomic-tip; Qiagen). The extracted DNA was concentrated by using centrifugal filter devices (Centricon; Millipore) and resuspended in a UV endonuclease buffer containing 30 mM Tris (pH 8.0), 40 mM NaCl, and 1 mM EDTA. The DNA preparations were treated with a UV endonuclease from *Micrococcus luteus*, prepared by the method of Carrier and Setlow (3), at 37°C for 45 min. The reaction was stopped by addition of an alkaline loading dye preparation containing (final concentrations) 100 mM NaOH, 1 mM EDTA, 2.5% Ficoll, and 0.05% bromocresol green. The DNA samples were electrophoresed at 0.5 V/cm for 16 h on 0.5% alkaline agarose gels in an alkaline buffer containing 30 mM NaOH and 1 mM EDTA along with two molecular length standards, T4dC+T4dC/*Bgl*I digest mixture (7GT; Wako) and T4dC+T4dC/*Bgl*II digest mixture (8GT; Wako). After electrophoresis, the gels were stained in a 0.5-μg/ml solution of ethidium bromide, photographed, and analyzed (Gel Doc 1000 Molecular Analyst; Bio-Rad). The midpoint of the mass of DNA was photographically determined by determining the median migration distance of each sample, which was converted into the median molecular length (L_{med}) of the DNA relative to the migration patterns of the molecular length standards. The average molecular length (L_n) of the DNA was obtained by using the equation of Veatch and Okada (29): $L_n = 0.6 \times L_{med}$.

The number of ESS per base was calculated as follows (9): ESS/base = $[1/L_{n(+UV)}] - [1/L_{n(-UV)}]$, where $L_{n(+UV)}$ and $L_{n(-UV)}$ are the average molecular lengths of UV-irradiated and nonirradiated samples, respectively.

The ESS remaining ratio, the ratio of the number of ESS during fluorescent light exposure to the number of ESS before fluorescent light exposure, was defined as follows: ESS remaining ratio = [(ESS/base) at t]/[(ESS/base) at t_0], where t is the time of exposure to the fluorescent light irradiation and t_0 is zero time.

Photorepair treatment of DNA in vitro. A solution of *E. coli* photolyase was prepared from nonirradiated *E. coli* by using the method of Friedberg and Hanawalt (10). Briefly, 5×10^9 cells of *E. coli* K-12 were lysed by sonication (20 passes at 70% output; model W185 sonifier; Branson) on ice and centrifuged (120,000 × *g*, 60 min), and this was followed by ammonium sulfate precipitation and chromatography purification by using a 25-ml phenyl-Sepharose column (CL-4B; Sigma) and a 20-ml hydroxylapatite column (Bio-Gel HT; Bio-Rad). The purified photolyase solution was confirmed not to be contaminated with other DNA repair enzymes for dark repair by repair treatment of ESS in vitro without exposure to fluorescent light. Some of the photolyase solution was directly exposed to the MP UV lamp in vitro at a dose of 6.3 mJ · cm^{-2} to obtain MP UV-exposed photolyase. Separate from the photolyase preparation, the genomic DNA of *E. coli* was extracted from an *E. coli* suspension previously exposed to an MP UV lamp in vivo at a dose of 6.3 mJ · cm^{-2}. The extracted DNA was suspended in the UV endonuclease buffer solution as described above and was mixed with the intact or the MP UV-exposed photolyase; this was followed by immediate exposure to the fluorescent light in vitro at 37°C for 45 min. Subsequently, the DNA-photolyase mixtures were subjected to the ESS assay as described above to determine the number of ESS after the photorepair treatment in vitro.

RESULTS

Inactivation by LP, MP, or MPF UV lamp. Figure 1 shows typical gel images of ESS assay mixtures for *E. coli* exposed to an LP, MP, or MPF UV lamp, indicating that higher UV doses

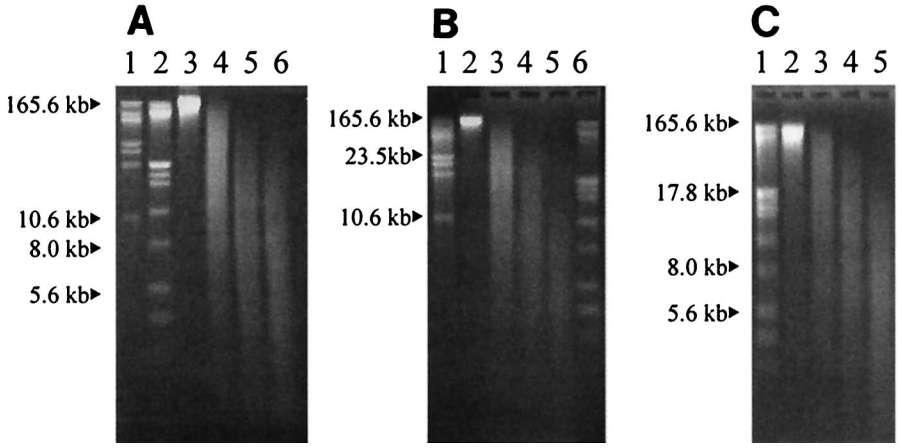

FIG. 1. Gel images for ESS assays of *E. coli* during exposure to LP, MP, or MPF UV lamps. (A) Exposure to LP UV. Lanes 1 and 2, standard markers; lane 3, no UV; lanes 4 to 6, UV doses of 1.9, 3.8, and 5.7 mJ · cm^{-2}, respectively. (B) Exposure to MP UV. Lanes 1 and 6, standard marker; lane 2, no UV;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

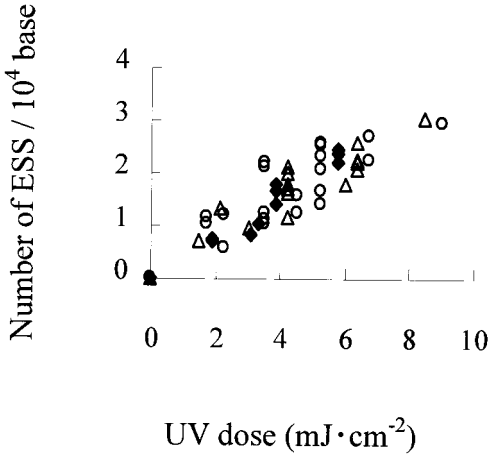

FIG. 2. Numbers of ESS in *E. coli* after exposure to an LP UV lamp (♦), an MP UV lamp (△), or an MPF UV lamp (○). The data are the results of five independent exposures to each type of lamp.

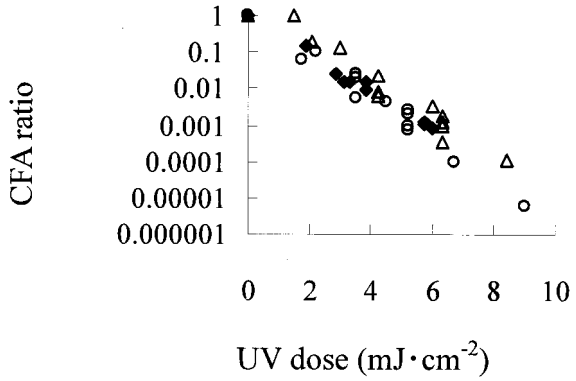

FIG. 3. CFA ratios for *E. coli* after exposure to an LP UV lamp (♦), an MP UV lamp (△), or an MPF UV lamp (○). The data are the results of five independent exposures to each type of lamp.

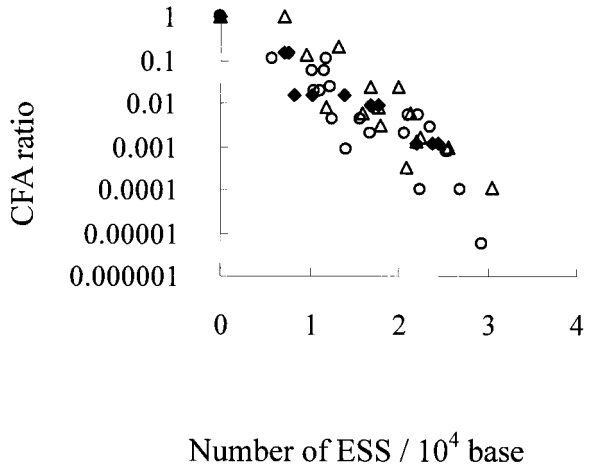

FIG. 4. Relationships between the numbers of ESS and the CFA ratios for *E. coli* after exposure to an LP UV lamp (◆), an MP UV lamp (△), or an MPF UV lamp (○). The data are the results of five independent exposures to each type of lamp.

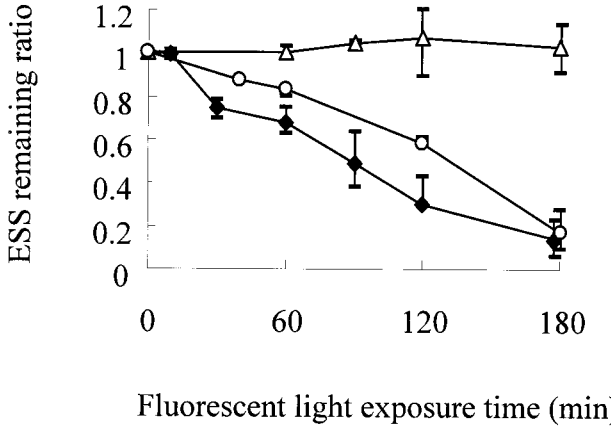

FIG. 6. ESS remaining ratios after exposure to fluorescent light after LP UV (◆), MP UV (△), or MPF UV (○) inactivation. The symbols indicate the means from two or three independent experiments, and the bars indicate the maximum and minimum values.

the CFA ratio recovered so that on average it was equivalent to 0.9- and 1.7-log inactivation, respectively, after exposure to fluorescent light for 3 h. After 3-log inactivation by MP UV irradiation, on the other hand, the CFA ratio showed little recovery and on average was equivalent to 2.3-log inactivation after 3 h of exposure to fluorescent light. Characteristics of photoreactivation after exposure to LP, MP, or MPF UV are summarized in Table 1. The relationships between the ESS remaining ratio and the CFA ratio after LP, MP, or MPF UV inactivation are shown in Fig. 9.

DISCUSSION

During UV exposure, no clear difference was observed among LP, MP, and MPF UV lamps in terms of the ESS and CFA profiles for UV doses determined with the biodosimeter, as shown in Fig. 2 and 3. This suggests that the mechanisms by which LP, MP, and MPF UV lamps inactivate bacteriophage Qβ and *E. coli* were similar in terms of ESS formation as well as in terms of the decrease in CFA. In addition, Fig. 2 and 3 suggest that the UV-A light included in the MP and MPF UV lamp emissions did not cause concomitant photoreactivation during inactivation procedures under our experimental conditions. This is probably because the time of exposure to the MP or MPF UV lamps was too short to utilize the UV-A light for repair, considering that the time commonly required to complete photoreactivation is 1 to 3 h. Photoreactivation seems to be more dependent on the time of exposure to photoreactivating light than to the irradiance of the light, probably because the limiting factor in the photorepair mechanism is the frequency of photolyase attachment to the dimers (14).

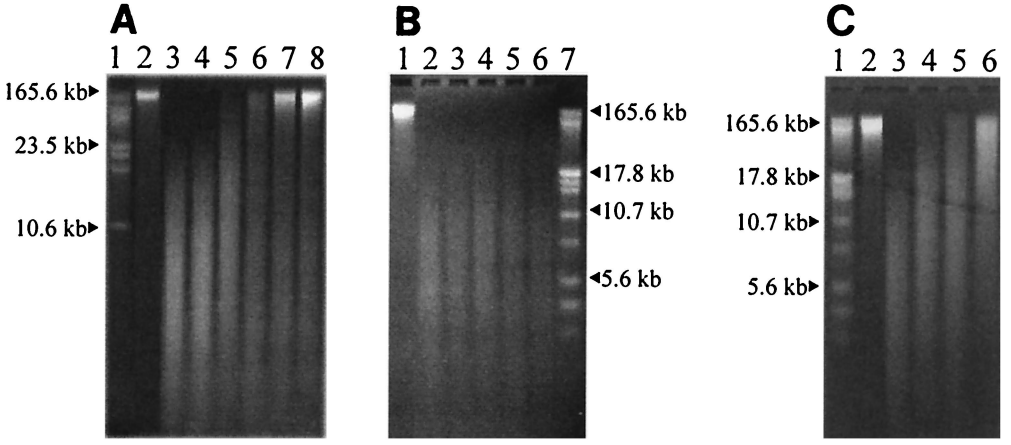

FIG. 5. Gel images for ESS assays of *E. coli* after exposure to fluorescent light after LP, MP, or MPF UV inactivation. (A) Exposure to LP UV. Lane 1, standard marker; lane 2, no UV; lane 3, UV dose of 5.7 mJ · cm^{-2}; lanes 4 to 8, UV dose of 5.7 mJ · cm^{-2}, followed by exposure to fluorescent light for 30, 60, 90, 120, and 180 min, respectively. (B) Exposure to MP UV. Lane 1, no UV; lane 2, UV dose of 6.3 mJ · cm^{-2}; lanes 3 to 6, UV dose of 6.3 mJ · cm^{-2}, followed by exposure to fluorescent light for 60, 90, 120, and 180 min, respectively; lane 7, standard marker. (C) Exposure to MPF UV. Lane 1, standard marker; lane 2, no UV; lane 3, UV dose of 5.4 mJ · cm^{-2}; lanes 4 to 6, UV dose of 5.4 mJ · cm^{-2}, followed by exposure to fluorescent light for 60, 120, and 180 min, respectively.

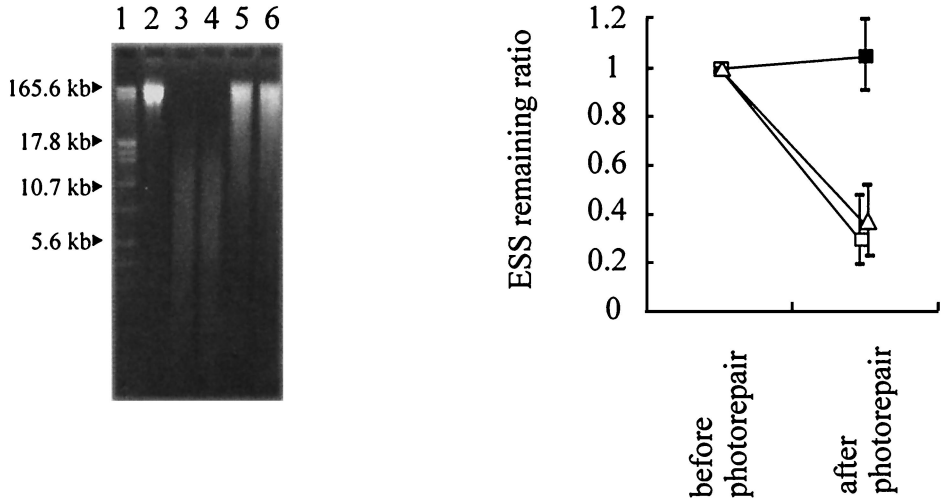

FIG. 7. Photorepair of ESS in vivo (■), in vitro with intact photolyase (□), or in vitro with MP-exposed photolyase (△) after MP inactivation. Lane 1, standard marker; lane 2, no UV; lane 3, MP UV dose of 6.3 mJ · cm^{-2}; lane 4, MP UV dose of 6.3 mJ · cm^{-2}, followed by photorepair in vivo; lanes 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.

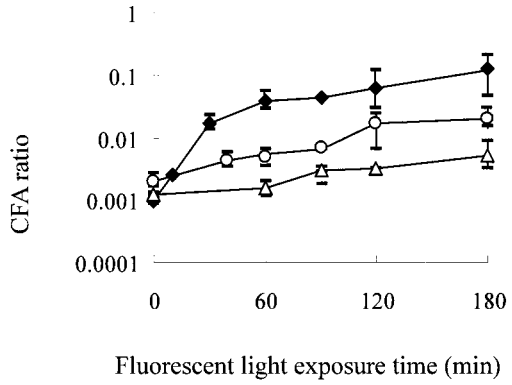

FIG. 8. CFA ratios after exposure to fluorescent light after LP UV (◆), MP UV (△), or MPF UV (○) inactivation. The symbols indicate the means from two or three independent experiments, and the bars indicate the maximum and minimum values.

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.

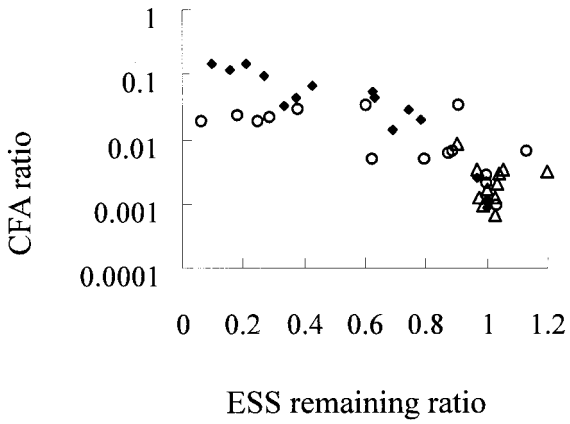

FIG. 9. Relationships between the ESS remaining ratios and the CFA ratios after exposure to fluorescent light after LP UV (♦), MP UV (△), or MPF UV (○) inactivation. The data indicate three independent results for each type of lamp.

UV resulted in less recovery of CFA than LP UV, although the levels of repair of ESS were equivalent after exposure to LP UV and after exposure to MPF UV, suggesting that the contribution of ESS repair to CFA recovery was less after exposure to MPF UV than after exposure to LP UV. This implies that exposure to MPF UV induced more damage besides pyrimidine dimer damage than exposure to LP UV irradiation induced; the latter reduced the recovery of CFA even after the repair of ESS. Among the MPF UV emissions, UV-A (320 to 400 nm) may play an important role in this respect because UV-A indirectly damages organisms through active species. As discussed above (Fig. 4), the ESS-CFA relationships of LP UV and MPF UV were not significantly different in terms of inactivation procedures, and it was therefore assumed that the culturability was regulated mostly by pyrimidine dimers and was not greatly affected by other damage during inactivation. On the other hand, damage in addition to pyrimidine dimer damage was thought to play an important role in the recovery of culturability during photoreactivation procedures. These two results can be reasonably explained by considering that pyrimidine dimer damage and other damage were simultaneously produced by exposure to MPF UV but only pyrimidine dimer damage could be photorepaired by exposure to fluorescent light. Simultaneous formation of pyrimidine dimers and other compounds may have occurred during exposure to MP UV as well, although even pyrimidine dimers could not be photorepaired in this case because of the disorder with photolyase, as discussed above.

In summary, the MP UV lamp was found to be more effective than the LP UV lamp for reducing subsequent photoreactivation of *E. coli* both in terms of photorepair of ESS and in terms of recovery of CFA. Among the emissions of the MP UV lamp, wavelengths from 220 to 300 nm were found to reduce the subsequent photorepair of pyrimidine dimers, possibly by causing a disorder with endogenous photolyase, while wavelengths between 300 and 580 nm were found to play an important role in reducing the recovery of culturability by inducing damage other than pyrimidine dimer damage. It was therefore concluded that inactivating light at a broad range of wavelengths was effective for reducing subsequent photoreactivation of *E. coli*, which could be an advantage that MP UV lamps have over conventional LP UV lamps from the viewpoint of photoreactivation control.

ACKNOWLEDGMENT

We thank Hiroshi Mitani (Department of Integrated Bioscience, University of Tokyo) for his contribution to the photorepair treatment in vitro.

REFERENCES

1. **Abbaszadegan, M., M. N. Hasan, C. P. Gerba, P. F. Roessler, B. R. Wilson, R. Kuennen, and E. V. Dellen.** 1997. The disinfection efficacy of a point-of-use water treatment system against bacterial, viral and protozoan waterborne pathogens. Water Res. **31:**574–582.
2. **Bukhari, Z., T. M. Hargy, J. R. Bolton, B. Dussert, and J. L. Clancy.** 1999. Medium-pressure UV for oocyst inactivation. J. Am. Water Works Assoc. **91:**86–94.
3. **Carrier, W. L., and R. B. Setlow.** 1970. Endonuclease from *Micrococcus luteus* which has activity toward ultraviolet-irradiated deoxyribonucleic acid: purification and properties. J. Bacteriol. **102:**178–186.
4. **Chandrasekhar, D., and B. Houten.** 2000. *In vivo* formation and repair of cyclobutane pyrimidine dimers and 6–4 photoproducts measured at the gene and nucleotide level in *Escherichia coli.* Mutat. Res. **450:**19–40.
5. **Clancy, J. L., T. M. Hargy, M. M. Marshall, and J. E. Dyksen.** 1998. UV light inactivation of *Cryptosporidium* oocysts. J. Am. Water Works Assoc. **90:**92–102.
6. **Craik, S. A., D. Weldon, G. R. Finch, J. R. Bolton, and M. Belosevic.** 2001. Inactivation of *Cryptosporidium parvum* oocysts using medium- and low-pressure ultraviolet radiation. Water Res. **35:**1387–1398.
7. **Craik, S. A., G. R. Finch, J. R. Bolton, and M. Belosevic.** 2000. Inactivation of *Giardia muris* cysts using medium-pressure ultraviolet radiation in filtered drinking water. Water Res. **34:**4325–4332.
8. **Didier, C., J. P. Pouget, J. Cadet, A. Favier, J. C. Beani, and M. J. Richard.** 2001. Modulation of exogenous and endogenous levels of thirodoxin in human skin fibroblasts prevents DNA damaging effect of ultraviolet A radiation. Free Radical Biol. Med. **30:**537–546.
9. **Freeman, S. E., A. D. Blackett, D. C. Monteleone, R. B. Setlow, B. M. Sutherland, and J. C. Sutherland.** 1986. Quantitation of radiation-, chemical-, or enzyme-induced single strand breaks in nonradioactive DNA by alkaline gel electrophoresis: application of pyrimidine dimers. Anal. Biochem. **158:**119–129.
10. **Friedberg, E. C., and P. Hanawalt.** 1988. DNA repair: a laboratory manual of research procedures, vol. 3, p. 461–478. Marcel Dekker, Inc., New York, N.Y.
11. **Friedberg, E. R., G. C. Walker, and W. Siede.** 1995. DNA repair and mutagenesis, p. 92–107. ASM Press, Washington, D.C.
12. **Gehr, R., and H. Wright.** 1998. UV disinfection of wastewater coagulated with ferric chloride: recalcitrance and fouling problems. Water Sci. Technol. **38:**15–23.
13. **Giese, N., and J. Darby.** 2000. Sensitivity of microorganisms to different wavelengths of UV light: implications on modeling of medium pressure UV system. Water Res. **34:**4007–4013.
14. **Harm, W.** 1980. Biological effects of ultraviolet radiation, p. 31–39. Cambridge University Press, New York, N.Y.
15. **Huffman, D. E., T. R. Slifko, K. Salisbury, and J. B. Rose.** 2000. Inactivation of bacteria, virus and *Cryptosporidium* by a point-of-use device using pulsed broad spectrum white light. Water Res. **34:**2491–2498.
16. **Jagger, J.** 1981. Near-UV radiation effects on microorganisms. Photochem. Photobiol. **34:**761–768.
17. **Kalisvaart, B. F.** 2001. Photobiological effects of polychromatic medium pressure UV lamps. Water Sci. Technol. **43:**191–197.
18. **Kamiko, N., and S. Ohgaki.** 1989. RNA coliphages Qβ as a bioindicator of the UV disinfection efficiency. Water Sci. Technol. **21:**227–231.
19. **Kim, S. T., K. Malhotra, J. S. Taylor, and A. Sancar.** 1996. Purification and partial characterization of (6–4) photoproduct DNA photolyase from *Xenopus laevis*. Photochem. Photobiol. **63:**292–295.
20. **Oguma, K., H. Katayama, H. Mitani, S. Morita, T. Hirata, and S. Ohgaki.** 2001. Determination of pyrimidine dimers in *Escherichia coli* and *Cryptosporidium parvum* during UV light inactivation, photoreactivation, and dark repair. Appl. Environ. Microbiol. **67:**4630–4637.
21. **Oppenheimer, J. A., J. G. Jacangelo, J.-M. Laine, and J. E. Hoagland.** 1997. Testing the equivalency of ultraviolet light and chlorine for disinfection of wastewater to reclamation standards. Water Environ. Res. **69:**14–24.
22. **Oppezzo, O. J., and R. A. Pizarro.** 2001. Sublethal effects of ultraviolet A radiation on *Enterobacter cloacae*. J. Photochem. Photobiol. B Biol. **62:**158–165.
23. **Petersen, A. B., R. Gniadecki, J. Vicanova, T. Thorn, and H. C. Wulf.** 2000.

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.