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Inactivation of Enterococci and Fecal Coliforms from Sewage and Meatworks Effluents in Seawater Chambers

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Inactivation in sunlight of fecal coliforms (FC) and enterococci (Ent) from sewage and meatworks effluents was measured in 300-liter effluent-seawater mixtures (2% vol/vol) held in open-topped chambers. Dark inactivation rates (k_Ds) were measured (from log-linear survival curves) in enclosed chambers and 6-liter pots. The k_D for FC was 2 to 4 times that for Ent, and inactivation was generally slower at lower temperatures. Sunlight inactivation was described in terms of shoulder size (n) and the slope (k) of the log-linear portion of the survival curve as a function of global solar insolation and UV-B fluence. The n values tended to be larger for Ent than for FC, and the k values for FC were around twice those for Ent in both effluent-seawater mixtures. The combined sunlight data showed a general inactivation rate (k) ranking in effluent-seawater mixtures of meatworks FC > sewage FC > meatworks Ent > sewage Ent. Describing 90% inactivation in terms of insolation (S_{90}) gave far less seasonal variation than T_{90} (time-dependent) values. However, there were significant differences in inactivation rates between experiments, indicating the contribution to inactivation of factors other than insolation. Inactivation rates under different long-pass optical filters decreased with the increase in the spectral cutoff wavelength (λ_{50}) of the filters and indicated little contribution by UV-B to total inactivation. Most inactivation appeared to be caused by two main regions of the solar spectrum—between 318 and 340 nm in the UV region and >400 nm in the visible region.

Microbiological water quality standards in coastal waters receiving effluent discharges have traditionally been based on specified counts of total or fecal coliform (FC) bacteria, depending on the use of the waters for contact recreation and shellfish harvesting. Thus, FC inactivation rates have been one of the key parameters in ocean outfall design. However, recently, guidelines based on enterococci (Ent), a subset of the fecal streptococci (FS), have been proposed or adopted in a number of states in the United States (11), in Canada (9), in Australia (3), and in New Zealand (34). These guidelines are based on the results of epidemiological studies conducted for the United States Environmental Protection Agency, which suggest that Ent are better predictors of gastrointestinal illness risk than FC in marine recreational waters (6, 7).

The shift to the alternative fecal indicator has created a need for more information on Ent inactivation in coastal waters. Although FC inactivation rates have been extensively investigated (20, 21, 25, 27, 38), there is much less information available for Ent. Wherever FC and Ent have been compared (although most workers have described FS inactivation, the majority of isolates on the media used were probably Ent), Ent have generally shown greater persistence (17–19, 54). However, interpretation of these results is often difficult because, for example, an artificial light source was used, incident solar radiation was not measured, or the spectral transmission characteristics of the containers and effluent-seawater mixtures were not defined.

Bacterial inactivation in seawater involves many factors, the most important being solar radiation (10, 23, 32). Inactivation may also be affected by nutrient availability (17), salinity (2, 17, 28, 39, 50), temperature (17, 20, 43, 50), pH (14, 50), and microbial predation (35, 44, 45). An additional complication is the ability of some bacterial cells to remain viable in seawater, even though they cannot be cultured on standard selective media (15, 26, 59). However, selective media are universally used in water monitoring and were employed in the United States Environmental Protection Agency epidemiological investigations, suggesting that culturable fecal indicator counts are valid predictors of disease risk. Thus, the emphasis of research into microbial inactivation in surface water continues to be the effects of sunlight on counts of culturable cells.

Laboratory studies, as compiled by Calkins and Barcelo (8), indicate that the UV-B band (280 to 320 nm) is the most intrinsically bactericidal portion of the solar spectrum, causing direct (photobiological) damage to the DNA (although this is often repairable). The action spectrum is lower by 4 orders of magnitude in the UV-A band (320 to 400 nm) and decreases further with increasing wavelength. A reported action spectrum for an *Escherichia coli* wild type, a reference solar irradiance spectrum, and the expected spectrum of lethal action for *E. coli* (the product of the other two curves) are presented in Fig. 1A. The predicted maximum inactivation of this bacterium in sunlight due to photobiological damage is around 305 nm in the UV-B band.

Above 320 nm, the action spectrum deviates from the pattern of the DNA absorption spectrum, suggesting the increasing contribution to inactivation of other (photochemical) mechanisms. Photochemical damage occurs when sunlight is absorbed by a sensitizer, which enters an excited state and initiates damaging reactions (57). Photosensitized reactions at the wavelengths found in sunlight are usually more injurious in the presence of oxygen (56), from which the excited sensitizer may form a number of reactive species, including singlet oxygen and hydrogen peroxide. The resulting cell damage (photooxidation) can be caused by both internal (endogenous) and external (exogenous) sensitizers. In seawater, an increase in sunlight attenuation occurs with decreasing wavelength (31),

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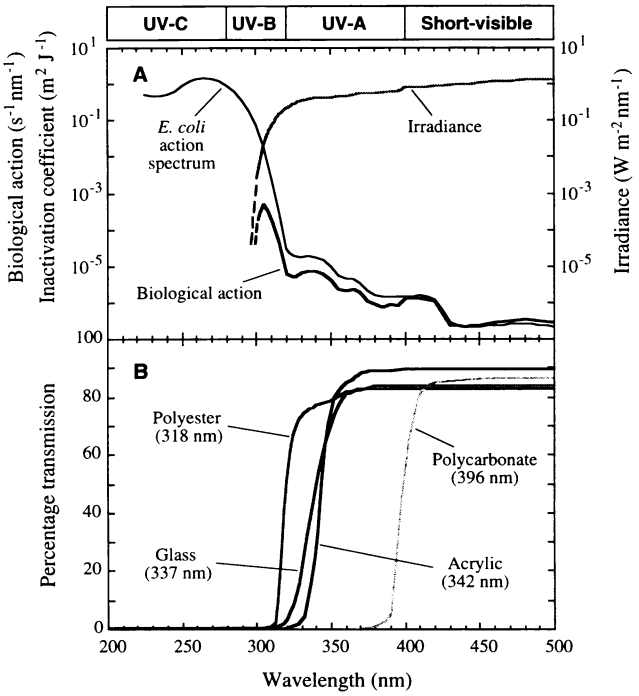

FIG. 1. (A) Action spectrum for *E. coli* (56) compared with typical irradiance for New Zealand (5) (poorly defined for wavelengths <300 nm) and derived *E. coli* biological action (product of the action spectrum and irradiance). (B) Spectral transmission curve of the optical filters used as chamber covers, including spectral cutoff (λ_{50}, wavelength at which the percentage of light transmission is halved) for each filter (obtained by scanning the filter materials from 200 to 800 nm, against an air reference, with a PU 8800 spectrophotometer).

which is likely to further increase the contribution to inactivation of photooxidation, compared with direct photobiological damage. The cytoplasmic membrane is the usual target for endogenous (41) and exogenous (52) sensitizers in *E. coli*, although endogenous sensitizers may also cause DNA damage (52). Membrane damage appears to render the cell more susceptible to damage from factors such as salinity (40).

The type of effluent may also be expected to affect fecal indicator inactivation rates. In New Zealand, in addition to urban sewage effluents, several major meat processing (meatworks) plants discharge effluent (after various levels of treatment) into coastal waters (58). Sewage and meatworks effluents have different physicochemical and spectral characteristics (12, 13)—which imply differences in sunlight attenuation and in photosensitizing action—and different microbial compositions (46). These differences suggest that bacterial inactivation in meatworks and sewage effluents should be investigated separately.

This paper describes an investigation into the comparative inactivation in seawater of FC and Ent from raw sewage and meatworks effluent by use of large, open-topped chambers, located outdoors beside the laboratory facilities. This approach allowed the processing of large numbers of samples and the use of data from nearby solar radiation monitoring stations. It also assisted in the comparison of the two effluent types and in the establishment and control of different experimental treatments, particularly the use of a range of optical filters to investigate the effects on inactivation of specific UV-B and visible wavelength bands. The optical filter experiments complemented the concurrent study by Davies-Colley et al. (16), in which submersible silica flasks were used to investigate fecal indicator inactivation, particularly the effects of sunlight attenuation with depth in the water column.

MATERIALS AND METHODS

Experimental facilities. The experimental site was located in a suburb of Christchurch (latitude 43°S), New Zealand, adjacent to the laboratory used for the bacteriological analyses.

Seawater-effluent mixtures were contained in white, plastic, open-topped chambers (600 mm wide by 900 mm long by 680 mm deep) filled to a depth of 560 mm (volume, approximately 300 liters). Because replicate chambers were not used, experimental procedures were designed to minimize between-chamber variability. The chambers were placed in a swimming pool, which was filled with 13,000 liters of fresh water, to a level about 100 mm below that of the seawater inside the chambers. The pool thereby acted as a thermal water jacket. In practice, there was <0.5°C temperature difference between chambers, and temperatures were usually maintained to within 2°C of the target temperature (that prevailing in nearby coastal waters). A 24-W submersible bilge pump on the bottom of each chamber was used to stir the seawater-effluent mixture. A timer simultaneously switched on the pumps for 3 min every half hour (dye tests showed complete mixing of the chamber contents in approximately 2 min). A plastic sampling tube opened 400 mm below the water surface in each chamber, with the other end being connected to a rubber seal on a sampling manifold next to the swimming pool. Samples were collected through application of a vacuum to a sampling bottle attached to the appropriate seal.

Bacterial inactivation in the dark was usually investigated in chambers double lined on the outside with aluminum foil and covered with a foil-lined lid. However, in one experiment, the effects of temperature (10° and 20°C) on dark inactivation were studied with 6-liter, foil-lined, enclosed pots, held in temperature-controlled air incubators. The effluent-seawater mixture in each pot was stirred for 3 min every half hour with a plastic paddle, and samples were extracted with a plastic tube and syringe.

To investigate the contributions of different regions of the UV-visible spectrum to inactivation, different chambers were covered with plate glass, polyester, acrylic, and polycarbonate. These four materials acted as long-pass optical filters, screening out different regions of the spectrum (spectral transmission curves are presented in Fig. 1B). The maximum transmission of the filter materials was around 85 to 90%, because of reflection at the filter-air interfaces.

Experimental procedures. The experimental program, which ran from September 1989 until February 1991, is outlined in Table 1. Experiments 1 to 3 ran for 2 days. All subsequent experiments ran for 1 day only. The dark experiments ran for around 10 h.

Two days before each experiment, about 2,000 liters of seawater was collected from a local coastal area (Sumner Wharf) with low background FC and Ent counts—around 1 CFU/100 ml for both indicators. The water was stirred (to ensure uniform water clarity) and pumped into the chambers in the swimming pool.

Two types of effluent were collected approximately 18 h before each experiment. Coarse-screened, raw sewage effluent was collected from the Bromley Sewage Treatment plant, which serves the city of Christchurch. Meatworks effluent was collected from a save-all tank receiving raw effluent from beef-killing chains at a local meat processing plant (Primary

TABLE 1. Summary of experimental program[a]

Expt no.	Season, weather	Water temp (°C)	Light regime[b]
1	Spring, fine	12.5	S
2	Spring, fine	14.0	S
3	Summer, high cloud	17.2	S
4		17.0	D
5		12.6	D
6		10 and 20	D
7	Winter, low cloud	9.1	S
8	Summer, fine	16.6	S
9	Summer, low cloud	19.4	S, D, 337, 342, 396
10	Summer, low cloud	18.5	S, D, 318, 342, 396

[a] Both sewage and meatworks effluents were used in experiments 1 to 8; sewage only was used in experiments 9 and 10.

[b] S, sunlight exposed; D, dark. Numbers are the λ_{50}s (in nanometers) of optical filters.

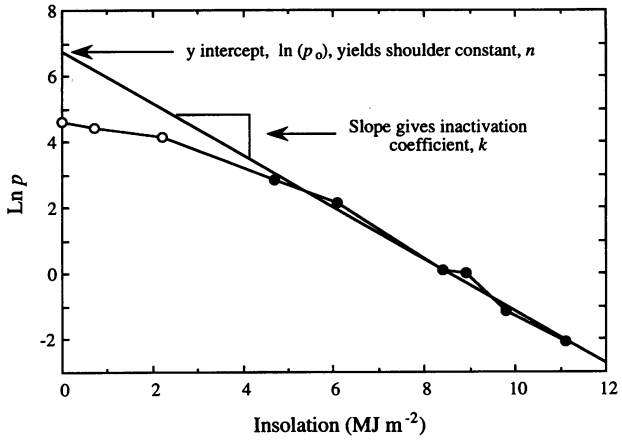

FIG. 2. Calculation of light inactivation parameters for full (○) and linear (●) curves. Example data are ln transformations of the percentage survival (p) of fecal coliforms, as a function of insolation, from experiment 10.

Producers Co-operative Society, Belfast, New Zealand). Both effluents were stored overnight at about 6°C (tests showed no significant overnight change in FC or Ent counts).

At the start of each experiment, effluent was added to each chamber of seawater, to give a concentration of 2% (vol/vol). After being stirred with the bilge pumps for 3 min, initial samples were collected in sterile glass bottles, by the tube and manifold system. Subsequent samples were collected hourly. To minimize cross-contamination between samplings, the manifold seals were rinsed with 70% ethanol and then rinsed twice with sterile water. The first 100 ml drawn into the evacuated sample bottle was discarded, and then a 500-ml sample was collected. The manifold was about 1 m above the chambers, so residual liquid in the tubes quickly flowed back into the chambers.

Samples were quickly transferred to the laboratory and held in the dark at about 15°C. Holding time between collection and analysis was typically ≤30 min, and bias from variations in holding time was expected to be negligible (16).

Laboratory analyses. Seawater-effluent mixtures were analyzed for Ent and FC by membrane filtration (Sartorius CN; 0.45-μm pore size), with dilution series of phosphate buffer (53), as required. Enumeration of Ent was by incubation on mE agar (Difco) at 41 ± 0.5°C for 48 h, followed by transfer to esculin-iron agar (EIA) for a further 20 min at 41°C (53). FC were incubated on mFC agar (Gibco) at 44.5 ± 0.5°C for 24 ± 2 h (1). Counts were expressed as CFU per 100 ml.

Speciation of Ent isolates from the beginnings and ends of five experiments was carried out, and the results have been reported separately (46).

Solar radiation and temperature measurements. Global (i.e., diffuse plus direct) solar irradiance and erythemal UV-B irradiance data were obtained from sensors located, respectively, at Christchurch Airport (5.5 km northwest of the site) and the New Zealand National Radiation Laboratory (3.4 km east of the site). Global solar irradiance was measured with an Eppley Pyranometer, which records hourly solar insolation (MJ m^{-2}). UV-B was measured with an erythemal sensor—one of a national sunburn network (48) used to monitor sunlight wavelengths responsible for erythema (skin reddening). This sensor records average UV-B irradiance every 10 min. The output signal was calibrated as an equivalent irradiance at 310 nm (i.e., the monochromatic irradiance at 310 nm, in mW m^{-2}, that would give the same erythemal response).

Although the action spectrum for erythema (and the response of the sensor) may be different from that for bacterial inactivation, the erythemal sensor output was used because it was the only available nearby monitor of short wavelength, solar UV radiation, which is intrinsically the most bactericidal part of the solar spectrum (Fig. 1).

To maintain parity with local sea temperatures, the target temperature for each experiment was set to the 1987 mean sea surface temperature for the relevant month. Chamber temperature was monitored hourly with a probe suspended 200 mm below water level in one chamber.

Calculation of inactivation parameters. A linear regression line was fitted to the (ln-transformed) counts from the dark chambers (all containers in experiments 4, 5, and 6 and dark controls in experiments 9 and 10) to derive the dark inactivation rate coefficient (k_D), in ln units per hour.

In tanks exposed to sunlight, the percent survival (p) at exposure time t was defined as $p = 100(N/N_0)$, where N is the CFU count and N_0 is the initial count. Sunlight inactivation parameters (Fig. 2) were evaluated from plots of ln p versus insolation (global solar radiation integrated from time 0 to t) or UV-B fluence (time-integrated UV-B irradiance). In experiments 9 and 10 (Table 1), CFU percentages were corrected for dark inactivation, with the equation $p_s = p \exp(k_D t)$. The sunlight inactivation curves (e.g., those shown in Fig. 2) usually displayed a recognizable shoulder, so a two-parameter multitarget kinetic expression (29, 55) was fitted to the data:

$$p_s = 100\{1 - [1 - \exp(-k_s S)]^{n_s}\} \quad (1)$$

where S is insolation, k_s (m^2 MJ^{-1}) is an inactivation coefficient, and the exponent n_s is a dimensionless parameter quantifying the size of the shoulder. The inactivation coefficient was obtained from the final slope of the inactivation curve ($k_s = -\Delta \ln p/\Delta S$), by use of the linear regression of ln$p$ versus S, omitting points in the shoulder region (open symbols in Fig. 2) where present. The parameter n_s (the shoulder constant) was evaluated as $n_s = p_0/100$, where $\ln(p_0)$ is the y axis intercept of the regression line. The shoulder constant may be interpreted as the average number of targets needing to be hit before a CFU is inactivated (29).

To enable comparison with other studies, the S_{90}, UV_{90}, and T_{90} values (respectively, the insolation, UV-B fluence, and time taken to achieve a 90% reduction in CFU count) were

TABLE 2. Mean dark inactivation coefficients for cold and warm temperature ranges

Effluent type[a]	Expt no.	Temp range[b]	Mean dark inactivation of[c]: FC k_D, h^{-1}	FC T_{90} (h)	Ent k_D, h^{-1}	Ent T_{90} (h)
Sewage	5, 6	Cold	0.020 (116)	115	0.005 (72.2)	461
	4, 6	Warm	0.028 (88.8)	82.3	0.008 (7.53)	288
Meatworks	5, 6	Cold	0.019 (4.20)	121	0.008 (78.2)	288
	4, 6	Warm	0.039 (58.3)	59.1	0.009 (47.9)	256

[a] 2% (vol/vol) in seawater.
[b] Cold, 8 to 10°C; warm, 15 to 20°C.
[c] Percent CVs for k_Ds are given in parentheses.

also calculated for dark and sunlight experiments, as applicable. S_{90} values were calculated by substitution in equation 1 of $p = 10\%$ and solving for S (similarly for UV_{90}). T_{90} values were calculated by plotting p against time and solving for $p = 10\%$.

Because replicate chambers were not used, confidence intervals of the membrane filtration analysis were estimated, on the basis of the procedures given by the American Public Health Association (1), to obtain some measure of the variability of the CFU percentages.

RESULTS

Dark inactivation. Mean inactivation coefficients (k_Ds) and 90% inactivation times (T_{90}s) for the three experiments (experiments 4, 5, and 6) in which both effluents were held in the dark are presented in Table 2. The results were subdivided into cold (8 to 10°C) and warm (15 to 20°C) seawater-effluent mixtures. For each effluent, the results show a consistent inactivation rate ranking of FC > Ent and warm > cold. However, with one exception, analysis of the combined inactivation curve data by the total regression method described by Zar (60) showed that these differences were not statistically significant at the 95% level. The exception was Ent inactivation in sewage-seawater (warm > cold; $P < 0.02$). There were no consistent patterns of dark inactivation between effluents.

Dark (overnight) inactivation was also recorded in each of the three experiments run over 2 days (experiments 1 to 3), i.e., between the last sample on day 1 and the first sample on day 2. Overnight inactivation rates tended to be higher than those recorded in the dark-only experiments (experiments 4 to 6). Typical 2-day results (from experiment 3) are presented in Fig. 3.

Inactivation in sunlight. Sunlight inactivation curves for FC and Ent in sewage-seawater mixtures, for the six spring and summer experiments (Table 1), are shown in Fig. 4A. The equivalent results for meatworks effluent-seawater mixtures are presented in Fig. 4B, for experiments 1, 2, 3, and 8 only (experiments 9 and 10 did not include meatworks effluent). The curves for experiment 7 (winter) are not presented, because they would not be discernible on the graphs (total insolation was less than 4 MJ m^{-2}). The stippled zones indicate reasonable concordance of the inactivation curves for each indicator, with Ent persisting longer than FC in both effluent-seawater mixtures. The zones overlap in, and beyond, the shoulder regions of the curves, up to an insolation of around 10 MJ m^{-2}.

Calculated mean inactivation coefficients and shoulder constants for insolation and UV-B fluence are presented for sewage and meatworks effluent-seawater mixtures in Table 3. To allow comparison between effluents, only data from the five experiments in which both effluents were studied were used in the calculations. The mean shoulder constants (n_S) for sewage-seawater mixtures were slightly lower for FC than for Ent, but for FC in meatworks effluent-seawater they were over twice those for Ent, primarily because of unusually large shoulders recorded for FC in experiment 3. The mean k values for FC in Table 3 (i.e., the slopes of the log-linear portion of the survival curves) were around twice those for Ent for both effluents.

Table 4 shows that, with the exception of an anomalous result for meatworks effluent-seawater in experiment 7 (winter), the cumulative radiation required to inactivate 90% of Ent, as indicated by the mean S_{90} and UV_{90} values, was higher than that for FC, but for each indicator there was little difference between the effluents. The winter T_{90}s were higher than the mean summer T_{90}s for both indicators and effluent-seawater mixtures.

The combined data from the linear portions of the inactivation curves in the sunlight experiments were analyzed by the linear regression method described by Zar (60). This analysis showed an overall inactivation rate ranking of meatworks FC > sewage FC > meatworks Ent > sewage Ent. Comparison of the slopes showed that, overall, FC were inactivated significantly more rapidly than Ent in both raw sewage ($P < 0.001$) and meatworks effluent ($P < 0.001$). Both Ent and FC

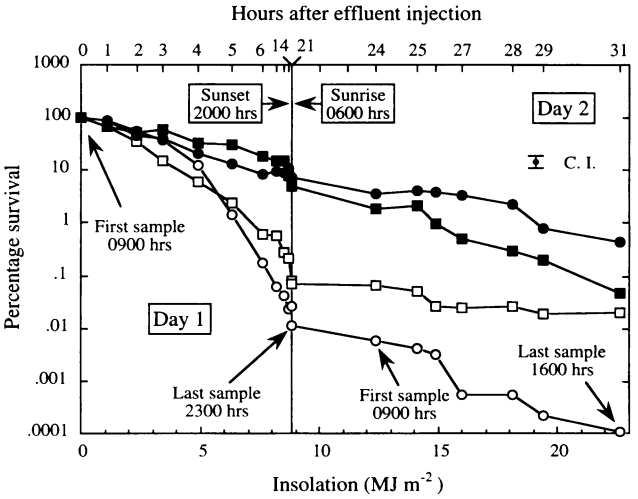

FIG. 3. Inactivation of fecal coliforms from sewage (□) and meatworks effluent (○) and enterococci from sewage (■) and meatworks effluent (●) over a 2-day period. Data are from experiment 3; C.I., representative 95% confidence interval of the membrane filtration analysis.

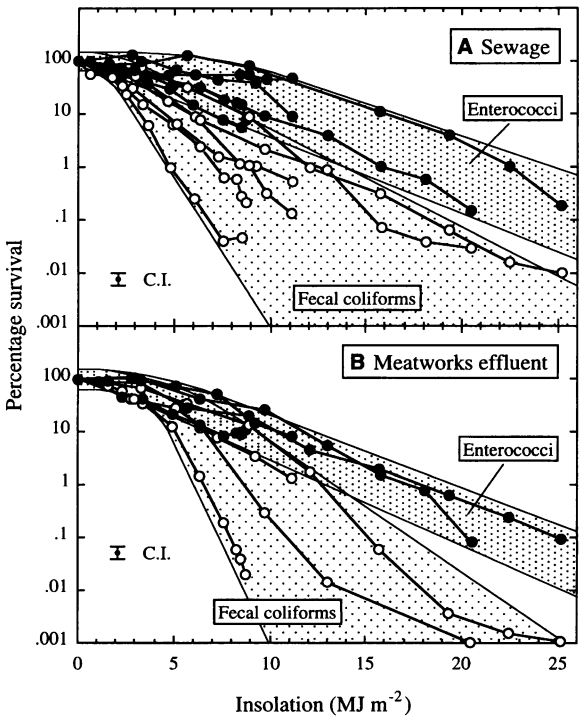

FIG. 4. Inactivation in seawater, as a function of insolation, of fecal coliforms (○) and enterococci (●) in experiments 1 to 3 and 8 to 10 for (A) untreated sewage and (B) meatworks effluent (experiments 9 and 10 did not include meatworks effluent). C.I., representative 95% confidence interval of the membrane filtration analysis. The stippled zones indicate the degree of concordance of the inaction curves for each indicator (see the text).

were inactivated more rapidly in meatworks effluent-seawater than in sewage-seawater. This difference was significant for FC ($P < 0.05$) but not for Ent ($P < 0.5$).

Analysis of the linear portions of the sunlight inactivation curves by the pooled regression method described by Zar (60) showed that, for each indicator and each effluent-seawater mixture, the slopes were significantly different between experiments (from $P < 0.01$ to $P \ll 0.005$), demonstrating the influence of factors other than sunlight on inactivation.

In each of the 2-day experiments, e.g., experiment 3 (Fig. 3), similar rates of Ent inactivation in sunlight were recorded on both days. However, FC inactivation tended to be lower on day 2, particularly in the sewage effluent-seawater mixture.

Contributions of different spectral regions to inactivation. In both optical-filter experiments (Table 1), FC and Ent inactivation rates decreased with increasing spectral cutoff (λ_{50}) of the optical filters—full sun > 318 nm (polyester) > 337 nm (glass) > 342 nm (acrylic) > 396 nm (polycarbonate) > dark. The inactivation curves for FC and Ent for experiment 10 are presented in Fig. 5.

In Fig. 6, k_S values, corrected for ≈85% transmission in the long-pass region of the spectrum, are plotted against the λ_{50} of the filters, for both optical-filter experiments. The flat curve between the full sun (reference solar cutoff wavelength, 290 nm—see the irradiance curve in Fig. 1A) and the 318-nm (polyester) filter (experiment 10) indicates that there was little contribution to total inactivation of either indicator by UV-B radiation. In contrast, the marked drop in the curve between the 318-nm (polyester) and 342-nm (acrylic) filters in experiment 10 indicated a strong contribution to inactivation from the intervening UV-A region centered on 330 nm. However, the very sharp drop between the 337-nm (glass) and 342-nm (acrylic) filters in experiment 10 may reflect the broader cutoff characteristics of the glass filter (Fig. 1B), which permitted the transmission of some relatively bactericidal radiation in the vicinity of 330 nm. The longer-wavelength UV-A between the 342-nm (acrylic) and 396-nm (polycarbonate) filters contributed about 25% of total FC inactivation, but only 7% of Ent inactivation. Visible light (>400 nm) contributed about 40% of the total inactivation of both indicators.

DISCUSSION

Dark inactivation and temperature effects. The lack of statistically significant differences (at the 95% level) between most of the k_D values in Table 2 was due to the high scatter in the dark data (most of the coefficients of variation (CVs) in Table 2 are considerably higher than those in Table 4) combined with the shallow slope of the survival curves (i.e., slow inactivation). Nevertheless, the observed inactivation ranking of FC > Ent is in agreement with other studies. For example, Evison (17) showed that FS were more resistant than FC to dark inactivation and that this was related to greater tolerance of seawater salinities. Ent may also be more resistant to lytic organisms and to predation by protozoans, which appear to consume different bacterial species with different efficiencies (37). For example, González et al. (24) attributed longer persistence of *Enterococcus faecalis* than of *E. coli* in dark vessels to slower digestion of the former organism by protozoan ciliates and flagellates.

The warm > cold rankings of the k_D values in Table 2 are also in agreement with other studies (20, 22), although the cold and warm temperature ranges (representing those in nearby coastal waters) may have been too close to provide statistically

TABLE 3. Sunlight inactivation parameters for FC and Ent for sewage and meatworks effluents (insolation and UV-B fluence)

Effluent type[a]	Sunlight inactivation parameter for[b]:							
	FC				Ent			
	Insolation		UV-B		Insolation		UV-B	
	k_S (m^2 MJ^{-1})	n_S	k_{UV} (m^2 kJ^{-1})	n_{UV}	k_S (m^2 MJ^{-1})	n_S	k_{UV} (m^2 kJ^{-1})	n_{UV}
Sewage	0.515 (22.8)	1.45	0.583 (26.8)	1.42	0.310 (19.6)	2.58	0.357 (68.0)	2.80
Meatworks	0.759 (54.7)	7.21	1.017 (35.2)	16.7	0.373 (27.1)	2.65	0.481 (75.1)	5.13

[a] 2% (vol/vol) in seawater.

[b] The data are the means of experiments 1, 2, 3, 7, and 8 (in which both effluents were studied); arithmetic means were used for the k values, and geometric means were used for the n values; k, inactivation rate of linear portion of survival curve; n, shoulder constant (Fig. 2); percent CVs for k values are given in parentheses.

TABLE 4. Summary of insolation and UV-B fluence levels and time required to achieve 90% inactivation of FC and Ent

Effluent type[a]	Expt no	Level of indicated parameter required to achieve 90% inactivation of[b]: FC			Ent			S_{90} Ent/ S_{90} FC
		S_{90} (MJ m^{-2})	UV_{90}[c] (kJ m^{-2})	T_{90} (h)	S_{90} (MJ m^{-2})	UV_{90}[c] (kJ m^{-2})	T_{90} (h)	
Sewage	7[d]	4.54		8.5	6.04		12.0	1.33
	1,2,3,8[e]	6.34 (50.7)	4.66 (13.0)	2.9 (47.0)	11.82 (19.4)	10.98 (33.5)	6.4 (40.9)	2.10
	1,2,3,7,8	5.98 (48.5)	4.66 (13.0)		10.66 (30.6)	10.98 (33.5)		1.95
Meatworks	7[d]	4.85		8.8	3.82		7.3	0.79
	1,2,3,8[e]	6.22 (24.5)	5.15 (19.6)	3.1 (32.1)	10.12 (16.9)	8.36 (12.8)	5.2 (33.5)	1.68
	1,2,3,7,8	5.94 (24.4)	5.15 (19.6)		8.86 (35.9)	8.36 (12.8)		1.50

[a] 2% (vol/vol) in seawater.
[b] Data are arithmetic means for insolation (S_{90}), UV-B fluence (UV_{90}), and time (T_{90}) for the experiments in which both sewage and meatworks effluents were studied. Percent CVs are given in parentheses.
[c] No UV data were available for experiments 1 and 7.
[d] Winter experiment.
[e] Summer experiments.

significant differences. Increases in inactivation at higher temperatures have been attributed to increases in the activity of predatory and lytic organisms (20) and to the detrimental effects of increased metabolism at low nutrient levels (22). Overall, the FC and Ent T_{90}s in sewage-seawater (Table 2) were similar to those reported by Gameson (20) for FC and FS at the same temperature ranges.

Although dark (overnight) inactivation in the 2-day sunlight experiments (e.g., Fig. 3) tended to be more rapid than that recorded in the dark-only experiments, the bacteria had already been exposed to direct solar radiation. In addition, the "dark" period of overnight inactivation probably included some exposure to diffuse early-morning sunlight on day 2, between sunrise and the first sample.

Inactivation in sunlight. The T_{90}s for FC in Table 4 fall within the reported averages for New Zealand waters, for the 0900 to 1500 h period, for both winter and summer (4). Overall, the Table 4 data show that Ent had higher 90% inactivation values (S_{90}s, UV_{90}s, and T_{90}s) than FC. The mean FC k values were around twice those of Ent (Table 3), which is similar to the results obtained by Gameson (21) for FC and FS and by Davies-Colley et al. (16) for FC and Ent.

The greater persistence of Ent, compared with FC, in

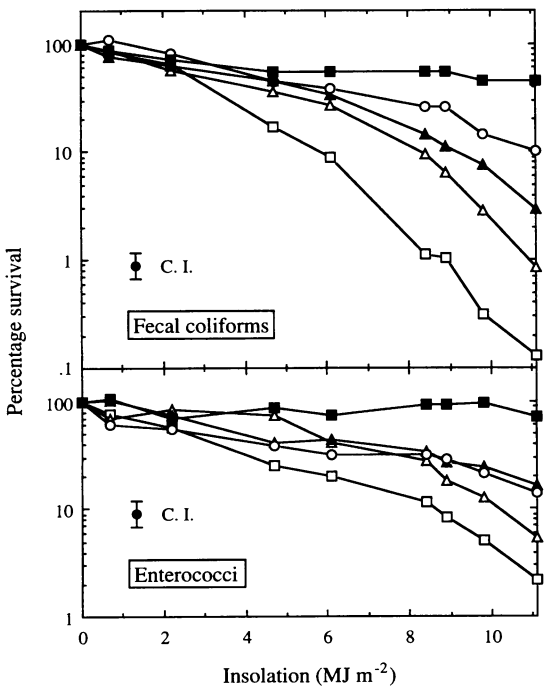

FIG. 5. Inactivation in seawater of fecal coliforms and enterococci in sewage-seawater in the dark (■) and full sun (□) and under 396-nm (polycarbonate, ○), 342-nm (acrylic, ▲) and 318-nm (polyester, △) optical filters. Data are from experiment 10. C.I., representative 95% confidence interval of the membrane filtration analysis.

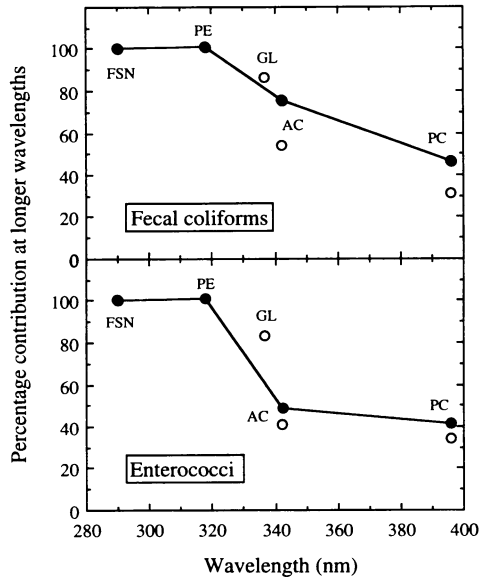

FIG. 6. Cumulative distribution of contributions of different regions of the solar UV spectrum to inactivation of fecal coliforms and enterococci. Plotted data are inactivation k_S values (Table 5) for Experiment 10 (●), expressed as a percentage of the k_S value in the full sun (FSN) chamber and plotted against λ_{50} (spectral cutoff) wavelengths of the polyester (PE, 318-nm), acrylic (AC, 342-nm), and polycarbonate (PC, 396-nm) optical filters. Data from experiment 9 (○), in which glass (GL) was used instead of polyester, are presented for comparison. The k_S values have been corrected for transmission losses due to surface reflection in the long-pass region of the spectrum for each filter.

sunlight-exposed seawater is in agreement with most reported studies (17–20, 23, 28, 36, 47, 54). However, the reasons for this difference are unclear. Cell shape and size may confer some physical advantage over the FC group. Cells of *E. coli* (the principal species) are rod shaped, ranging in diameter from 1.1 to 1.5 μm and in length from 2.0 to 6.0 μm, whereas Ent are spherical and <2.0 μm in diameter (49). Thus, a suspension of FC cells may present greater overall surface area to sunlight effects than the equivalent number of Ent cells. In addition, FC cells tend to exist singly, whereas Ent cells frequently occur in pairs or chains. Thus, the multiple targets (29) represented by the cell aggregates may have contributed to the generally larger shoulders in the Ent curves. Depending on chain length, some Ent cells may also be partially shielded from sunlight effects by adjacent cells. The association of bacterial cells with particles, which has been shown to partially protect coliform bacteria from UV disinfection in wastewater treatment experiments (42), may have influenced inactivation rates. However, the extent to which Ent and FC could be differentially protected by particles appears to be unknown.

Different susceptibilities to various endogenous and exogenous sensitizers may also contribute to differences in sunlight-induced inactivation rates between FC and Ent. Because the cell membrane is the principal target of photooxidation, differences in cell wall structure between FC and Ent may be significant. The gram-negative FC cells have a complex cell wall structure, with an inner and outer membrane separated by a periplasm, whereas the gram-positive Ent have more compact cell walls, containing a higher proportion of peptidoglycan. The functions of cell wall structures in both groups are not fully understood, but it is known that, in gram-negative organisms, the periplasm is the site of enzymes involved in important electron transport processes. In *E. coli*, cell wall damage from exposure to near-UV radiation has been shown to inhibit these processes (51), cause leakage of certain ions (33), and cause cells to suffer osmotic stress in saline conditions (40). There appears to be little equivalent information available on the effects of sunlight-induced cell wall damage to gram-positive bacteria.

In the 2-day experiments (e.g., Fig. 3), the generally lower rates of FC inactivation recorded on day 2, compared with day 1, may indicate the presence of a sunlight-resistant FC population or a sunlight-protected population associated with particles (42). It is also possible that, by day 2, in spite of stirring of the mixtures every half hour, dissolved oxygen concentrations in the seawater-effluent mixtures had fallen to an extent which decreased the photooxidative impact of the sunlight. Similar factors may explain why, at higher summer insolations (experiments 2 and 8), in both seawater-seawater mixtures (Fig. 4), the FC survival curve tended to flatten towards the end of the sampling period. However, some FC cell repair (photoreactivation) of any direct photobiological damage may also have occurred during exposure to longer solar wavelengths in the late afternoon (29, 30). Whatever combination of these factors may have been involved, it appears that, within the irradiance ranges studied, they differentially affected FC, because the log-linear portions of the Ent curves showed little change in slope in either the 1- or 2-day experiments.

The effects of effluent type on inactivation. The greater variability in k values for FC and Ent from meatworks effluent, as indicated by the higher CVs in Table 3, may reflect the greater physicochemical and microbial variability of this effluent. The sewage collected from the Bromley plant was the integrated and well-mixed output of a large city (population > 200,000), whereas the composition of the meatworks effluent probably varied between experiments, depending on the animal slaughtering and processing schedule at the plant.

The generally more rapid inactivation of FC and Ent in meatworks effluent-seawater than in sewage-seawater cannot be attributed directly to greater exposure to inactivating wavelengths (i.e., to photobiological damage), because spectrophotometric absorbance measurements indicated consistently greater attenuation of UV and visible wavelengths in the former effluent. However, the presence of different exogenous sensitizers in the two effluents may have resulted in different photooxidative impacts on the indicators. In addition, different microbiological characteristics, such as population age (older cells tend to be more resistant than younger cells to many environmental factors) and species composition, may have affected inactivation rates. The average age of the bacteria (i.e., the elapsed time between excretion and analysis) was likely to have been between 1 h and 2 days for the meatworks effluent, compared with up to 9 days for the sewage effluent. Sinton and Donnison (46) have found that meatworks effluent has a high proportion of *Enterococcus durans*, which appears to be inactivated more rapidly than *Enterococcus faecium* and *E. faecalis*, which dominate in sewage effluents.

Contributions to inactivation of UV and visible components of sunlight. The spectrum of biological action for *E. coli* in Fig. 1 (i.e., the product of spectral irradiance and the action spectrum) suggests that solar UV-B radiation should account for around 2 orders of magnitude more inactivation than UV-A and short-visible wavelengths. However, Fig. 6 indicates that most inactivation was caused by sunlight in two spectral bands—one centered on 330 nm (UV-A) and one at $\lambda > 400$ nm (short visible). This suggests that wavelength dependence of inactivation in effluent-seawater mixtures may differ from published action spectra (8), most of which are obtained with pure cultures in buffer solutions. However, the 50% point (± 15%) on the curves in Fig. 6 is close to 360 nm, i.e., about half the inactivation was attributable to wavelengths above 360 nm, and half was attributable to wavelengths below 360 nm. These findings are in general agreement with those of Gameson and Gould (23), who reported that 50% of inactivation of coliforms in sewage-seawater mixtures is attributable to wavelengths >370 nm and 25% is attributable to wavelengths >400 nm. The results are also broadly consistent with those of Davies-Colley et al. (16), who showed that the depth dependence of inactivation was very different from the depth profile of UV-B radiation (at about 310 nm) but matched the depth profile of radiation at about 360 nm in the UV-A band.

The finding that UV-B contributed little to overall inactivation for both indicators in the effluent-seawater mixtures (Fig. 6) is supported by indirect evidence. If UV-B had been responsible for most of the inactivation, UV-B fluence should have predicted inactivation better than total (global) solar insolation, whereas in Table 3, the CVs were generally higher for k_{UV} than for k_S.

Differences in inactivation rates between experiments. The statistically significant differences in inactivation rates between sunlight experiments indicate that, although inactivation parameters such as S_{90}s are likely to have broader seasonal and geographical applicability than T_{90}s, there is still an important contribution to inactivation that is not explained by the direct measurement of insolation. In our study, this contribution may have included variables such as the spectral composition of sunlight (such as that associated with cloud cover; Table 1), seawater temperature (Table 1), microbial composition of the effluents and natural seawater populations, clarity of the effluent-seawater mixtures, and dissolved oxygen concentrations. A similar explanation for varying inactivation kinetics

has been advanced by Davies-Colley et al. (16). There is a need for more information on the synergistic relationship between these factors and sunlight-induced inactivation of different fecal indicators and on the wavelength dependence of inactivation in surface waters.

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