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Sunlight Inactivation of Enterococci and Fecal Coliforms in Sewage Effluent Diluted in Seawater

ROBERT J. DAVIES-COLLEY,[1]* ROBERT G. BELL,[1] AND ANDREA M. DONNISON[2]

NIWA Ecosystems, National Institute of Water and Atmospheric Research Ltd.,[1] and Environmental Management Section, Meat Industry Research Institute of New Zealand Inc.,[2] Hamilton, New Zealand

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Inactivation (loss of culturability) by sunlight of enterococci and fecal coliforms within sewage effluent diluted in seawater was investigated in field experiments. In most experiments, 500-ml flasks of pure silica were used to confine activated sludge effluent diluted to 2% (vol/vol) in seawater. Inactivation of bacteria in these flasks (diameter, 0.1 m) was faster than in either open chambers (depth, 0.25 m) or patches of dyed effluent (depth of order, 1 m), probably because of the longer light paths in the latter two types of experiment, which caused greater attenuation of sunlight. Inactivation of 90% of enterococci generally required 2.3 times the insolation required for 90% inactivation of fecal coliforms, because of both the presence of larger initial shoulders on survival curves and a lower final inactivation rate. Two parameters are required to model inactivation of enterococci, a shoulder constant as well as a rate coefficient. The depth dependence of inactivation rate for both fecal indicators matched the attenuation profile of UV-A radiation at about 360 nm. Inactivation by UV-B radiation (290 to 320 nm), which penetrates much less into seawater, is of minor importance compared with the UV-A and visible radiation in sunlight, contrary to expectations in consideration of published action spectra for bacterial inactivation.

The coliform group, especially fecal coliforms (FC), has been the traditional fecal pollution indicator. However, following the epidemiological studies of Cabelli and coworkers (5–8), there has been a revival of interest in the enterococcus group (Ent, a subset of the fecal streptococcus group [FS]). Ent counts (based on culturable enterococcus cells) appear to be a better predictor of the risk to swimmers of contracting gastrointestinal illness, caused mainly by enteric viruses in sewage-contaminated waters (5, 8). In recent years, various government agencies in the United States, Canada, Australia, and New Zealand have recommended enterococcal guidelines for coastal recreational waters.

Considerable research on inactivation (loss of culturability) kinetics of the FC group (and its main constituent species, *Escherichia coli*) in coastal waters has been carried out (15, 16, 18, 19). However, comparative inactivation rates for other fecal indicator bacteria (13) and their response to bactericidal agents, particularly solar radiation, are less well-known. An understanding of the inactivation kinetics of fecal indicators is fundamental for soundly based sewage outfall design and for monitoring to ensure that public health is safeguarded.

Repeatedly, solar radiation has been shown to be the dominant influence on culturable densities of fecal indicator bacteria in open waters, the inactivation rate in sunlight being, typically, 2 or more orders of magnitude greater than that in the dark (17). Solar radiation varies markedly on a range of time scales from minutes to seasons, depending on the solar elevation and weather (cloud) conditions. This implies that inactivation rates will also vary greatly, particularly over a diurnal cycle (3, 4), so that the traditional inactivation parameter, the time for 90% inactivation (T_{90}), is less meaningful than the sunlight exposure producing 90% inactivation (17).

Factors and phenomena besides sunlight thought to cause or influence inactivation in seawater include (27) predation by protozoans (31), osmotic stress in moving from fresh to saline waters (particularly when combined with sunlight-induced membrane damage [29]), lysis by bacteriophage, association with particles, suboptimal water temperature, and nutrient deficiencies. Some of these factors, together with effluent and receiving water clarity and high oxygen concentrations (9, 41), may interact with sunlight (23, 31, 36). Measured counts may also be affected by loss of culturability on laboratory media of still-viable cells (42).

Much of the previous work on fecal indicator inactivation by radiation has been carried out in laboratories, with either artificial or natural light (13, 16, 36, 40). Because of the difficulties in applying the results to field conditions where environmental factors such as water clarity play a major role, field experiments have increasingly become the preferred approach for investigating fecal indicator bacterial inactivation (31), in spite of logistical difficulties. Two main types of field experiment are discussed by Harremoës (21), Gameson (16), and Bell et al. (2). In confined experiments, sewage effluent-seawater mixtures (usually in dilution ratios of 1 to 10%) are held in containers or permeable chambers in the sea or in shore-based tanks (34). Effluent release experiments involve discharge of effluent labelled with a conservative tracer (e.g., fluorescent dye or radioactive isotope). Tracer concentrations must be measured in the resulting effluent field to correct bacterial counts for physical dispersion (21).

Below the sea surface, sunlight is attenuated at a rate dependent on the water clarity (a function of wavelength). Hence water clarity becomes an important factor influencing fecal indicator inactivation rates. For example, in estuarine waters of low clarity, Rhodes and Kator (31) found that *E. coli* inactivation at a depth of 0.25 m was significantly reduced compared with that at the surface, and at both 0.5 and 1 m, the inactivation rates were similar to dark rates. Other workers using in situ containers (for examples, see references 14 and 38) have also noted a depth dependence for fecal indicator bacteria inactivation rates, and they attributed this to light

* Corresponding author. Mailing address: NIWA Ecosystems, National Institute of Water and Atmospheric Research, P.O. Box 11-115, Hamilton, New Zealand. Phone: 64-7-856-7026. Fax: 64-7-856-0151. Electronic mail address: colley@eco.cri.nz.

attenuation. Depth dependence of inactivation should reflect wavelength dependence, inasmuch as shorter wavelengths penetrate less than longer wavelengths in the UV and short wavelength-visible range (16), but, hitherto, this has not been explicitly investigated.

The present investigation compares sunlight inactivation of Ent to that of the traditional FC indicator in field experiments. Sewage effluent, diluted in seawater, was confined in UV-transparent silica flasks (2) for most experiments. This method was verified by comparison with labelled effluent release experiments and with outdoor chamber experiments similar to those of Sinton et al. (34). The silica flask method lent itself to investigation of depth dependence of inactivation in relation to the penetration into water of UV and short wavelength-visible radiation in sunlight.

MATERIALS AND METHODS

Field techniques. The field experiments used sewage effluent from a municipal activated-sludge plant, which was diluted with seawater before being exposed to sunlight. Inactivation of FC and Ent by sunlight was measured over several hours by comparing counts of culturable bacteria with counts in dark controls. In the period 1990 to 1992, nine experiments were carried out over the middle of the day, approximately 3.5 h either side of local solar noon. The experiments were conducted in summer months (November to March in New Zealand), at which time sewage contamination of waters is of greatest concern for bathers. (Sewage contamination is of relevance to shellfish harvesting throughout the year.) Weather and sky conditions ranged from bright days to dull days with some rain, and instantaneous irradiance (flux of radiant energy from the sun per unit of horizontal surface area) ranged from about 200 to 1,100 W m^{-2}. A variety of water environments providing a range of water clarities were used for the experiments, including a relatively clear estuary (Tauranga Harbour), a clear oligotrophic lake (Taupo), and a swimming pool (all near latitude 38°S).

Confined experiments. In most experiments, diluted activated sludge effluent was confined in 500-ml spherical flasks made from pure silica, which is transparent to all wavelengths in the solar spectrum at sea level. The development of the flask technique is discussed in detail by Bell et al. (2). The effluent was added to about 15 liters of seawater to yield a 2% mixture by volume, which was stirred with a motor-driven paddle for 20 min prior to dispensation into the flasks. Flasks were filled completely except for a gas bubble of about 1 ml (a bubble formed by gas evolution even when flasks were initially filled completely by overflow).

Seawater was obtained offshore from Tauranga (North Island, New Zealand) and filtered through a membrane of 0.22-μm pore size to remove solids (these could interfere with the inactivation experiments, e.g., by settling within the quiescent volume contained in the flasks) and microbiota (mainly to preserve the seawater). The slight reduction of light attenuation with solids removal was accounted for by measurement of light penetration of the experimental mixtures. Typically, average irradiance within the 2% effluent-in-seawater mixture within the flasks (diameter, 0.1 m) was about 98% of the incident irradiance at 360 nm. Particle effects on inactivation were found to be insignificant over the duration of our experiments.

The activated sludge effluent was obtained from the Tauranga City sewage treatment plant and held at 4°C for about 20 h before use. Following activated-sludge treatment and overnight refrigeration, it seems reasonable to assume that many of the indicator bacteria cells would be in a resting state and therefore more resistant to environmental stresses, including sunlight, than actively growing cells (23).

The flasks containing the sewage-seawater mixture were fixed, with laboratory clamps, on frames moored in place at different depths, including virtually at the water surface (centers at 0.075 m), within various water bodies (2). At time zero, the flasks were exposed by removal of their aluminum foil covering, except for those that were left covered as dark controls. In two experiments, the depth dependence of fecal indicator inactivation in relation to water clarity (spectral attenuation of irradiance in the UV and short-visible range) was examined (2).

In one experiment, carried out in a swimming pool, the inactivation in silica flasks (500 ml) was compared directly with that in 50-liter chambers, similar to but smaller than the 300-liter tanks (600 mm wide by 900 mm long by 680 mm deep) used by Sinton et al. (34). The effluent-seawater mixture was stirred continuously in two 50-liter chambers (350 by 550 mm and 25 mm deep), one of which was completely foil covered as a dark control and the other of which was exposed to sunlight.

Effluent patch experiments. In two separate experiments, inactivation was also measured in dyed patches of activated sludge effluent released into seawater. In the first of these experiments (12 December 1990), 5 liters of rhodamine WT dye (20% by weight in an aqueous solution) was mixed in 6.8 m^3 of effluent contained in a liquid waste tanker (initial concentration of dye = 170 g m^{-3}). The tanker was driven onto a barge, which proceeded to the experimental site, and the dyed sewage was discharged into the waters of Tauranga Harbour over a period of about 6 min through a 5-m-wide, multiple-port diffuser at a depth of 2.5 m (initial dilution = 870:1). In a second experiment (18 February 1992), 0.5 m^3 of activated sludge effluent contained in a monsoon bucket was dyed with 4.5 liters of rhodamine WT and released almost instantaneously by helicopter (initial dilution = 105:1) into the Pacific Ocean about 4 km from the coast north of Tauranga, from about 1.5 m above the water surface. The initial dye concentration in the effluent for this experiment was high (2,070 g m^{-3}) to ensure visibility of the patch for several hours in the presence of rapid subsequent dispersion in open seawater. Samples were taken for bacteriological analysis from the dispersing patches with a tube sampler to depth integrate from the water surface to 1 m of depth. To correct for physical dispersion, dye concentrations in these samples were measured with a Turner 112 digital fluorometer.

The contributions to light absorption of the various components of the first patch experiment immediately after injection are shown in Fig. 1. The absorption mainly of green light by rhodamine (dominating total absorption in the range 480 to 600 nm) is responsible for the strong magenta color and powerful red-orange fluorescence. At wavelengths >600 nm, absorption by seawater itself dominated. Absorption by the sewage effluent was negligible throughout the UV-visible spectrum. (Indeed, even when much more concentrated—2% (vol/vol)—in flasks, the sewage effluent contributed no more than 25% of the absorption of the mixture at any wavelength.) At wavelengths <480 nm, in the region of the spectrum thought to contribute most to bacterial inactivation (34), absorption by natural constituents of the seawater was higher than that by rhodamine WT, even at the start of the experiment when the dye was most concentrated. To test for artifacts arising from the light absorption by rhodamine WT dye, including possible photosensitizing action, inactivation of FC and Ent in dyed effluent diluted in seawater and contained in a stirred, open chamber (final rhodamine WT concentration =

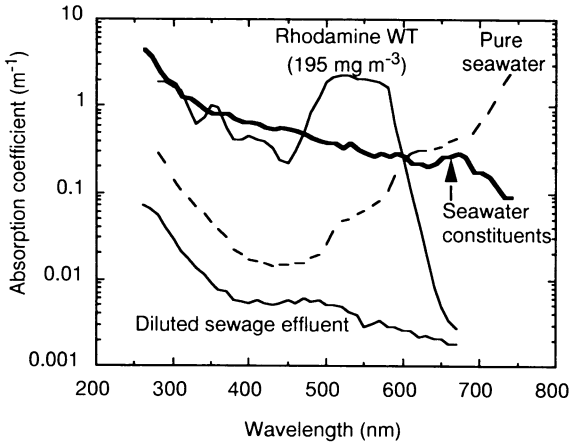

FIG. 1. Absorption spectra of seawater (and its constituents), diluted sewage effluent, and rhodamine WT dye. Data are for a patch of activated sludge effluent, dyed with 170 g m^{-3} of rhodamine WT, soon after release into the waters of Tauranga Harbour on 12 December 1990 (initial dilution = 870:1). Each absorption spectrum was calculated from spectrophotometric measurements on the separate components following procedures in Davies-Colley et al. (11) and adjusted for concentration of that component within the patch (e.g., initial concentration of rhodamine WT in the patch = 170 g m^{-3}/870 = 195 mg m^{-3}).

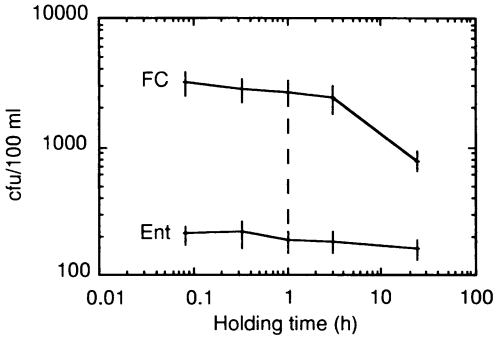

FIG. 2. Counts of bacterial indicators as a function of holding time, after sampling but before membrane filtration. Activated sludge effluent, diluted 1:50 in seawater and contained in silica flasks, was exposed to sufficient insolation to produce approximately 90% inactivation (2.1 MJ m^{-2} and 3.8 MJ m^{-2}, respectively, for FC and Ent). The vertical bars indicate confidence intervals about the mean for membrane filters counted in duplicate (typically ±20% for both indicators). The standard holding time adopted in our experiments was 1 h.

37 mg m^{-3}) was compared with that in an identical chamber, without dye. Inactivation was slightly more rapid in the dyed chamber, suggesting that rhodamine WT may be slightly more efficient as a photosensitizer than seawater constituents (per unit absorption) but that bias in our experiments would be small.

Radiation measurements and calculations. Total solar irradiance (300 to 2,800 nm) was measured on site with a Li-Cor LI-200SA pyranometer (Li-Cor, Inc., Lincoln, Nebr.) calibrated to standard (thermopile-based) instruments. Irradiance was logged as 10-min averages during the experiments. Total insolation, S, was calculated from time integrals of the logged irradiance over the exposure times. As noted by Sinton et al. (34), published lethal action spectra for bacteria (for an example, see reference 40) suggest that most sunlight inactivation would be by the intrinsically very biocidal UV-B component (290 to 320 nm), so UV-B irradiance, comprising <1% of total solar irradiance, was also logged on site. The UV-B sensor was an International Light SED 240 photo sensor combined with an ACTS 270 filter, which mimics the skin-burning action spectrum in the 300- to 320-nm range (26) and is identical to instruments used in New Zealand's national skin burn (erythemal) network (35). Irradiance measured with these instruments is expressed as the 310-nm-equivalent monochromatic irradiance which would cause skin burn in the same time.

Penetration into the water column of UV and short-visible wavelengths was measured with a Li-Cor LI-1800UW submersible spectroradiometer at 5-nm intervals over the range 300 to 550 nm. In addition, light penetration was calculated from laboratory spectrophotometric measurements of light absorption and scattering in water and diluted effluent samples, according to procedures in Davies-Colley et al. (11) (Fig. 1 shows examples of such absorption spectra). The irradiance attenuation coefficient, K_d, was estimated from the measured absorption and scattering coefficients (symbols a and b, respectively) with an empirical equation given by Kirk (25) (for a solar altitude of 45°):

$$K_d = \frac{a}{0.847}(1 + 0.170b/a)^{1/2} \quad (1)$$

Equation 1 shows that K_d depends strongly on absorption and weakly on scattering. Dependence of K_d on solar altitude is weak and was ignored (25). The average irradiance, $\bar{E}$, over water depth, z_m, as a proportion of the surface irradiance, E_0, was then estimated using with the Morowitz (28) equation:

$$\frac{\bar{E}}{E_0} = \frac{[1 - \exp(-z_m K_d)]}{z_m K_d} \quad (2)$$

For example, average irradiance was 98% of the incident irradiance at 360 nm in the UV-A range within silica flasks but was appreciably lower in stirred chambers and effluent patches because of their greater depth. (The choice of 360 nm as the wavelength for this comparison is explained below in Discussion.)

Sampling and bacteriological analysis. Samples for bacteriological analysis, including contents of the silica flasks (500 ml), were transferred to acid-washed, polyethylene bottles and placed in dark, insulated boxes at ambient temperature (31) for transport to the laboratory (by helicopter in experiments remote from the analytical laboratory). Sample analysis commenced 1 h ± 10 min after collection. A longer holding time (up to 3 h) was unavoidable for a few samples analyzed towards the end of effluent patch experiments. In our systems, counts tended to decrease with increased holding time (Fig. 2), possibly because of loss of cellular ionic integrity following radiation damage to membranes (29). The decline in counts was slow for FC and very slow for Ent (Fig. 2), suggesting that slight deviations from the target 1-h holding would not greatly bias our results.

Stirred chambers were sampled by dipping a sterile plastic jar below the water surface. The dyed effluent patches were sampled with a plastic tube (diameter, 50 mm) to obtain a depth-averaged sample from the top 1-m surface layer. The chamber and patch samples (up to 5 liters—volume increased as dye and bacterium concentrations decreased) were trans-

ferred to acid-washed plastic containers for transport to the laboratory together with flask samples.

FC, Ent, and *E. coli* were analyzed by membrane filtration (0.45-μm pore size, gridded, Sartorius type ECN). For FC and *E. coli*, the mTEC procedure was used (1) (Difco agar) with enhanced resuscitation that consisted of placing filters on nonselective medium (tryptic soy broth [Difco] solidified with 1% agar [Davis]), for 4 h at 35°C (22). Following resuscitation, filters were transferred to mTEC agar (at ambient temperature) and incubated for 20 h at 44.5°C. Colonies that were yellow prior to the urease assay for *E. coli* were counted as FC (30, 32). Ent were counted by the mE method (1) (mE and EIA agars [Difco]). For both indicators, half-log-interval test volumes were filtered in duplicate whenever sample volume permitted (37). The 95% confidence interval for both indicators (1), based on counts of around 50 colonies per filter, in duplicate, was typically ±20%. Method accuracy was assessed with reference bacterial cultures: (i) by the mTEC method, with *E. coli* PK1 (a wild strain isolated from sewage), and (ii) by the mE method, with *Enterococcus faecalis* NZRM 1106 (12). At least 20 colonies of each indicator were verified after each experiment (37). No correction of counts for nontarget colonies was found to be necessary.

Data analysis. Linear regression lines were fitted to dark control counts (ln-transformed) and plotted against elapsed time t from the start of the experiment. The intercept of this line at $t = 0$ was taken as the best estimate of the initial count, $\hat{N}_0$, rather than a single direct count, so as to gain the extra precision inherent in multiple counts. The slope of this regression line was used to estimate the dark inactivation coefficient, k_D (ln units per hour). Counts in the sunlight-exposed samples (N), expressed as a percentage of the estimated initial count ($p = 100N/\hat{N}_0$), were assumed to decline because of the independent action of two processes: inactivation by solar radiation and (slow) dark inactivation (17). Accordingly, the culturable percentages that would be expected in the absence of dark inactivation were calculated as $p_S = p \exp(k_D t)$, where t is exposure time. Sunlight inactivation curves ($\ln p_S$ versus insolation, S) were typically log-linear after an initial lag or shoulder region, and data were fitted to the so-called multitarget expression (20):

$$p_S = 100\{1 - (1 - e^{-k_S S})^{n_S}\} \quad (3)$$

where k_S is the slope of the log-linear region of the inactivation curve and n_S is an index of the size of the initial shoulder.

In practice, this fitting was done by linear regression of $\ln p_S$ versus S for all points other than those in the shoulder region (34). The slope of the regression line yielded k_S, and the intercept was taken as $\ln(100 n_S)$. The shoulder constant, n_S, in equation 3 can be interpreted as the average number of targets (cells) aggregated into a single CFU (20). Equivalent parameters were also computed for the UV-B fluence (the time integral of UV-B irradiance).

The overall persistence of bacterial indicators is commonly indexed by the time required to reduce culturability by 90% (T_{90}). However, since inactivation rate, and therefore persistence, vary appreciably with solar irradiance, we calculated instead the insolation required to reduce culturability by 90% (S_{90}) (16), by substituting $p_S = 10\%$ in equation 3 and solving for S. T_{90} values corresponding to these S_{90} values can be calculated as

$$T_{90} = S_{90}/\langle \bar{E} \rangle \quad (4)$$

where time-averaged irradiance, $\langle \bar{E} \rangle$, is known.

Similarity of inactivation kinetics was examined by testing homogeneity of regression line fits to the log-linear portions of inactivation curves by methods given by Zar (43).

RESULTS

Table 1 summarizes the calculated inactivation parameters for both indicators (FC and Ent), in terms of both total insolation and UV-B fluence.

An example of data for a typical inactivation experiment is shown in Fig. 3. Plots of counts versus insolation for flasks exposed virtually at the water surface (centers at 0.075 m) are shown, together with counts in an open-topped, stirred chamber containing the same 2% (vol/vol) effluent-seawater mixture. As expected, the inactivation both in the exposed flasks and in the exposed chamber was much faster than in the dark.

The inactivation curve for FC in Fig. 3 was nearly exponential from $t = 0$, but an initial lag appearing as a broad shoulder ($n_S \gg 1$) was observed on Ent inactivation curves in this and other experiments. The Ent inactivation rate (beyond the shoulder region) was about 75% that for FC (the k_S values were 0.89 and 1.26 m^2 MJ^{-1}, respectively, a difference that is significantly different [$P < 0.02$] in this and other experiments). Because FC were typically present in the activated-sludge effluent at about an order-of-magnitude-higher count than Ent, the more rapid inactivation of the former indicator results in a crossover of inactivation curves. This occurred at an insolation level of about 2.3 MJ m^{-2} (and a UV-B level of 2,500 J m^{-2} on corresponding UV-B fluence curves [data not shown]) as indicated in Fig. 3B. Similar findings have been reported by others (e.g., Fujioka et al. [14]).

Figure 3 shows that the inactivation curves for the chamber are slightly lower than for the flasks—for both indicators. The corresponding difference in inactivation rate was significant for FC ($P < 0.02$), but not for Ent.

Figure 4 gives the inactivation curves for silica flasks at the water surface in six separate summer experiments (with water temperature averaging around 20°C). The inactivation curves all follow a fairly similar pattern for each indicator group. The FC curves had small shoulders (if any), and the inactivation usually followed a first-order trend down to less than 1% of the original count. In some experiments, the FC inactivation rate slowed at large insolation exposures (<1% culturable) as shown by a concave-up shape in the inactivation curves. Ent curves all showed substantial shoulders followed by first-order inactivation at rates less than those for FC (overall, the FC inactivation rate was significantly greater than the Ent inactivation rate [$P < 0.02$]).

The coefficients of variation (Table 1) for inactivation coefficients, calculated from the first-order portions of the curves in Fig. 4, are relatively small (FC, 22% for insolation and 16% for UV-B fluence; Ent, 17% for insolation and 29% for UV-B fluence). The dispersion of inactivation curves, particularly for Ent, relates more to the variability in size of the shoulder (n value) than to variation in slope (k value). The overall spread of the inactivation curves is similar in plots against UV-B fluence (curves not shown). Apparently, the kinetics of sunlight inactivation are reasonably reproducible for these two fecal indicators in activated-sludge effluent diluted in seawater. However, a test for multiple comparisons of linear regression lines following chapter 18 of reference 43 showed the log-linear portions of the different inactivation curves in Fig. 4 to be significantly different ($P < 0.001$) for both indicators, suggesting the influence of uncontrolled factors affecting the inactivation kinetics.

Figure 5 compares inactivation in two sewage effluent patch

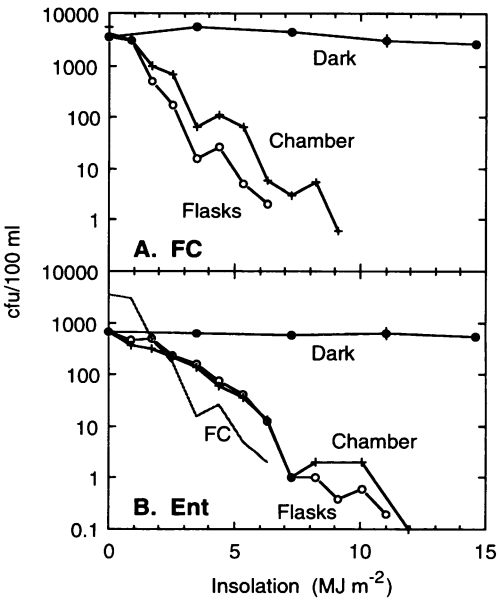

FIG. 3. Inactivation curves for FC (A) and Ent (B) in silica flasks and in stirred chambers. Data are from an experiment conducted in a swimming pool in Hamilton. The vertical bar on one of the dark points indicates the typical confidence interval on all the counts (±20% for both indicators). Results for a dark chamber (not shown) were very similar to those for the dark (foil-covered) flasks. The FC inactivation curve for flasks from panel A is superimposed on panel B to indicate the crossover phenomenon. (Survival curves plotted versus UV-B fluence exhibited a similar pattern and, therefore, are not shown.)

TABLE 1. Sunlight inactivation kinetic parameters for FC and Ent derived for surface silica flasks (summary statistics), chamber, and patch experiments in terms of both insolation and UV-B fluence

Type of expt	Mean sunlight inactivation kinetic parameter ± SD (CV) for[a]:									
	FC						Ent			
	Insolation		UV-B fluence				Insolation		UV-B fluence	
	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}
Silica flasks	1.059 ± 0.235 (22)	1.32 ± **0.761** (89)	0.954 ± 0.151 (16)	1.04 ± **0.910** (113)			0.686 ± 0.116 (17)	4.30 ± **0.864** (105)	0.543 ± 0.158 (29)	3.02 ± **0.960** (123)
Chamber (7 November 1990)	0.907	0.98	0.767	0.73			0.768	2.07	0.619	1.27
Dye patch (12 December 1990)	0.551	0.67[b]	0.306	0.66[b]			0.436	0.84[b]	0.240	0.83[b]
Dye patch (18 February 1992)	0.254	0.83[b]	0.199	0.73[b]			0.314	0.84[b]	0.282	0.79[b]

[a] Parameters have been corrected for dark inactivation. k, inactivation rate of first-order section of survival curve; n, shoulder constant, equivalent to the exponential of the y axis intercept of the first-order section of the curve divided by 100. Sample means, standard deviations (SD), and coefficients of variation (CV [percent]) are given for the inactivation coefficients in six comparable summer experiments with silica flasks. For the shoulder constants (n_S and n_{UV}), sample geometric means, SDs (based on the ln-transformed values and given in boldface), and CVs {[exp(SD^2) − 1]$^{0.5}$} are given.

[b] Shoulder constants for patch survival curves are spurious to some extent, because effluent releases were not truly instantaneous and were not sampled until several minutes after release, when initial mixing had taken place.

experiments with that in flasks. Inactivation was slower in the second (18 February 1992) patch than in the earlier experiment (12 December 1990), in which dye concentrations were lower. In both patches, FC inactivation was significantly slower than in flasks ($P < 0.05$). Inactivation curves for Ent in the patches lacked shoulders, but again, k_S values were lower than in flasks. Unfortunately, counts of Ent generally lacked precision (as shown by wide confidence intervals in Fig. 5), because after initial dilution, bacterial densities were low in both experiments (e.g., starting count = 110 CFU/100 ml in the first patch experiment), so little can be concluded from curve shapes. The inactivation rates for Ent in the patches were lower than those for the flasks, but the difference was not significant, as a result of the small number of datum points combined with low precision.

Figure 6 gives the inactivation curves for both indicators in silica flasks exposed at different depths in Lake Taupo, a water body optically similar to type III ocean water according to the optical classification of Jerlov (24), on the basis of the irradiance attenuation coefficient at 350 nm, $K_d(350)$, of 0.28 m^{-1}. The inactivation curves for both indicators show a strong trend with depth, inactivation being slower at greater depths. A similar depth series of inactivation curves has been reported by Davies-Colley et al. (10) for an experiment carried out in Tauranga Harbour, a water appreciably less clear than Lake Taupo [$K_d(350\ nm) = 0.70\ m^{-1}$, similar to type I coastal water [24]).

The inactivation coefficients from Fig. 6 are plotted versus depth in Fig. 7, for comparison with depth profiles of spectral irradiance at selected wavelengths measured by spectroradiometer. The depth dependence of inactivation coefficients for both indicators compares closely with the depth profile of

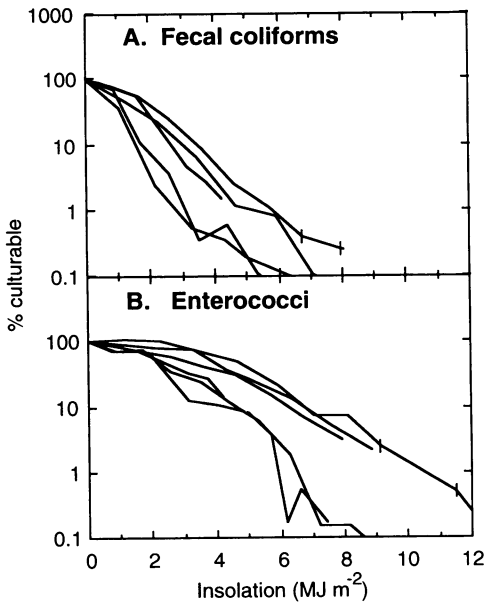

FIG. 4. Inactivation curves for six separate summer experiments with silica flasks exposed at the water surface. (A) FC; (B) Ent. In one experiment, only Ent data (no FC data) were obtained. The vertical bars indicate representative confidence intervals on all the counts (±20%). (Patterns for plots versus UV-B fluence are not shown but were very similar.)

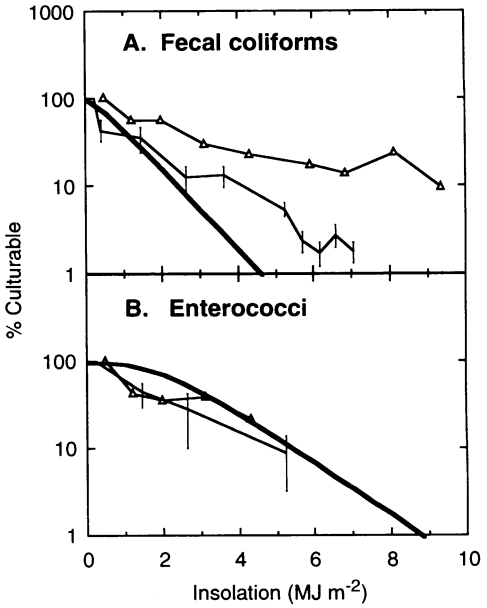

FIG. 5. Average inactivation curves for silica flasks exposed to sunlight at the water surface (heavy solid lines), constructed from mean values of the inactivation parameters for insolation (Table 1) with the multi-target expression (equation 3) for six separate summer experiments. (A) FC; (B) Ent. (Patterns are very similar for UV-B fluence.) Inactivation curves for two experiments with unconfined patches of sewage effluent are overlain for comparison. The vertical bars on the data for the first patch experiment (12 December 1990) indicate the confidence intervals on the counts. Data for the second patch experiment (18 February 1992) are indicated by open triangles (confidence intervals were broadly similar to those for the first patch experiment).

irradiance at about 350 to 360 nm. UV-B irradiance, e.g., that at 310 nm, was attenuated much more rapidly and followed a steeper profile than the inactivation coefficient.

DISCUSSION

Reproducibility of inactivation kinetics. The inactivation kinetics of both FC and Ent in surface silica flasks were reasonably reproducible, as shown by relatively low coefficients of variation for the inactivation coefficients for both insolation and UV-B fluence (Table 1). However, the individual inactivation curves for the same indicator were significantly different in different experiments, suggesting significant levels of between-experiment variability in factors affecting the inactivation (other than sunlight). The unexplained variation in inactivation kinetics was similar for both UV-B fluence and total insolation, suggesting that neither measure of sunlight exposure was ideal. The depth dependence of inactivation suggests that UV-A wavelengths in sunlight may be responsible for most inactivation, as is discussed below. Thus some of the unexplained variation in inactivation kinetics may relate to variation in spectral quality of sunlight, which results in an imperfect correlation of bactericidal irradiance with either measured UV-B irradiance or total irradiance.

Sinton et al. (34) found an even higher level of variation between inactivation curves for repeat experiments, possibly reflecting a greater level of variability of factors such as average irradiance levels, clarity of experimental mixtures (which affects the chamber technique more than the flask technique, in which the light path is much smaller), microbial composition of effluents, and dissolved oxygen concentration (which may have been more variable in diluted raw effluents than in diluted activated sludge effluent), as well as variation in the spectral quality of solar radiation.

Inactivation rates in different experimental systems. The slightly slower inactivation in the chamber than in the (smaller) flasks (Fig. 3) can probably be attributed to attenuation of radiation in the deeper containers. The average irradiance at 360 nm, over the 0.25-m depth of effluent-seawater in the chamber, was about 88% of that in the flasks, which compares, broadly, with the corresponding ratios of inactivation coefficients (chamber to flasks) of 72% for FC and 87% for Ent. Sinton et al. (34) measured a still lower rate of inactivation (about 50% of that in our surface flasks) in deeper (0.56-m) stirred chambers with correspondingly greater irradiance attenuation.

The lower rate of inactivation in dyed effluent patches (and chambers) than in our silica flasks might be attributed initially to some artifact of the silica flask method, causing faster inactivation in these (unstirred) containers. But the fact that the inactivation rates in the two effluent patch experiments were different and those in both were lower than that in the stirred chamber suggests the operation of some factor within the patches (and chamber) rather than in the silica flasks. A probable explanation for the slower inactivation in the patches than in the surface-moored flasks is the greater attenuation of bactericidal irradiance through the mixed depth of the patches.

There is some quantitative support for this explanation. In the first patch experiment carried out in Tauranga Harbour, the vertical mixing of the dyed effluent was restricted to a depth averaging 1 m over intertidal flats. Attenuation of bactericidal radiation of <480 nm within this patch was largely controlled by the attenuation of harbor water. (That is, the contribution to attenuation of radiation by rhodamine WT dye

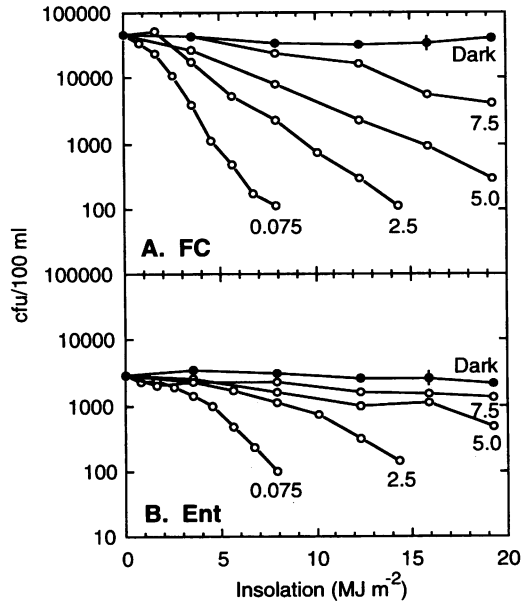

FIG. 6. Depth dependence of inactivation of fecal indicator bacteria in Lake Taupo (26 February 1991, summer). (A) FC; (B) Ent. The vertical bar on one of the dark points indicates the typical confidence interval on all the counts (±20% for both indicators). Labels on curves are depths in meters at which silica flasks were exposed. (Patterns for UV-B fluence are similar.)

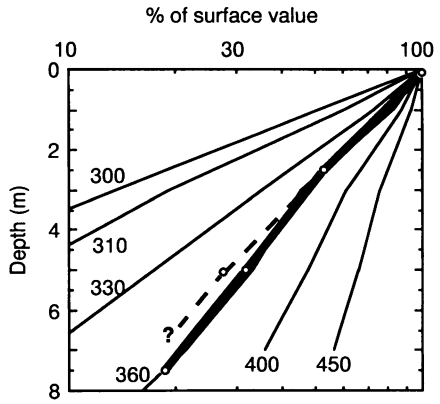

FIG. 7. Comparison of the depth dependence of dark-corrected inactivation rate coefficients (k_S) for fecal indicator bacteria with the spectral irradiance profiles measured in Lake Taupo at the time of the 26 February 1991 experiment (thin curves). The heavy line is for FC, and the dashed curve is for Ent. The value for Ent at a depth of 7.5 m is difficult to estimate because the inactivation curve did not move beyond the shoulder region (see Fig. 6B). Labels on the spectral irradiance profiles are wavelengths (in nanometers).

in this region of the spectrum was relatively small compared with attenuation by seawater constituents. Figure 1 shows that the absorption of the dye was mostly less than that of seawater constituents at the start of the run—before subsequent dispersion further reduced dye concentrations.) The irradiance attenuation coefficient at 360 nm [$K_d(360)$] was about 1.5 m^{-1}, which yields an average irradiance of 52% of the surface value over the 1-m patch depth by equation 2. This compares closely with the ratio of inactivation coefficients: that for FC in the patch was about 51% of the mean value for surface flasks over five experiments.

Inactivation in the second patch was slower, suggesting greater attenuation of radiation over the depth of this patch, partly due to the greater dye concentration in the first hour following release. Unfortunately, quantitative analysis of these data is difficult for two reasons: firstly, because the vertical turbulent mixing of this patch (released in deep, offshore water) was unconstrained and patch depth continued to increase with time, and, secondly, because the effluent was dyed with a high concentration of rhodamine WT (initially, 20 g m^{-3}) which dominated light attenuation within the patch until rapid dispersion reduced concentration to around 120 mg m^{-3}, about 50 min after release. We may note, however, that the concave-up shape of the inactivation curves for this patch (Fig. 5) is consistent with the time trend towards lower average irradiance over the increasing depth of the patch as vertical mixing proceeded.

Taken together, the comparisons of inactivation kinetics both in stirred chambers and in effluent patches with that in silica flasks suggest that the flask method does not suffer pronounced artifacts related to confinement without stirring, at least in short-term experiments (<7 h) with activated sludge effluent (2, 34). We attribute the generally lower rates of inactivation in patches and chambers to the attenuation of biocidal solar radiation by the effluent-seawater mixtures over differing, but appreciable, depths in these experiments. This highlights the need to measure the spectral attenuation of bactericidal wavelengths of sunlight down the water column in order to compare the inactivation kinetics measured in different types of field experiments.

Shape of inactivation curves. Inactivation curves for Ent routinely displayed lag periods (shoulders), except in the patch experiments. In contrast, inactivation curves for FC lacked large shoulders. Sinton et al. (34) obtained similar small shoulders for raw sewage effluent in deep (0.56-m) chambers, but FC shoulders were generally large (n_S = 7.25) (and variable) for meatworks effluent. Such shoulders on bacterial inactivation curves have usually been attributed to the extensive operation of enzymatic repair at low fluences, with inactivation of repair mechanisms and consequently faster inactivation at higher fluences (23). However, a more plausible explanation, in our view, is that these shoulders reflect the degree of aggregation of bacterial cells into single CFU. The shoulders on our Ent inactivation curves were probably related to the normal occurrence of Ent in chains rather than as individual cells (33). Gram-positive cocci, conforming to the general description of enterococci, were identified microscopically in the Tauranga activated-sludge effluent occurring in chains, typically of 4 to 6 cells in length. A chain of cells will produce only one colony on a membrane filter, and all of the cells so aggregated must be inactivated to prevent colony formation. Consideration of such aggregation of cells leads directly to multitarget kinetics for inactivation (20) as given in equation 3. The finding that chains frequently comprised 4 to 6 cells accords with the geometric mean value of the shoulder constant for Ent in our experiments (n_S = 4.3).

Comparative inactivation of the two indicators. Ent persisted much longer in seawater than FC in our experiments because of both the broad shoulder on inactivation curves and the lower inactivation coefficient. The overall persistence in surface flasks as indexed by S_{90} values (16) averaged 2.5 MJ m^{-2} of insolation (and 2.5 kJ m^{-2} of UV-B) for FC, compared with 5.8 MJ m^{-2} (and 6.2 kJ m^{-2} of UV-B) for Ent. Therefore, on average in summer, Ent required 2.3 times more radiation

exposure than FC for 90% inactivation. With typical New Zealand summer midday irradiances of about 1.2 kW m^{-2}, corresponding T_{90} values calculated by equation 4 are 35 min for FC and 81 min for Ent.

The S_{90} values reported by Sinton et al. (34) for raw sewage averaged 6.0 MJ m^{-2} for FC and 10.7 MJ m^{-2} for Ent, about twice our values. This agrees with calculations made with equation 2, showing that average irradiance in the chambers at 360 nm was about half the incident irradiance.

Gameson (16) reported S_{90} values for FC in eight separate tracer-labelled patches of raw sewage effluent (median S_{90} = 1.29 kWh m^{-2} = 4.6 MJ m^{-2}; range, 0.60 to 2.7 kWh m^{-2} or 2.2 to 10 MJ m^{-2}). The lower end of this range is comparable with our mean S_{90} value of 2.5 MJ m^{-2} for FC in surface flasks. Our demonstration that depth dependence of inactivation relates to penetration of spectral irradiance at around 360 nm suggests that the variability, and higher central tendency, of Gameson's S_{90} is related to variable, and sometimes appreciable, attenuation of bactericidal solar radiation within sewage effluent patches in different experiments.

Environmental persistence during daylight hours has been widely reported for coliform bacteria (including FC) compared with the other main alternative indicators, FS and Ent. Traditionally, T_{90} values are reported, but these can vary markedly with sunlight irradiance during and between experiments (3). For example, the T_{90} values we obtained for FC and Ent in an experiment on a dull rainy day were 3.5 times higher than the average for sunny conditions, yet the S_{90} values were similar. However, the ratio of T_{90} for sunlight exposures of FS to those of FC is less variable and provides a useful index of relative persistence. This ratio varies between approximately 0.9 and 4.0 in the literature (for examples, see references 13 and 16), but most values lie in the range 1.3 to 2.0 (i.e., FS are consistently more persistent than FC in seawater). A mean value of 1.5 for the T_{90} ratio (FS to FC) was obtained by Evison (13) in laboratory experiments. In the five summer experiments in the present study, the mean ratio for S_{90} (and, therefore, for T_{90}) was higher (2.3), on the basis of Ent (a subset of FS) in activated-sludge effluents. These results are also consistent with the ratio of 2.0 for both S_{90} and T_{90} obtained by Sinton et al. (34) for chamber experiments with raw sewage. Therefore, greater persistence of Ent than FC appears general in sewage effluent-seawater mixtures.

The greater persistence of Ent than FC may explain why Ent predicts gastrointestinal infection of swimmers better than the traditional coliform indicator (6, 8). Enteric viruses, including, presumably, gastrointestinal agents such as Norwalk viruses, are known to be generally more resistant to sunlight than FC (14). However, viruses do not usually exhibit large shoulders in their inactivation curves, so the detailed kinetics of Ent inactivation, as opposed to their overall persistence, are not expected to match closely those of viral pathogens.

Two parameters are needed to model the sunlight inactivation curves for Ent, an inactivation coefficient and a shoulder constant. In contrast, FC inactivation in sewage-seawater mixtures is more nearly first order ($n \approx 1$) and can be modelled reasonably accurately with just the one parameter, k_S. In this respect, the inactivation kinetics of the increasingly favored Ent indicator is more complex, and possibly more variable, and may be more difficult to model realistically for prediction of inactivation in seawater receiving sewage effluent discharges.

Depth dependence of inactivation of fecal indicator bacteria. The depth dependence of inactivation rate (k_S) appears to match the attenuation of UV-A radiation at about 360 nm. Davies-Colley et al. (10) have analyzed the inactivation curves for a similar experiment in Tauranga Harbour (a water of much lower clarity than Lake Taupo), where the depth dependence also closely matched that of irradiance at 360 nm.

The correspondence of the depth dependence of inactivation with the profile of irradiance at 360 nm does not necessarily mean that most inactivation is by UV-A radiation in the vicinity of 360 nm. In fact, Sinton et al. (34) have shown that most inactivation occurs in two regions of the spectrum to either side of 360 nm, one centered on about 330 nm in the UV-A range and the other in the visible region at >400 nm. However, we may note that half the inactivation in Fig. 7 of reference 34 occurs at wavelengths of < 360 nm for both indicators, showing that the wavelength and depth dependence of inactivation are consistent. A similar result was obtained by Gameson and Gould (17) for total coliforms in petri dish mixtures, half the inactivation being attributable to wavelengths of <370 nm.

The empirical depth dependence of inactivation, which matches the depth profile of 360-nm radiation rather than that of UV-B (290 to 320 nm), implies that UV-B does not dominate sunlight inactivation of fecal indicator bacteria in effluent-seawater mixtures, contrary to published action spectra (for an example, see reference 40). Our finding that inactivation is imperfectly predicted from erythemal UV-B fluence is also consistent with the notion that UV-B radiation is comparatively unimportant in sewage effluent-seawater mixtures. Experiments with optical filters placed over stirred chambers to selectively screen different regions of the solar UV spectrum confirm that UV-B contributes little to inactivation (34).

Apparently, the FC within our sewage effluent-seawater mixtures are much less susceptible to UV-B than the *E. coli* in buffered suspensions used by Webb and Brown (39, 40) to develop action spectra for bacterial inactivation. There seem to be two possible explanations: (i) the sewage indicator bacteria are much more proficient at repairing UV-B-induced radiation damage (mainly pyrimidine dimerization in the DNA) than the *E. coli* strains used by Webb and Brown (39, 40), or, more likely, (ii) the bacteria are very effectively physically screened from UV-B radiation, presumably because of association at a microscopic scale with some UV-absorbing components of the sewage effluent-seawater mixtures that are not present in sterile buffer. Studies need to be carried out to explain why sunlight inactivation of indicator bacteria in sewage effluent-seawater mixtures is not dominated by the UV-B component.

UV-A and short-wavelength visible radiation, in contrast, seem to be much more damaging to bacteria than expected from published action spectra, probably reflecting the photosensitizing action by light-absorbing components of seawater and effluent—leading to cell damage by photooxidation (9, 41). A range of potential phototoxic species are known to be produced photochemically in natural waters, including peroxides, superoxide, hydroxyl radicals, and singlet oxygen (9, 17). However, only very short-lived species such as singlet oxygen can be primarily responsible for inactivation of bacteria in our systems, since on removal from sunlight, dark inactivation during holding is very slow (Fig. 2). We suggest that, ideally, UV-A radiation would be monitored during future experiments on inactivation of bacteria by sunlight.

Implications for coastal sewage discharges. To predict the rate of inactivation of fecal indicator bacteria discharged from an outfall, it is advisable (31) to carry out field measurements of fecal indicator inactivation (surface and at depth) near the proposed outfall site, under a range of ambient water and sky conditions. From knowledge of the incident and underwater ambient light climate for a particular site and the results of in situ inactivation experiments at various depths, a realistic set of

depth-dependent inactivation parameters can be derived for modelling the impact of sewage outfall discharges (3, 16). If in situ studies are not feasible, the mean parameter values reported in Table 1 can probably be used, at least in temperate areas, together with measurements or predictions (on the basis of established optical principles [11]) of the light penetration at 360 nm, to predict inactivation in the dispersing effluent plume. Models of daylight bacterial inactivation should incorporate parameters based on solar radiation, either S_{90} or the multitarget expression parameterized by the shoulder parameter n and the inactivation rate coefficient k_S. Various scenarios of typical inactivation on winter versus summer and dull versus bright days can be simulated, on the basis of characteristics of the incident light climate, and with account taken of water clarity and mixing depth of the effluent plume.

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