Depth-dependence of Enterococcus inactivation by solar UV in seawater

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ABSTRACT

The inactivation of two faecal indicator bacteria, enterococcus and the traditional faecal coliform indicator, were measured in silica flasks exposed to sunlight at different depths in the water column. Enterococci were found to be appreciably more persistent than faecal coliforms (e.g., T90 of about 70 minutes for enterococci compared with about 23 minutes for faecal coliforms, under midday, midsummer irradiances at the water surface). The slower inactivation of enterococci is largely a result of the pronounced shoulder occurring on inactivation curves, in contrast to the more-nearly exponential inactivation of faecal coliforms. Inactivation rate declined rapidly with depth in waters, the actual depth-dependence being dependent on ambient water clarity as measured by spectral attenuation coefficients. The profile of inactivation rate with depth in the water column matched that of UV-A irradiance at about 360 nm, suggesting a simple means by which this depth-dependence can be accounted for in the modelling of bacterial inactivation in sewage effluent plumes.

INTRODUCTION

The faecal coliform (FC) group is the most widely used indicator of faecal contamination of waters. However, enterococcus (a subset of the faecal streptococci group), is now regarded as a preferred indicator, being more closely related to incidence of gastro-intestinal symptoms in swimmers [3–6]. Many water management authorities round the world are beginning to adopt enterococcus standards or guidelines for coastal waters used for contact recreation.

Since the pioneering report of Gameson and Saxon [11], sunlight has become generally recognised as the single most important environmental factor causing inactivation of faecal indicator bacteria during daylight hours. There is a fairly large amount of information on the sunlight inactivation kinetics of the coliform group of organisms, including faecal coliforms, in seawater [9]. However
inactivation rates and mechanisms for other faecal indicator bacteria, including the faecal streptococcus group, are not so well known. The inactivation rate is required for predictive calculations of faecal indicator bacteria numbers arising from discharge of sewage effluents [2].

The work of photobiologists, summarized by Jagger [13], suggests that all the UV radiation (290–400 nm) in the solar spectrum at sealevel, together with violet to blue visible sunlight (400–500 nm), contributes to the sunlight inactivation of enteric bacteria. Gameson and Gould [10] report experimental results indicating that approximately half the inactivation of coliforms at the water surface is attributable to wavelengths each side of 370 nm (comparable information appears not to be available for the streptococcus group). Seawater attenuates light selectively, with attenuation increasing approximately exponentially below 500 nm, owing to the ubiquitous presence of yellow substance (aquatic humus) [14]. Therefore, below the sea surface, total inactivation rate is expected to decline with decline in UV irradiance, and the longer UV-visible wavelengths are expected to increase in importance at the expense of short-wave UV.

As a contribution to the estimation of parameters needed for the modelling of marine outfall performance in terms of the now-favoured enterococcus indicator, experiments have been carried out on the sunlight inactivation of faecal indicator bacteria in sewage effluent diluted in seawater. The effluent/seawater mixture was contained in spherical flasks made of pure silica, which, unlike some other container materials, is transparent to all solar UV-visible radiation present at sealevel. The comparative survival of Enterococcus (Ent) and FC, in exposed silica flasks, in mixed chambers, and in simultaneously-released slugs of effluent, is reported in more detail elsewhere [7,22]. In this paper we emphasise the depth-dependence of inactivation rate that was conveniently measured by exposing silica flasks at different depths, and then discuss the ramifications for modelling the performance of sewage effluent outfalls in coastal waters.

METHODS

Activated-sludge effluent, diluted with seawater and contained in flasks of pure silica, was exposed to sunlight at different depths in waters, and the progression of inactivation of faecal indicator bacteria with solar irradiation was followed by sampling flasks at different times. Seawater for the silica flask experiments was obtained offshore from Tauranga, New Zealand, and sterile-filtered (0.22 μm). The activated-sludge effluent (almost entirely domestic sewage) was obtained from the Tauranga City treatment works and held at 4°C overnight before use in the experiments. The faecal indicator bacteria count was estimated from membrane filter incubations during this time (no discernable decline in bacterial counts occurred overnight).

On the morning of experiments, the effluent sample was diluted in filtered seawater and vigorously stirred for 20 minutes before being dispensed into the (500 mL spherical) silica flasks. These flasks, initially covered in aluminium foil, were mounted on frames at different depths in the water column, depending on water clarity at the various (harbour and clear lake) sites. At time zero, the contents of the flasks were exposed by stripping off their aluminium
foil covering. At intervals thereafter, usually every 20 minutes, flasks were sampled and their contents transferred to polypropylene bottles placed in dark, insulated boxes at ambient temperature [20] for transport to the laboratory. Analysis commenced 1 hour ± 10 min after collection.

During the experiments, usually of about 3 to 4 hours duration near solar noon, total solar irradiance was measured with a LI-200SA pyranometer, and erythemal (skin-burning) UV-B irradiance was measured using an International Light SED 240 detector combined with an ACTS 270 filter. The latter sensor/filter combination is identical to those in New Zealand's national skin-burn network [18]; the spectral response mimicking the skinburning action spectrum [17]. (Originally it was hoped that the skin-burning climatology data, now becoming available from this network, could be used to predict bacteria inactivation in outfall modelling studies.) The preamplified UV-B signal, and the pyranometer signal, were applied to different channels of a LI-1000 datalogger which sampled every 5 seconds and logged the average over 10 minute intervals.

The attenuation of spectral irradiance with depth was measured by scanning at 5 nm intervals in the 300 - 550 nm range with a Li-Cor LI-1800UW submersible spectro-radiometer. At intervals during the experiments, salinity and temperature were also measured.

Faecal coliforms (FC) and enterococci (Ent) were analysed by membrane filtration. The mTEC procedure [1] was used for FC after enhanced resuscitation: placement of filters on non-selective medium for 4 h at 35°C. Colonies that were yellow prior to the urease assay for E. coli, were counted as FC [19,21]. Enterococci were enumerated using the mE method [1] (mE and EIA agars - Difco).

Seven separate experiments have been carried out using the silica flask method. The technique has been broadly validated against experiments with open chambers and field releases of effluent slugs [7,22], so it seems unlikely that significant artefacts affecting bacterial survival arise from containment of the diluted effluent in these short duration experiments (lasting up to 4 hours).

RESULTS AND DISCUSSION

Figure 1 shows inactivation (or "survival") curves for enterococcus in an experiment using silica flasks moored at the surface (flask centres at 0.075 m depth) and at 0.66, 1.33, and 2.07 m depth in the relatively clear estuarine waters of Tauranga Harbour. The survival data in Fig. 1 are plotted against UV-B fluence, but, over seven experiments, there was little evidence that UV-B was an appreciably better predictor of sunlight inactivation than total solar irradiation. At first we found this surprising, given that published action spectra for bacterial killing (e.g. Webb & Brown [24]) show UV-B to be >4 orders of magnitude more intrinsically biocidal than UV-A and visible wavelengths, but it is consistent with our other findings (discussed below).
Two main features are evident in the enterococcus survival curves in Fig. 1: firstly, a broad "shoulder", arising from a lag in the inactivation, and secondly, the progressive decrease in inactivation rate with depth. The inactivation curve for faecal coliforms, measured by the same technique (Figure 1), shows a small shoulder and a mild degree of non-linearity, but is much more nearly exponential. The inactivation rate of enterococci, after the initial lag period, is about two thirds that of the faecal coliforms. But the appreciable shoulder, together with the slower inactivation rate, means that enterococcus is much more persistent than the traditional indicator, as has also been reported by Fujioka et al. [8]. To illustrate, the $T_{90}$ for enterococcus at the water surface near mid-day on a clear day in summer at 37° South is about 70 minutes, compared to about 25 minutes for faecal coliforms.

The survival curves for the bottle series exposed at different depths show the expected progression. (No correction for dark inactivation is required since this was negligible over the short duration of the experiments – Fig. 1) This depth dependence is plausibly explained by the decrease in biocidal irradiance with depth. We can compare the depth-dependence of inactivation rate with spectral irradiance profiles in the water column (Figure 2.) The inactivation rate profile most nearly matches the spectral irradiance profile of 360 nm radiation in the centre of the UV-A range.
Figure 2.
Depth-dependence of inactivation rate (heavy curve) compared with spectral irradiance profiles (thin curves). The inactivation rates were taken from the exponential portions of the survival curves in Figure 1. The spectral irradiance profiles were measured with a spectro-radiometer at the experimental site during the 23 November, 1990 experiment. (The departure of the irradiance profiles from linearity reflects the optical stratification of the water column.)

This depth-, and therefore, wavelength-dependence, is broadly consistent with the results of survival experiments with optical filters used to screen various regions of the UV-visible range of solar irradiance [22]. Although UV-B radiation is intrinsically much more biocidal than other regions of the solar spectrum at sealevel [13], the UV-A to visible may be more significant in sunlight inactivation because of its much greater wavelength-integrated irradiance. The broad match of the inactivation rate profile with depth with that of UV-A irradiance at about 360 nm is also consistent with the finding [10] that about half the total inactivation of faecal coliforms could be attributed to wavelengths either side of 370 nm. The action spectrum for killing of enterococci is not known, as far as we are aware, so, at present, the biological action (spectral irradiance convolved with the action spectrum) can not be calculated for direct comparison with the observed inactivation rate as a function of depth.

CONCLUSIONS – IMPLICATIONS FOR COASTAL OUTFALL DESIGN AND MONITORING

The greater persistence of Ent than FC, measured either as $T_{90}$ or as inactivation rate constants, may explain why the former faecal indicator bacteria predicts gastro-intestinal infection of swimmers better than the traditional indicator [3–6]. Viral pathogens are known to be generally more resistant than FC to most known environmental agents, including solar UV, e.g. [8]. However, viruses, lacking enzymatic repair capability, do not usually exhibit large shoulders in their survival curves, so the detailed kinetics of enterococcus
inactivation, as opposed to their overall persistence, are not expected to match closely to that of viral pathogens.

The large "shoulder" in the Ent survival curves suggests "multi-hit" kinetics [12], whereby several "hits" are required to inactivate a cell. In fact, as regards direct damage to the DNA, repair mechanisms are so efficient that many hundreds of lesions may occur for every one left unrepaired. A more plausible explanation is that the large shoulder reflects efficient repair of UV damage, up to a point, beyond which repair mechanisms are themselves significantly damaged by the radiation [13,23]. Photobiological work on the streptococci group is highly desirable to elucidate such mechanisms and further support use of this faecal indicator bacteria as an alternative to the traditional FC.

Whatever the reason for the pronounced shoulder on enterococcus survival curves, this feature does appear to pose a practical problem for modelling. An accurate description of the kinetics of inactivation requires two parameters, one to quantify the size of the shoulder as well as one to quantify the eventual exponential decay.

The finding that the depth dependence of inactivation of enterococcus matches the attenuation of UV-A irradiance may simplify the modelling of the behaviour of this faecal indicator bacteria in effluent plumes. Obviously the clarity of effluents will generally have to be taken into account as well as that of the receiving seawater [2]. The spectral attenuation, and therefore the bacterial inactivation, within the plume can be calculated if the optical properties of both the effluent and receiving water, as well as the dilution ratio, are known.

REFERENCES