A review of the factors affecting sunlight inactivation of micro-organisms in waste stabilisation ponds: preliminary results for enterococci

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ABSTRACT

Waste stabilisation ponds (WSP) are efficient, cost-effective methods of treating wastewater in rural and remote communities in Australia. It is recognised that sunlight plays a significant role in their disinfection, however, due to the poor penetration of light in turbid waters it has been hypothesised that other mechanisms may also contribute to disinfection in WSPs. To date, studies have reported various and conflicting results with regards to the relative contributions of UVA, UVB, PAR and environmental factors including pH, DO and photo-sensitisers on micro-organism disinfection. Initially we investigated the role of these environmental factors on the solar disinfection of enterococci in buffered distilled water to control for potential confounding factors within the wastewater. Die-off rate constants were measured, in sterile buffered distilled water at varying pH and dissolved oxygen concentrations, for enterococci irradiated with UVA and UVB. Enterococci were found to be predominantly inactivated by UVB ($p < 0.001$), however, UVA was also observed to increase inactivation rates relative to the dark control ($p < 0.001$). DO and pH were found to have no effect on inactivation rate when enterococci were irradiated with UVB ($p > 0.05$), however, when irradiated with UVA, both DO and pH were observed to further increase inactivation rates ($p < 0.01$).

Key words | dissolved oxygen, pathogen removal, pH, ultraviolet light, waste stabilisation ponds

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INTRODUCTION

Waste stabilisation ponds (WSPs) provide an efficient, cost-effective method of treating sewage effluent in rural and remote communities in Australia. WSPs are considered to treat wastewater to a high level, particularly by achieving high rates of removal of pathogenic bacteria and viruses (Mayo 1995; Davies-Colley 2005). The final pond or ponds in a waste stabilisation pond series are the maturation or 'polishing' ponds. The main role of maturation ponds is pathogen removal, or disinfection of the water for discharge or reuse. Maturation ponds are designed to be shallow in depth (1–1.5 m) ostensibly to allow high penetration of sunlight through the water column (Shilton & Walmsley 2005). Solar irradiation is recognised as a main contributor to WSP water disinfection, with UVA (320–400 nm), UVB (280–320 nm) and photosynthetically active radiation (PAR) (400–700 nm) all contributing to micro-organism removal (Curtis *et al.* 1992; Davies-Colley *et al.* 1997, 1999; Muela *et al.* 2000). In fact, many studies have concluded that sunlight is the most important factor causing disinfection in WSPs, e.g. Mayo (1989, 1995). This conclusion is largely based on observations of rapid die-off in the uppermost regions of WSPs and may ignore the much less efficient contribution of 'dark' die-off observed in the lower regions of the water column where light is unable to penetrate. Knowing that light is greatly attenuated in WSP effluent (Curtis *et al.* 1994) leads us to question whether sunlight is indeed the most important factor when the integrated results of attenuation throughout the entire

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depth column are considered. It is currently unclear to what extent other environmental factors such as pH, DO and the presence of photo-sensitisers contribute significantly to disinfection in WSPs (Fallowfield *et al.* 1996; Kohn & Nelson 2007). In considering pathogen removal, it is also unclear to what extent faecal indicator organisms e.g. *E. coli*, enterococci or coliphages are representative of the pathogenic bacteria or viruses of concern (Chang *et al.* 1985; Davies-Colley *et al.* 1997; Tree *et al.* 1997) and also to what extent photo-sensitisers are effective in disinfection processes. The objective of this paper is to review the literature regarding the relative contribution of UV, DO, pH and the role of photo-sensitisers to disinfection in WSPs and to present preliminary results on the interaction between UV, DO and pH on the die-off kinetics of enterococci.

Factors affecting pathogen removal

Ultraviolet light

UV light can directly damage RNA, DNA and other cell constituents of micro-organisms, in processes termed direct photoinactivation. UVA, UVB and photosynthetically active radiation (PAR) have all been shown to contribute to the inactivation of micro-organisms in water. However, due to the differences in their energy, inactivation, mechanisms vary for the different wavelength regions of the solar spectrum (Curtis *et al.* 1992; Davies-Colley *et al.* 1997, 1999; Sinton *et al.* 1999, 2002; Muela *et al.* 2000, 2002; Kohn & Nelson 2007). In addition to processes of direct photoinactivation, UV light and to a lesser extent photosynthetically active radiation (PAR) are able to indirectly inactivate and damage micro-organisms via photo-oxidation. Photo-oxidation occurs with the formation of highly reactive oxygen species (ROS), which react with and damage/inactivate micro-organisms. In aquatic environments such as in a WSP, ROS can be produced by endogenous and exogenous sensitisers as well as by other reactions such as Fenton's reaction (Curtis *et al.* 1992; Gracy *et al.* 1999; Kohn & Nelson 2007). Sensitisers are light absorbing compounds that transfer their energy to other molecules leading to the formation of ROS. Endogenous sensitisers are found inside the cells of microbes, e.g. flavins and porphyrin derivatives while exogenous sensitisers are found outside the cell in the aquatic environment e.g. humic substances, photosynthetic pigments and dissolved organic matter (Curtis *et al.* 1992; Kohn & Nelson 2007).

Dissolved oxygen (DO)

High levels of DO occur in aquatic systems due to the photosynthesis of algae and macrophyte organisms. Sweeney *et al.* (2007) reported DO levels in the upper area of a WSP, which reached over 30 mg/L in summer. Due to light attenuation, however, DO stratification can vary significantly through the water column, with nearly all effective light being absorbed in this surface layer (Haag & Hoigne 1986). Maturation ponds are generally photosynthetically oxygenated due to the relatively high optical clarity of the effluent received from the facultative pond. It is hypothesised that an increase in DO would result in an increase in ROS formation and a corresponding increase in photo-oxidation.

pH

Significant diurnal changes in pH occur frequently within WSPs due to algal photosynthesis, which consumes and removes CO_2 from the water. This in turn affects the carbonate/bicarbonate buffering system (Equation (1)) leading to a decrease in hydrogen ions and a corresponding increase in pH (Paterson & Curtis 2005). Assimilation of NO_3 may contribute to further increases in pH (Fallowfield *et al.* 1996).

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3{-} \leftrightarrow H^+ + CO_3^{2-} \quad (1)$$

Consequently, high pH values are often observed in WSPs, with values varying diurnally within the range of 7–9.4 (McDonnell, 1989 (cited in Curtis *et al.* (1992)), Kayombo *et al.* 2002; Sweeney *et al.* 2007). It is hypothesised that an increase in pH would result in a decreased stability of the micro-organism cell with a subsequent increase in solar inactivation.

Photo-sensitisers

The photochemical properties of humic substances, which act as exogenous sensitisers, vary from source to source with their identity, location and concentration important variables in the photo-oxidation of micro-organisms (Curtis *et al.* 1992). Endogenous sensitisers also play an important role in the solar inactivation of bacteria (Reed 1997). However, it is unlikely viruses are affected by endogenous photo-oxidation as they lack a bound chromophore to act as the endogenous sensitiser (Kohn & Nelson 2007). It is hypothesised that the presence of sensitisers will positively affect solar inactivation of bacteria, but have no impact on the solar inactivation of viruses.

Review of the literature

A summary of the results of solar inactivation studies of various indicator micro-organisms investigating the role of environmental factors is shown in Table 1. These studies were all performed in dissimilar matrices and in small reaction vessels of approximately 5–6 cm deep. The authors are unaware of any studies to date (including those found in Table 1), which have dealt with sunlight inactivation of micro-organisms in actual WSPs or even in systems more closely representative of an actual WSP. Maturation WSPs are in the range of 1–1.5 m deep and hence are much deeper than the samples irradiated in the studies summarised in Table 1. The result of this is that it is likely only the surface depths of these ponds would receive enough solar radiation for sunlight inactivation to occur as reported in the summarised studies. In a WSP where the water generally contains a substantial amount of organic matter and is very turbid the light intensity decays very rapidly with depth. Light attenuation is wavelength dependent with light of shorter wavelength attenuated more greatly and penetrating less deeply than light of a longer wavelength (PAR > UVA > UVB). Kohn & Nelson (2007) observed that over 99% of UVB light at 290 nm was absorbed in the first 2.5 cm of WSP water and over 99% of visible light at 556 nm was absorbed in the first 8 cm of WSP water. Furthermore, Haag & Hoigne (1986) state that in most waters, nearly all effective light is absorbed by a depth of 1 m. It is therefore unknown whether the reported inactivation rates and processes in the reviewed studies would hold true in a real WSP.

The aim of the current study was to irradiate enterococci with environmentally relevant levels of UVA and UVB at various pH and DO initially in distilled water to quantify 'best case scenario' disinfection rates. This will allow a better understanding of disinfection processes in WSPs to be developed as well as the significance of the environmental factors; pH, DO and sensitisers on micro-organism inactivation.

METHODS

Ninety millimeters diameter, 500 mL vessels containing 300 mL buffered distilled water were inoculated with 300 μL enterococci (10^9 CFU/ml) under the conditions shown in Table 2. Vessels were irradiated with two sets of solar light regions (UVB and UVA) using UVB (Sankyo Denki, Japan) and UVA (NEC, China) lamps at environmentally relevant levels and a dark control at 20°C for up to 48 h. Light received by each vessel was limited to penetration through a UV penetrable 66 mm diameter quartz window in the lid of the vessel. During the incubations, irradiance was measured at a set reference point. The irradiance at the liquid surface of each vessel was calculated with this value by using the predetermined relationship to the reference point. Numbers were quantified over 48 h or until undetectable using Enterolert® (Idexx Corp.). Die off rate constants (K) were calculated from linear regression of semi log plots of the number of organism at time t (N_t) divided by the number of organisms at time zero (N_0) against time. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

The results from solar disinfection experiments with enterococci irradiated with UVB and UVA under varying pH and DO are shown in Figures 1 and 2, respectively. Statistically, at each pH no difference was observed between systems with high DO and those with low DO irradiated with UVB (Figure 2; $p > 0.05$). Furthermore, increasing the pH was found to have no effect on enterococci die-off irrespective of DO. Negligible die-off was observed for the dark controls irrespective of DO levels and pH.

Table 1 | Summary of previous experiments investigating the affect of the environmental factors (pH, DO and sensitisers) on solar inactivation rates

Affect of inactivation rates by Organism	**Faecal Coliforms**	***E. coli***				**Enterococci**		**FRNA**		**FDNA**
Increasing DO	↑*	↑	↑	↑	↑†	↑	↑	X	↑	X
Increasing pH	↑*	↑	↑	–	–	X	–	X	–	X
Presence of exogenous sensitisers	↑	↑	X	–	↑	↑	–	↑	↑	X
Matrix	WSP effluent and distilled water	HRAP effluent and saline solution	WSP effluent	Distilled water	River water and saline solution	WSP effluent	Distilled water	WSP effluent and distilled water	WSP effluent	WSP effluent
Irradiated by	Sunlight	Sunlight	Sunlight	Sunlight	Fluorescent lamps	Sunlight	Sunlight	Solar simulator	Sunlight	Sunlight
Reference	Curtis *et al.* (1992)	Benchokroun *et al.* (2003)	Davies-Colley *et al.* (1999)	Reed (1997)	Muela *et al.* (2002)	Davies-Colley *et al.* (1999)	Reed (1997)	Kohn & Nelson (2007)	Davies-Colley *et al.* (1999)	Davies-Colley *et al.* (1999)

*Measured only in the presence of exogenous sensitisers (WSP effluent).

†Only in the absence of exogenous sensitisers (saline solution).

Table 2 | Experimental conditions for enterococci disinfection experiments in buffered sterile distilled water

Vessel	pH	DO (ppm)
1–3	7.5	>8
4–6	8.5	>8
7–9	9.5	>8
10–12	7.5	<1
13–15	8.5	<1
16–18	9.5	<1

Die-off was significantly slower when organisms were irradiated with UVA under all conditions compared with UVB ($p < 0.001$). The fastest die-off of enterococci irradiated under UVA in Figure 2 occurred at pH 9.5 and high DO. At pH 8.5 and 9.5, decreasing DO resulted in a corresponding significant decrease in die-off ($p < 0.01$). This result is comparable to that observed by Reed (1997) where a faster die-off rate was achieved for enterococci as well as *E. coli* incubated in sunlight in aerobic distilled water at pH 6.8 compared with anaerobic distilled water at pH 6.8. At high DO, a significant increase in die-off was observed when the pH was increased from 7.5 to 8.5 and again on increasing pH to 9.5 ($p < 0.001$). At low DO, increasing pH from 7.5 to 8.5 resulted in a significant increase in die-off ($p < 0.01$). Increasing the pH to 9.5 had no further effect on die-off at low DO.

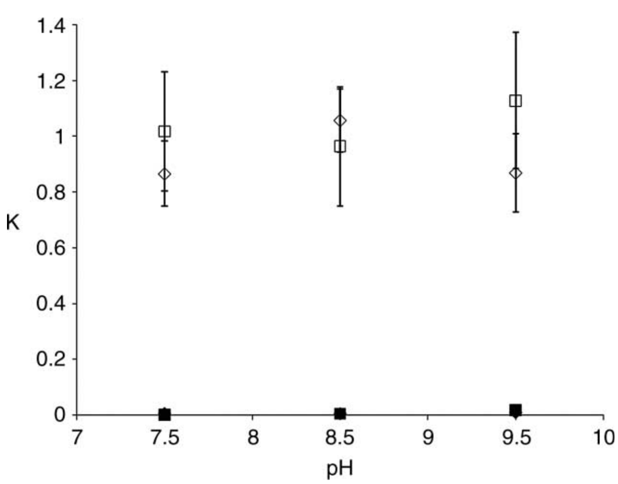

Figure 1 | Die-off rate constant (K) of enterococci in buffered distilled water determined over a 4 h incubation period. Irradiated with 1.1 J s^{-1} m^{-2} UVB at pH 7.5, 8.5 and 9.5 at high DO □ and low DO ◇ compared with dark control at pH 7.5, 8.5 and 9.5 at high DO ■ and low DO ◆.

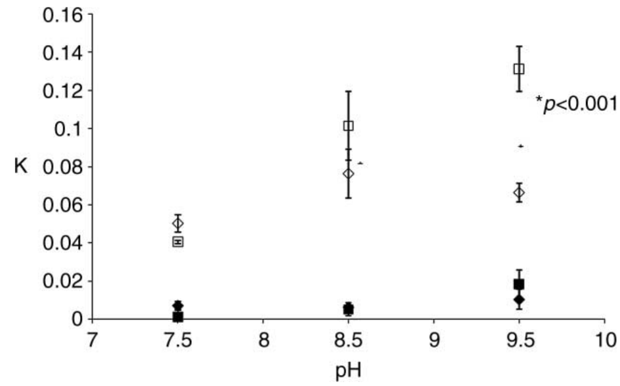

Figure 2 | Die-off rate constant (K) of enterococci in buffered distilled water determined over a 48 h incubation period. Irradiated with 23 J s^{-1} m^{-2} UVA at pH 7.5, 8.5 and 9.5 at high DO □ and low DO ◇ compared with dark control at pH 7.5, 8.5 and 9.5 at high DO ■ and low DO ◆.

From the above observations it is likely that the predominant disinfection mechanism for UVB under the current experimental conditions is direct inactivation by UVB. For UVA it appears that both endogenous photo-oxidation and direct inactivation by UVA are the predominant mechanisms under the given conditions.

CONCLUSION

The effect that variables including pH, DO and the presence of sensitisers have on the solar inactivation of micro-organisms is still somewhat unknown, with conflicting results reported in many of the limited number of studies in this area. Furthermore, there is disagreement in the literature on the predominant mechanisms contributing to the inactivation of certain micro-organisms. Further research is needed to examine the role these environmental factors have in solar inactivation and also a wider range of micro-organisms need to be examined. This is especially true for pathogenic viruses, as their survival in WSPs and other aquatic environments is currently unknown and their presence in water poses a threat to public health. Furthermore, a suitable indicator organism for pathogenic viruses has not as yet been identified. The results presented here indicate that enterococci die-off in distilled water is predominantly impacted by UV, but that DO and pH are also important in the presence of UVA and absence of UVB. This is likely to be important at depths where most UVB has been attenuated, but where UVA is still able to penetrate, such as in WSP effluents, which will be the focus of further study.

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