New insights into sunlight disinfection mechanisms in waste stabilization ponds

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Abstract Previous research on sunlight-mediated disinfection in waste stabilization ponds has identified three different mechanisms by which inactivation may occur: direct damage to nucleic acids; indirect damage via endogenous sensitizers; and indirect damage via exogenous sensitizers. In this paper we discuss new experimental results that provide additional insights into these mechanisms. We documented wide variability in the direct inactivation by UVB wavelengths of lab strains and field isolates of bacteriophage. Indirect inactivation of *E. coli* via endogenous sensitizers appeared to involve both singlet oxygen as well as Fenton-like reactions. Finally, the pH dependence of *E. coli* and *E. faecalis* inactivation in waste stabilization pond water suggests that different mechanisms cause damage.
Keywords sunlight; disinfection; WSP; reactive oxygen species

INTRODUCTION

Sunlight plays a key disinfection role in waste stabilization ponds (WSP). Previous research has identified three main types of mechanisms through which sunlight can damage microorganisms. The first mechanism involves the direct damage of cell components by sunlight in the ultraviolet (UV) region (Jagger 1985). All organisms can be damaged by UVB wavelengths (280-320 nm) that directly damage nucleic acids, although susceptibilities differ among organisms (Jagger 1985). The second and third mechanisms, endogenous and exogenous photoinactivation, respectively, are indirect processes, which can potentially be initiated by both UV and visible light. In the presence of oxygen, the excitation of sensitizers (light-absorbing compounds that transfer energy to other molecules) leads to the formation of reactive oxygen species (ROS), which can damage cell and virus constituents. In endogenous photoinactivation, sensitizers are located within the cell (e.g., flavins; ref. (Jagger 1985)), whereas in exogenous photoinactivation sensitizers are located outside the cell (e.g., humic substances; refs. (Cooper et al. 1989; Curtis et al. 1992; Hoigné et al. 1989)). ROS produced in surface water and inside bacterial cells include singlet oxygen ($^1O_2$), superoxide (O_2^-), hydroxyl radical ($OH\cdot$), peroxyl radical ($RO_2^{-\cdot}$) and hydrogen peroxide (H_2O_2) (Cooper et al. 1989). Common formation pathways for ROS involve absorption of a photon by a sensitizer molecule and subsequent reaction either by energy transfer or by electron transfer reactions with oxygen (Cooper et al. 1989; Hoigné et al. 1989). All organisms are potentially susceptible to ROS produced exogenously. Laboratory studies have shown, however, that MS2 coliphage and enterococci can be inactivated by exogenous ROS whereas *E. coli* are not susceptible at neutral pH (Curtis et al. 1992; Davies-Colley et al. 1999).

Bacteriophage are convenient models for studying sunlight inactivation because endogenous inactivation is presumably minimal due to the absence of endogenous chromophores. Consistent with previous research, we have observed faster inactivation of MS2 in WSP water than in buffered DI water (Kohn and Nelson 2007). These results suggest that inactivation was dominated by exogenous processes, in which damage by ROS formed by irradiation of chromophores in the WSP water was more important than light attenuation. We also demonstrated that singlet oxygen was the most important exogenous ROS causing damage to MS2 in WSP water (in the absence of UVB light) (Kohn and Nelson 2007). Furthermore, we found that most of the $^1O_2$ was produced by sensitizers in close association with the MS2 particles (Kohn et al. 2007). Thus, the exogenous inactivation rate of any particular organism is expected to be a function of its susceptibility to ROS, as well as the physical-chemical interactions between the organism and chromophores in the water.

The purpose of this paper is to review new data from our ongoing research that provides further insight into these three sunlight–mediated inactivation mechanisms.

EXPERIMENTAL METHODS

Inactivation experiments were conducted in custom 150-mL glass reactors exposed to simulated sunlight (Oriel 1000W Solar Simulator, Spectra Physics, model # 91194). Irradiance was measured using spectroradiometers (International Light, RPS 200 and RPS 380). Filters were used to adjust the solar spectrum (Spectra Physics, models # 81088, 81017) or block out all light below 320 nm (Spectra Physics, model # 81088, 81050). Depending on the experiment, reactors were filled with buffered DI water or WSP water, and spiked with the organism of interest.

RESULTS

Direct sunlight inactivation of viruses and bacteriophage

The goal of these studies is to characterize the variability in inactivation rates of viruses in the presence of UVB wavelengths. We have studied inactivation of lab strains and field isolates of bacteriophage in the absence of external sensitizer molecules. Under these conditions, direct damage to nucleic acids is typically the dominant (fastest) mechanism. We found that inactivation rates of lab strains varied dramatically for different phage types (Table 1). The inactivation rate of MS2 (linear ssRNA) was much slower than PRD1 (linear dsDNA), which was much slower than PhiX174 (circular ssDNA). Preliminary analysis of field isolates indicates that inactivation rates may group according to phage type, similar to the lab strains (data not shown). We plan to expand our database of field isolates, in addition to conducting further phylogenetic characterization of these isolates. Future experiments will also include measuring the inactivation rate of several human viruses. By comparing these to the various phage groups we hope to identify

appropriate indicators for human viruses under environmental conditions dominated by UVB light. These results are most relevant to clear surface waters; in most WSP, UVB light will be attenuated in the first few centimeters, and it is likely that indirect inactivation mechanisms will dominate the overall inactivation rates.

Table 1. First-order inactivation rates of laboratory phage under full-spectrum simulated sunlight

Phage	Nucleic acid	Genome length (kb)	k_{obs}	N
MS2	ssRNA	3.5 – 4.3	0.41-0.54	4
PRD1	dsDNA	147-157	1.19 – 1.30	3
PhiX174	ssDNA	4.4-5.4	7.31	1

Indirect inactivation of bacteria in the absence of external sensitizers

The goal of these studies is to better understand the mechanisms through which sunlight causes loss of culturability through endogenous mechanisms. Thus, during these experiments UVB wavelengths are blocked with a filter to eliminate direct damage of DNA. The test organism is *E. coli*, which has been a particularly useful model because many mutant strains are available that allow us to explore the role of ROS scavengers and DNA repair. One key finding is that an *E. coli* mutant lacking the recombination DNA repair enzyme recA was inactivated much more rapidly by sunlight than wild-type *E. coli* (Figure 1). Inactivation of the mutant strain was dramatically reduced by the addition of desferrioxamine, an iron chelator, and catalase, a hydrogen peroxide scavenger. This result suggests that sunlight may damage DNA via reactions between iron and hydrogen peroxide, such as via a Fenton-type reaction. A Fenton-like process is already known to be central to oxidative stress that occurs in aerobically respiring *E. coli* in the dark (Imlay 2003). Thus, the next step is to identify what sunlight does to exacerbate this type of oxidative stress. We have also found that *E. coli* was inactivated more rapidly when irradiated in D_2O than in H_2O, which suggests that singlet oxygen may play a role in photoinactivation as well (Figure 2).

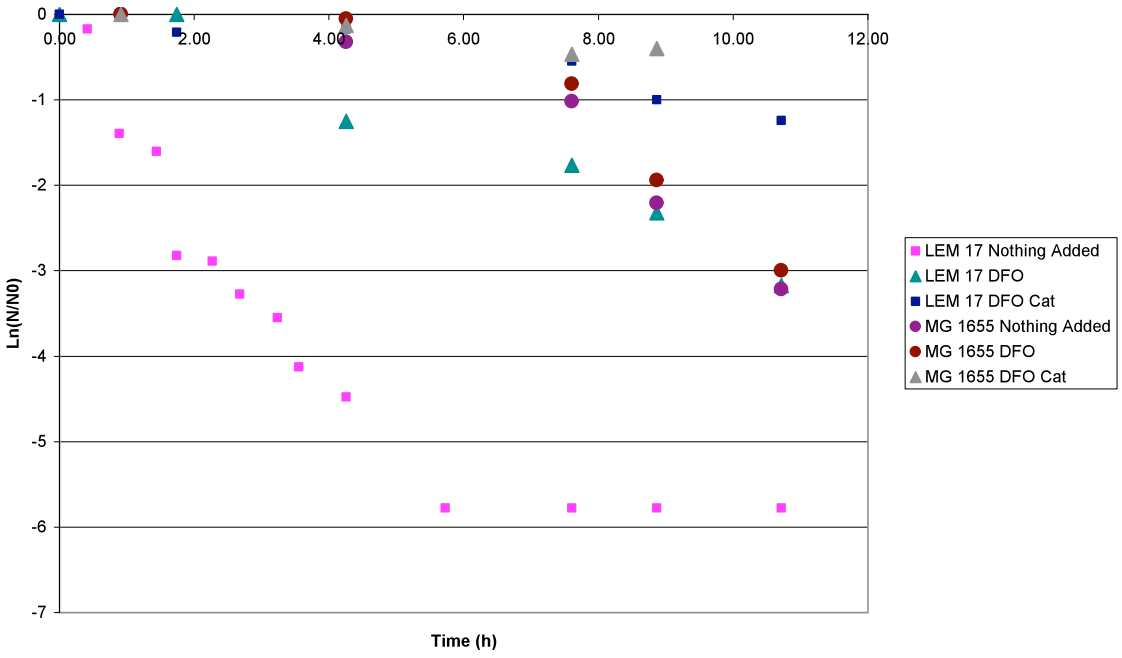

Figure 1. Inactivation of recA-deficient *E. coli* with and without addition of DFO and catalase.

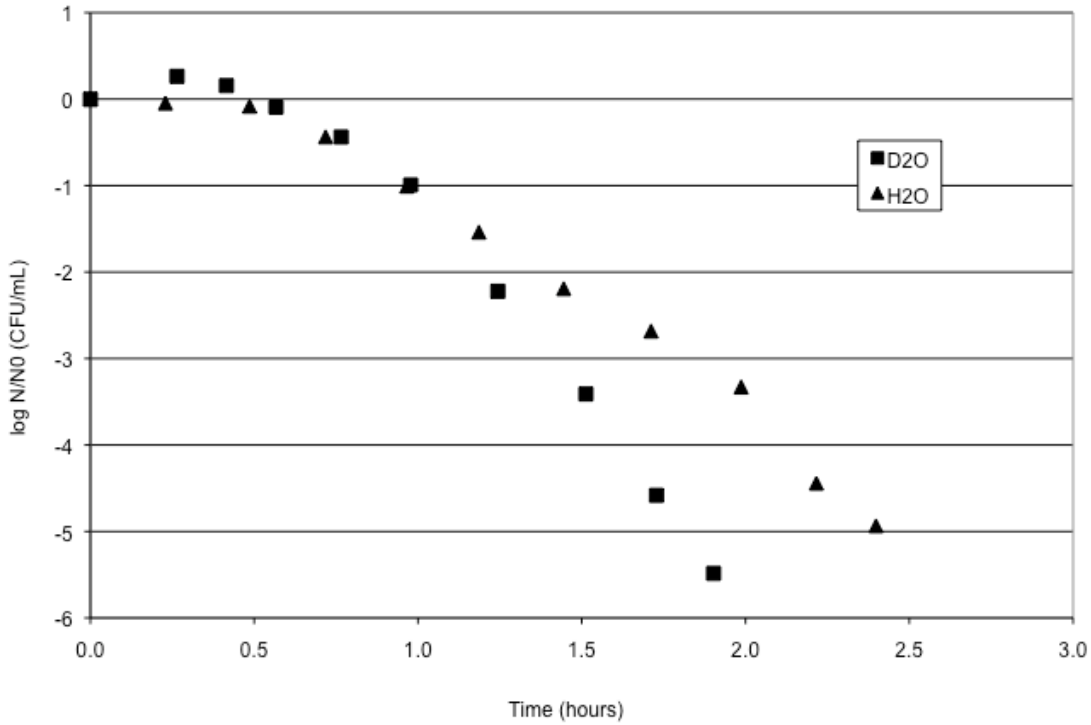

Figure 2. Inactivation of wild-type *E. coli* in D_2O versus H_2O.

Indirect inactivation in the presence of external sensitizers (WSP water)

Previous researchers have demonstrated that increased pH can dramatically effect the inactivation rate of *E. coli* in pond water (Curtis et al. 1992; Davies-Colley et al. 1999), while having no significant effect on *E. faecalis* (Davies-Colley et al. 1999). We characterized the effect of pH on these two bacteria over a much wider range both in the presence of pond water (external sensitizers) and in buffered DI water (Figure 3). The results are consistent with previous research, and further illustrate the dramatic differences between *E. coli* and *E. faecalis* inactivation mechanisms. The inactivation rate of *E. coli* was lower in pond water compared to DI, suggesting that the dominant effect of pond water was to screen light rather than sensitize inactivation. However, the inactivation rate increased dramatically with pH (k = 0.3 at pH 7; k = 1.2 at pH 10). In contrast, the inactivation rate of *E. faecalis* was higher in pond water than in DI, and was roughly similar between pH 7 and 10. Thus, these two indicator organisms are expected to respond very differently in WSP that experience significant pH increase during the day due to algal photosynthesis. The next important step is to understand the dominant mechanisms affecting bacterial pathogens, and how they are impacted by pH.

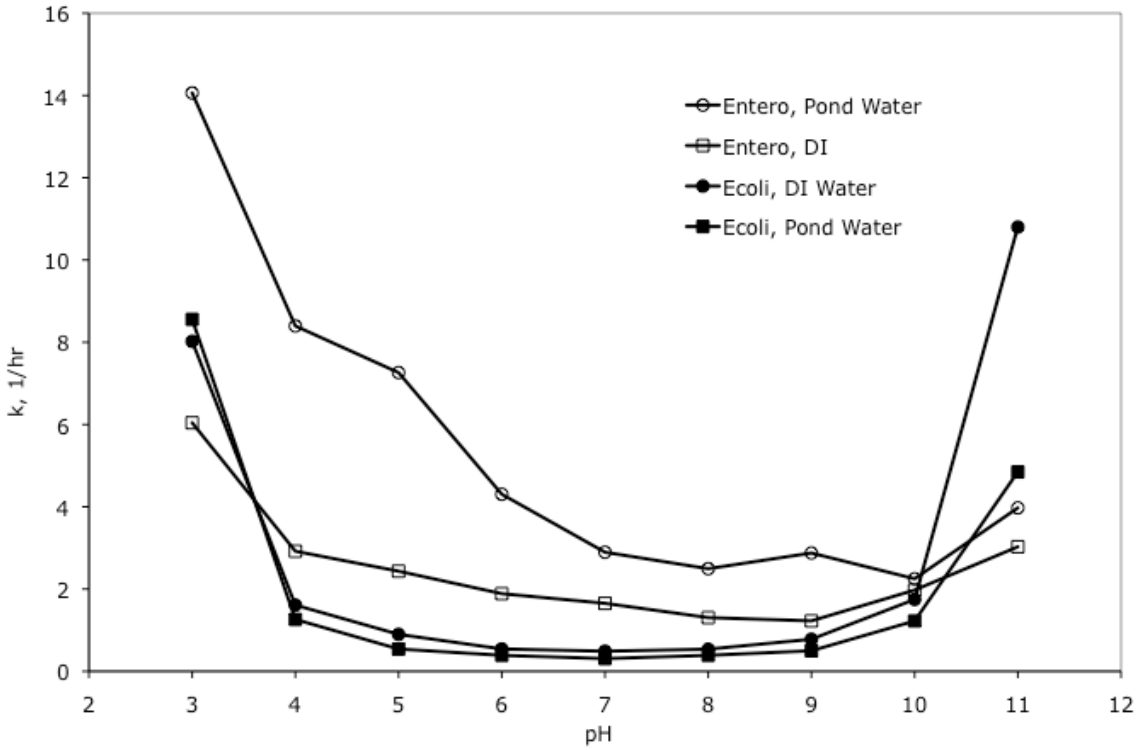

Figure 3. First-order inactivation rate of *E. coli* and *E. faecalis* by sunlight as a function of pH, in buffered DI water or pond water.

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