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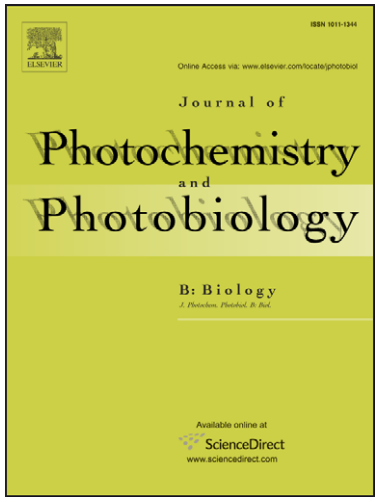

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The inactivation of phages MS2, ΦX174 and PR 772 using UV and solar photocatalysis

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Abstract

The photolytic and photocatalytic disinfection of three bacteriophages (MS2, ΦX174, PR772) under both artificial UV irradiation and natural sunlight was studied. A large variation was seen in the relative responses of phages to the two light sources, while solar photocatalysis showed promising disinfection capabilities for all three phages. Under artificial UV, the resistance of phages to both photolytic and artificial treatment was found to decrease in the following order: PR772>ΦX174>MS2. Indeed, 3-log inactivation of PR772 was not achieved after 25 hours of illumination in either the absence or presence of catalyst. Under solar treatment, PR772 was inactivated far more readily, 3-log inactivation being achieved within an hour in the presence of catalyst in two trials. It is thought that the variation in solar intensity during the trials had a major effect on disinfection rates, as kinetics for phages varied considerably under different weather conditions.

Keywords: photocatalysis, solar disinfection, titanium dioxide, bacteriophage, MS2, PR772, ΦX174.

1. Introduction

Primary water treatment can be undertaken without the need for energy and/or chemical consuming processes. For example, organic and biological contaminants can be removed by trickling the water through slow sand filters. However, the disinfection stage of drinking water treatment is considerably more energy-intensive. The most widely used method is chlorination, but this yields problems in terms of taste and the potential for the creation of toxic compounds, such as THMs [1]. Other options such as ozonation are also used, but this too has a high energy requirement. The use of sunlight to treat water has been researched extensively in recent years, although it is by no means a newly discovered phenomenon. Downes and Blunt [2] linked the effectiveness of sunlight to a bactericidal effect and conducted experiments that led them to suspect that UV light was responsible for disinfection. Disinfection using UV light is usually employed in industry with the use of either medium or low pressure lamps which optimize the production of artificial light across the spectrum of UV wavelengths lethal to microorganisms. However, in the past thirty years there has also been a lot of research into the use of natural sunlight to promote photolytic disinfection, especially for applications in developing countries [3]. Equally, there has been a lot of research into the area of Advanced Oxidation Processes (AOPs), including SPC-DIS (solar photocatalytic disinfection), which are used to produce strong oxidative agents. The most effective of these so far is photocatalysis, which is defined as the acceleration of a photoreaction in the presence of a catalyst [1]. More specifically, the use of TiO_2 photocatalysis for disinfection has been under investigation ever since Matsunaga and co-workers reported the complete sterilization of *Lactobacillus coli* when subjected to UV-irradiation in the presence of platinum-loaded TiO_2 [4].

E. coli has been used as a target organism in the vast majority of experiments looking into photocatalytic disinfection [5-12]. As well as *E. coli*, the inactivation of other bacteria has been investigated using TiO_2 as a photocatalyst [13-16]. Less research has been conducted on the photocatalytic inactivation of viruses, although *Poliovirus 1* [17], *phage MS2* [18], and *B. fragilis bacteriophage* [19] are among those which have been tested. For example, *Poliovirus 1* inactivation

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rate kinetics were shown to follow Chick's Law and the virus was inactivated more quickly than *E. coli* under the same conditions [17].

Viruses are unique in that they have no independent metabolic activities, rather they must infect living host organisms in order to reproduce. A virus consists of RNA or DNA (and not both), contained in a protective protein coat. The binding site for attaching a virus to a host is located on the protein coat of the virus. Viruses attach themselves to host cells and inject their genetic materials into the cells' cytoplasm [20]. They use the host cell's mechanisms to reproduce themselves and the new viruses move on to infect other cells. If the binding site of a virus is attacked by a disinfection method, it can no longer recognise receptor sites on host cells. Early work on UV treatment of viruses concluded that DNA is the primary target for UV, while it was theoretically possible that the loss of unique genetic information is the main cause of inactivation. However, experiments suggested that the prevention of intracellular development preceding phage-coded expressions is the actual biological mechanism accounting for most of the inactivation [21]. While *E. coli* is comprised of many complex systems, the inhibition of one of which can lead to deactivation, the simple, rigid structure of viruses makes them more difficult to disinfect in general [22].

Phage MS2 is a single-stranded, icosahedral RNA bacteriophage, which, like ΦX174, has overlapping genes. It appears to be the most widely studied bacteriophage in the field of UV and solar disinfection. MS2 was found to be highly resistant to UV treatment (filtered UV, of wavelength >295 nm) in a study conducted by Mamane *et al.* [23], with no disinfection at a wavelength greater than 295 nm. When H_2O_2 was introduced, however, MS2 was found to be more sensitive to treatment than T4 and T7 phages. The authors concluded that, in general, disinfection due to $^{\bullet}OH$ radicals is small compared to that due to UV irradiation, although for viruses some oxidative enhancements can improve disinfection efficiency. On the other hand, Sjogren and Sierka [18] proposed that hydroxyl radical oxidation is the primary cause of the inactivation of MS2. They also showed that the process can be enhanced by the addition of ferrous sulphate, which provides the iron required for the Fenton reaction to proceed.

Koizumi & Taya [24] conducted a study investigating the disinfection of phage MS2 in a bubble column reactor in the presence of TiO_2. While no disinfection took place at a light intensity of 22 W/m^2 in the absence of catalyst, they found that disinfection occurred in the presence of TiO_2 and proceeded linearly with respect to irradiation time. They also varied the concentration of TiO_2 and discovered a near-linear relation between catalyst concentration and disinfection rate constant up to a saturation value [24].Using the same apparatus, Sato & Taya [25] investigated the effect of different crystalline structures of TiO_2 on the disinfection of MS2. They found that an anatase:rutile ratio of 70 % (by wt.) was the most efficient.

In order to investigate the role of hydroxyl radicals in the photocatalytic disinfection of MS2 and *E. coli*, Cho *et al.* [22] performed experiments under UV light (300-420 nm) in which hydroxyl radical scavengers, *tert*-butanol and methanol were added to illuminated TiO_2 suspensions containing the microorganisms. In the absence of the scavengers, MS2 was shown to be far more resistant to photocatalytic disinfection than *E. coli*, with 0.95 and 2.25 log inactivations, respectively, after 120 minutes of illumination. When the scavengers were introduced, no inactivation of MS2 was seen at all, although some inactivation of *E. coli* still occurred, probably due to the effect of direct UV, other reactive oxygen species such as superoxide and hydrogen peroxide, and surface hydroxyl radicals that might survive the effects of the scavengers. The fact that no disinfection of MS2 occurred despite the existence of some radicals on the surface of TiO_2 particles led the authors to suspect that MS2 is inactivated by only the bulk phase hydroxyl radicals. The adsorption of MS2 onto TiO_2 particles is inhibited by electrostatic repulsion between the two. The influence of hydroxyl radicals was further supported when the authors performed further tests in which Fe^{2+} ions were introduced to induce the Fenton reaction, as discussed previously. In these tests, the inactivation rate of MS2 increased substantially in the absence of scavengers and there was still no disinfection in the presence of scavengers. Again in this study, the disinfection of all plaque-producing viruses investigated was

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shown to follow first-order kinetics. The photocatalytic disinfection of PRD1, MS2 and ΦX174 was investigated more recently by Gerrity *et al.* [26], who performed experiments at bench-scale and pilot-scale. They found that at bench-scale, disinfection was inhibited by the presence of catalyst and attributed this to the attenuation of light by the catalyst and the lack of adsorption of MS2 to TiO_2 particles. When the experiments were repeated at pilot-scale, the introduction of catalyst had very little influence on the disinfection rate. The authors also looked into the influence of the capsid protein components on photocatalytic disinfection and found that phages with higher levels of alanine, glycine and praline seemed to be more susceptible to treatment.

Phage ΦX174 is an icosahedral single-stranded DNA bacteriophage, which infects *E. coli*. Its circular, single-stranded DNA molecule was the first genome ever to be sequenced [27]. It was also the first example of a genetic element with overlapping genes. These provide efficient coding for all of its proteins and overlap due to the small size of the genome [28]. In a study investigating the sensitivity of microorganisms to different wavelengths of light, ΦX174 had no shoulder region in the dose response curves, which, as the author suggested, is to be expected from a single stranded virus [29]. Sommer *et al.* [30] discovered that ΦX174 was more susceptible to UV (253.7 nm) treatment than B40-8, which in turn was inactivated more readily than MS2. They suggested that the type of nucleic acid, RNA, in MS2 could be the reason for its high resistance, as the uracil in RNA may be less photoreactive than the thymine in DNA. However, other similar RNA viruses had been found to be inactivated relatively easily by UV, while there were some DNA viruses which were quite resistant. They therefore suggested that it was a combination of factors including size and type of virion as well as type of nucleic acid that dictated the susceptibility to treatment. The authors also concluded that when investigating the inactivation of viruses, a variety of strains should be used as indicators. This, they explained, was due to the fact that thus far it was not possible to predict the sensitivity of viruses to UV treatment from their size, structure and type of nucleic acid. They recommended that ΦX174 was too susceptible to UV treatment to be used as an indicator virus, but that MS2, as the most UV resistant bacteriophage in their study, may prove effective as a conservative indicator of UV virucidal activity. The inactivation kinetics of MS2 and ΦX174 were seen to follow a first order reaction, demonstrating a single hit process. It must be noted that this study was carried at UV-C wavelengths, as opposed to UV-A in the case of the results presented in this paper.

PR772 belongs to the *Tectiviridae* family of bacteriophages and was first isolated in the 1970s from a wastewater treatment system in South Africa [31]. They are icosahedral, double-stranded DNA bacteriophages, containing a lipid membrane beneath the shell. They infect Gram-negative bacteria carrying certain plasmids and are pilus-specific [32,33]. Among their hosts are *Escherichia* coli, *Salmonella typhimurium* and *Pseudomonas aeruginosa* [34]. Members of this family of phages are remarkably similar to each other, even though they have been isolated independently in extremely diverse geographical locations. This led Coetzee and Bekker to conclude that PR4 and PR772 were, in fact, the same phage [35]. Members of the *Tectiviridae* group are thought to be good indicators for adenoviruses, as they are similar in terms of genome replication, capsid architecture and vertex organization [32]. Unlike some other members of the family, such as PRD1, which has *Salmonella typhimurium* as a host, PR772 is grown on non-pathogenic *Escherichia coli*, thus giving it a practical advantage for laboratory testing. To the author's knowledge, no disinfection studies have been carried out on PR772, however a relatively similar somatic double-stranded bacteriophage, P22, was used in solar photolytic and photocatalytic studies by Davies *et al.* [36]. They obtained an S_{90} value (Global Solar Exposure required to achieve a 90 % reduction in phage concentration) of 1.5 MJ/m^2. This was not improved by the introduction of fixed or suspended TiO_2 and was significantly reduced when the transmittance of UV-B wavelengths was attenuated. Another similar double-stranded DNA bacteriophage, PRD1 (the genome sequences of PRD1 and PR772 were found to be 97.2% identical [32]), was shown to be 2-3 times less resistant to UV treatment than MS2 in an early study [37]. The characteristics of the three phages studied, as well as phage PRD1, are summarized in Table 1.

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Bacteriophage	MS2	ΦX174	PR772	PRD1
Size	26 nm	25 nm	53 nm	64 nm
Genome length	3,569 bp	5,386 bp	14,964 bp	14,927 nm
Genetic material	ssRNA	ssDNA	dsDNA	dsDNA
Topology	Linear	Circular	Linear	Linear
Infection	Male-specific	Somatic	Somatic	Somatic
Isoelectric point	3.5-3.9	6.6	4.0-4.2	3.0-4.2
Bacterial host	*Escherichia coli* 15597	*Escherichia coli* 13706	*Escherichia coli* BAA-769	*Salmonella typhimurium* LT2
UV Dose required for 4-log inactivation (mJ cm^{-2})	62	10	N/A	30

Table 1 Characteristics of bacteriophages used, as well as PRD1 (Adapted from [26,31,38])

This paper considers the photolytic and photocatalytic disinfection of the three phages described above, both under UV light at laboratory scale and under natural sunlight. Solar trials were conducted under varying weather conditions, in order to investigate the influence of solar intensity on disinfection rates. The findings revealed that the disinfection kinetics of the phages were greatly influenced by the nature of the light source and the variation in illumination intensity. Under artificial UV, the resistance of the phages to both photolytic and artificial treatment was found to decrease in the following order: PR772>ΦX174>MS2. Under solar treatment, however, phage PR772 was inactivated far more readily and MS2 was found to be more resistant.

2. Materials and Methods

2.1 Materials

2.1.1 Photocatalyst

Titanium dioxide was used as the photocatalyst in all of the experiments. The TiO_2 was supplied by the Degussa company and was of the type P25 Aeroxide (80:20 anatase:rutile). For experiments under artificial UV light, 50 mg of TiO_2 was measured out using a four-figure microbalance (Cahn C-33) and mixed with 1 L of distilled water in an autoclave bottle. The contents of the bottle were then sterilized by autoclaving [HiClave HV-25 autoclave (HMC, Japan)] for 40 minutes at 121 °C.

2.1.2 Viruses

The three viruses used in the experiments - MS2 (F-specific coliphage), ΦX174 (somatic coliphage) and PR 772 (*Tectiviridae* coliphage) - were supplied by ATCC in freeze-dried form. After hydration in suitable broths, the viruses were stored at 4 °C.

2.1.3 Host Bacteria

Three strains of *Escherichia coli* were used as the hosts for the phages. The bacteria were *E. coli 15597* (MS2), *E. coli 13706* (ΦX174) and *E. coli BAA-769* (PR772) and were all supplied by ATCC.

2.1.4 Growth Media

The media used were Escherichia broth, agar and top agar (MS2); nutrient broth, agar and top agar (ΦX174); and tryptic soy broth, agar and top agar (PR 772). These were all prepared according to ATCC guidelines.

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2.1.5 Reactors

Two compound parabolic collectors (CPCs) were used in the experiments, one laboratory scale [Figure 1 (a)] and one full-scale [Figure 1 (b)].

The laboratory scale compound parabolic reactor consisted of six parallel Pyrex tubes (9.6 mm internal diameter), each 250 mm long, connected by plastic tubing (which was covered during experiments to ensure that only the Pyrex tubes were illuminated). The half acceptance angle of the reflector was 90^0 and the concentration ratio was approximately 1. The closed loop reactor was connected in series to a submersible pump (2.8 L/min, giving a fluid velocity of 0.64 m/s) pumping from a 1 L reservoir which was located in the dark [Figure 2 (a)]. The Reynolds number for the flow rate was calculated to be 5450 meaning that the flow regime was in the smooth turbulent zone. The water undergoing treatment was continuously recirculated throughout the experiment.

To prove no photocatalyst settling would occur in the reservoir, the velocity gradient (G) was calculated to be 147 s^{-1}, indicating that the photocatalyst was well mixed. The settling velocity of the TiO_2 particles was worked out to be 1.37×10^{-9} m/s using Stoke's Law, compared to the fluid velocity of 0.64 m/s, meaning that no settlement could occur in the reactor system.

The full-scale CPC, obtained from AO SOL (Portugal), consisted of ten parallel glass tubes (23 mm internal diameter), each 1,504 mm long, connected by opaque plastic joints, which were 549 mm (left-hand side of CPC) and 389 mm long (right-hand side). The half acceptance angle of the aluminium reflector was 90^0 and the concentration ratio was 1. The reactor was gravity fed and was connected to two reservoirs, one above the top tube and one on the ground. A peristaltic pump (Watson Marlow, USA) was used to transfer water from the lower reservoir to the upper [Figure 2 (b)].

2.2 Methods

2.2.1 Viral propagation and titration

The host *E. coli* was rehydrated in the appropriate broth and streaked on an agar plate, which was incubated for 18 hours at 37 °C. A colony of the host *E*. coli for each virus was scraped from a plate into a conical flask containing 100 mL of appropriate broth and grown up in an orbital shaker at 37 °C for 18 hours. 200 µL of the appropriate *E. coli* was added to 3.5 ml of melted top agar, which were kept in sterile tubes at 50 °C in a water bath. The tubes containing the top agar and bacteria were then vortexed for 5 s and poured onto agar plates, pre-warmed at 37 °C in an incubator. The plates were allowed to cool at room temperature. The plates were quartered and two aliquots of 20 µL of each sample dilution were plated. The plates were stored overnight in an incubator at 37 °C and the plaques were counted.

When necessary, phages were amplified by preparing plates as outlined above and vortexing the top agar, plaques and *E. coli*, scraped off the plate after incubation. The supernatant was conserved and a titration was carried out as above to determine the concentration of phage.

2.2.2 Laboratory scale CPC

The reactor was placed in a non-fluorescent box, below a parallel UV-A light source consisting of four parallel UV tubes (Philips HB 175) and subjected to a light intensity of 17 W/m^2 [measured using a PMA2100 radiometer (Solarlight, USA)]. The reactor was first disinfected by pumping 0.1 M ethanol around it for five minutes. This was then followed by rinsing with four flushes of sterile water to ensure that no ethanol would remain in the system and lead to unwanted bacteria kill-off during the experimental periods. This rinsing procedure was proven to remove all traces of ethanol in a previous study on the same system [39].

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1 mL of each phage was added to 1 L of sterile water in the reservoir, containing the appropriate concentration of TiO_2. After priming the pump with the sample, the tubes at either end of the reactor system were placed in the reservoir, along with a thermometer. The reservoir was placed in a non-fluorescent chamber and the pump was turned on. From previous flow regime tracer studies on the same system using Rhodamine, it was determined that complete mixing of the phages occurred throughout the system after 140 seconds [39]. At this time, a 1 mL sample was taken from the reservoir, using a sterile transfer pipette, as the UV light source was switched on. Samples were then taken at the sample points described below. The sample points were: 0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420 and 1500 minutes. The photolytic and photocatalytic experiments were both carried out three times to ensure accuracy of results.

2.2.3 Full-scale CPC

Experiments were also performed in the full-scale CPC under natural sunlight. The CPC was tilted due south at 53°21'to the horizontal, which corresponds to the angle of latitude of the location at which the experiments were undertaken (Trinity College, Dublin, Ireland). The water was allowed to flow by gravity from a covered elevated reservoir through the CPC to a second covered reservoir situated at ground level [Figure 2 (b)]. The water was pumped back to the higher reservoir using a peristaltic pump (Watson Marlow 624S, USA) and the pump flow rate was adjusted to ensure a constant head was achieved. The UV-A and UV-B detectors were attached to the frame of the reactor, which meant they were also inclined at an angle corresponding to the angle of latitude. After ethanol was passed through the system for disinfection purposes, the reactor was rinsed using four flushes of distilled water, followed by the water being used in the experiment. The reactor was covered while phage samples were spiked into the lower reservoir. TiO_2 was also added at this point in the photocatalytic experiments. After five minutes, when complete mixing of the microorganism had occurred, the first sample was taken. 1 mL samples were then taken using a sterile transfer pipette at the following times: 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 420 minutes. A thermometer was placed in the lower reservoir and the temperature of the water was noted at each sample time.

2.2.4 Analysis

For a sample taken at time t, the phage concentration (N_t) was divided by the initial concentration (N_0) and the log of this value was plotted against time and/or cumulative dose. The cumulative UV dose was calculated using the following formula:

$$Q_{uv,n} = Q_{uv,n-1} + \frac{\Delta t_n UV_{G,n} A}{V_T} \qquad \text{(Eqn. 1)}$$

where Q_{UVn} and Q_{UVn-1} are the cumulative irradiated UV energy received per L of sample at times *n* and *n*-1; Δt_n is the time interval between sampling times; UV_{GN} is the average incident radiation on the irradiated area; A is the irradiated area; and V_T is the total circulating volume.

In the case of the UV lamp, the intensity was a constant 17 W/m^2 throughout the experiment, so the equation became:

$$Q_{uv,n} = \frac{t_n UV_{G,n} A}{V_T} \qquad \text{(Eqn. 2)}$$

where t_n is the time at which the sample is taken and UV_{GN} is a constant value of 15 W/m^2.

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Note: the solar experiments were repeated a number of times, as phage titration methods yield no results for some phages on occasion, usually due to the top agar and bacterial host overlay being too thin, making the visibility of plaques too poor for cell counts. Sufficient experiments were conducted such that there were two sets (maybe you should say 3 sets of results as they will only come back at you saying 2 is not enough!) of results for each phage in both the absence and presence of TiO_2.

3. Results and Discussion

3.1 UV photolytic and photocatalytic inactivation

PR 772 was found to be extremely resistant to both UV and UV photocatalytic treatment, with only minor decay witnessed in 25 hours of illumination [corresponding to 87.21 kJ/L (Figure 3)]. The inactivation rates in the absence and presence of catalyst were very similar, with the introduction of photocatalyst bringing about a slight decrease in inactivation rate. This would corroborate findings by Gerrity *et al.* [26], who used the similar phage PRD1, as reported in Section 1.1. They attributed the poor performance of the photocatalyst to the limited adsorption of the viruses onto catalyst particles and the attenuation of light by the TiO_2. ΦX174 was deactivated much more readily, with the introduction of catalyst improving the disinfection rate. MS2 was found in this research to be the most susceptible to both photolytic and photocatalytic treatment and again was found to disinfect more readily in the presence of catalyst. This is in contrast to previous findings by Sommer *et al.* [30], who found MS2 far more resistant to UV treatment than ΦX174. In that study, however, germicidal light (~253 nm) was used, as opposed to UVA, meaning that the influence of reactive oxygen species would be muted in relation to the direct DNA disruption. They also suggested that ΦX174 may be a poor indicator, due to its high susceptibility to UV treatment, a claim which these findings would not support. In the tests conducted during this study, the phages were all present simultaneously, meaning that they were all in competition for any reactive oxygen species produced. Thus, results may have differed if the experiments were carried out on each phage sample individually. The plots for each of the trials showed good linearity, with the exception of the MS2 plots. Linearity would be expected in plots based on tests on single stranded viruses under a constant UV source [29], but the MS2 plots in the absence and presence of a catalyst both show fast initial disinfection, followed by a tailing off period until the MDL is reached.

3.2 Solar photolytic and photocatalytic inactivation

The results of all experiments conducted under UV (in the bench-scale CPC) and sunlight (in the full-scale CPC) are compiled in Table 2 and compared in Figure 4. The time and cumulative UV dose to reach 1-log and 3-log inactivation in each case are presented. The days on which solar experiments took place ranged from completely overcast to very sunny.

3.2.1 MS2

Under sunlight, the inactivation rate of MS2 was far slower than it had been under UV light, with respect to both time and UV dose received [Figure 4 (a)]. However, when photocatalyst was introduced, the initial inactivation rates were very similar to those seen in UV tests. Whereas there was a tailing off period during UV photocatalytic disinfection however, the solar photocatalytic inactivation continued at the same rate until the MDL was reached. This linearity is more in keeping with previous research into the disinfection of phage MS2 by photocatalysis [18]. The higher disinfection rate in the presence of catalyst can be attributed to the far greater number of hydroxyl radicals produced during TiO_2 photocatalysis than during solar treatment in the absence of catalyst. As reported in Section 1.1, according to Cho *et al.* [22] the photocatalytic disinfection of MS2 takes place solely due to the influence of bulk phase hydroxyl radicals, which induce the denaturing of the capsid protein. Sjogren and Sierka [18] deduced that adsorption of MS2 to TiO_2 particles is not favored at neutral pH, due to surface charges. Even the MS2 particles that would be forced into contact with TiO_2 particles by the

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water due to their hydrophobic nature would be situated at parts of the catalyst particle unsuitable for contact with hydroxyl radicals. Therefore, it would be expected that MS2 would be less readily inactivated if a fixed catalyst was used instead of suspended TiO_2.

3.2.2 ΦX174

Disinfection of ΦX174 in the full-scale CPC under sunlight was more efficient than in the bench-scale CPC under UV light, both in the presence and absence of catalyst [Figure 4 (b)]. The solar intensity on all four days on which ΦX174 results were obtained was greater than the intensity received during the UV experiments, as is displayed in Table 2. It would appear that as well as cumulative dose, the intensity of light received is an important parameter for disinfection. That is, a short exposure to high intensity light is more efficient than a long exposure to lower intensity light, even if the cumulative dose is the same. It must also be remembered when comparing the UV and solar experiments that they were conducted in different reactor systems, with the full-scale system being a commercial production, optimized for optical efficiency and having a higher proportion of the circulating volume illuminated at any time. It is interesting that during both of the solar photolytic experiments the UV intensity decreased dramatically in the final two hours, but that disinfection proceeded rapidly. This might suggest that irreversible damage was done to some viruses during the high intensity period and that they subsequently gradually lost their viability.

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Phage	TiO_2 concentration [mg/L]	Light source	Time for 1-log inactivation [min]	Time for 3-log inactivation [min]	UV Dose for 1-log inactivation [kJ/L]	UV Dose for 3-log inactivation [kJ/L]	Peak UV intensity [W/m^2]	Average UV intensity [W/m^2]
MS2	0	UV lamp	~10	~60	~0.6	~3.5	17	17
ΦX174	0	UV lamp	~180	~900	~10.4	~52.3	17	17
PR772	0	UV lamp	~1000	nr	~58.1	Nr	17	17
MS2	50	UV lamp	~5	~15	~0.3	~0.9	17	17
ΦX174	50	UV lamp	~22	~345	~1.3	~20.1	17	17
PR772	50	UV lamp	~1300	nr	~75.6	Nr	17	17
MS2	0	Natural sunlight	~170	nr	~17	Nr	39.3	22.5
MS2	0	Natural sunlight	~105	nr	~5	Nr	18.2	6.6
ΦX174	0	Natural sunlight	~40	~190	~5	~18	39.3	22.5
ΦX174	0	Natural sunlight	~23	~120	~2	~14.5	45.5	22.7
PR772	0	Natural sunlight	~92	~145	~13	~16.5	45.5	22.7
PR772	0	Natural sunlight	~179	nr	~6	Nr	18.2	6.6
MS2	50	Natural sunlight	<5	<5	<0.25	<0.25	44.4	19.3
MS2	50	Natural sunlight	<5	<10	<0.5	<1	41.8	33.4
ΦX174	50	Natural sunlight	~20	~30	~1	~2.6	41.8	33.4
ΦX174	50	Natural sunlight	<5	<10	<1	<1	40.4	19.3
PR772	50	Natural sunlight	~20	~32	~2	~4.5	46.9	26.8
PR772	50	Natural sunlight	~32	~52	~3	~5.4	41.8	33.4

Table 2 Time and UV Dose required to reach 1-log and 3-log inactivation in each of the experiments. (Note: values are interpolated and values under UV light are averages from a combination of three repeat experiments). nr = not reached.

3.2.3 PR772

PR772 exhibited the most pronounced differences between inactivation rates under solar and UV light [Figure 4 (c)]. Under UV light, 3-log disinfection was not achieved with or without photocatalyst after 25 h, or 87.21 kJ/L, of irradiation. In the solar experiments in the presence of photocatalyst, this was reached after just 32 and 52 minutes, or 4.5 and 5.4 kJ/L, respectively (these figures were interpolated from the results). Indeed, 5-log disinfection was achieved after 60 and 100 min, or 7 and 13 kJ/L. In

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the first photocatalytic trial, the average UV dose received was roughly 1.5 times that received during the photolytic experiments, while in the second trial, the average dose was twice the photolytic value. This would seem insufficient to explain the vast discrepancy in disinfection rates encountered. It is hypothesized that the vastly superior disinfection rates are linked to the high peak solar intensities received (46.9 and 41.8 W/m^2), as well as the optical efficiency and proportion of volume illuminated in the large-scale reactor and the fact that the solar spectrum is far wider than the spectrum emitted by the UV lamp. Another factor which could be investigated in further studies would be the influence of temperature, as peak values of 33° and 37° were measured in the solar tests, while the temperatures did not rise above 27° under artificial UV light. It is an encouraging finding for the efficiency of solar photocatalysis that a species which was so resistant to UV photocatalytic treatment was so readily inactivated under sunlight. It must also be remembered that these tests, although conducted on sunny days, were performed at a significantly more northerly latitude (53°21') than most previous solar photocatalytic tests.

3.2.3 Comparison of disinfection kinetics under sunlight

In Figure 5, the disinfection rates of the phages under solar treatment are compared. In the UV experiments, the viruses were seen to show the following relative resistance: PR772>ΦX174>MS2. Under natural sunlight, however, the inactivation rates were much more similar for each phage. Indeed, as Figure 5 (a) shows, it is difficult to draw a comparison among the phages in terms of efficiency of disinfection in the absence of catalyst. If the UV dose required for 1-log disinfection in each case is compared (Table 2), resistance declines in the following order: MS2 (Trial 1)>PR772 (Trial 1)>PR772 (Trial 2)>MS2 (Trial 2)≈ ΦX174 (Trial 1)>ΦX174 (Trial 2). These findings would suggest that ΦX174 is the most susceptible to solar treatment, but further investigation shows that both ΦX174 trials and the first PR772 trial were the only ones in which 3-log disinfection was reached. It is thought that the variation in intensity during the experiments had a major influence on the disinfection rate as the experiments progressed. In Figure 5 (b), it can be seen that the introduction of a catalyst brought about much more defined, linear inactivation kinetics. Again, this could be attributed to the production of hydroxyl radicals being less dependent on solar intensity than direct solar disinfection. Again, as was found under artificial UV light, the MS2 phage was the most easily inactivated by photocatalysis, with PR772 the most resistant.

4. Conclusions

The bacteriophages studied, MS2, ΦX174 and PR772, showed varying disinfection characteristics under UV and solar treatment. Under artificial UV, the resistance of phages to both photolytic and artificial treatment was found to decrease in the following order: PR772>ΦX174>MS2. Under solar treatment, PR772 was inactivated far more readily and it is thought that the variation in solar intensity during the trials had a major effect on disinfection rates, as kinetics for phages were quite different under different weather conditions.

The large variation in the disinfection characteristics of the different organisms under different experimental conditions suggests that a wide range of organisms should be used as indicators in similar studies. It also highlights the dangers of deeming particular phages as unsuitable indicator organisms on the basis of single experiments. For example, based on the solar experiments, PR772 may have been dismissed as too susceptible to photolytic and photocatalytic treatment to be of use as an indicator, yet it was by far the most resistant phage in the experiments conducted under artificial UV light. The findings in this paper that MS2 was the most easily inactivated goes against the consensus seen in the literature (Section 1.1), which again shows that phages may behave very differently when experimental conditions are not exactly the same.

The findings presented above highlight the importance of conducting preliminary in-situ experiments prior to the implementation of solar photocatalytic reactor systems for the disinfection of drinking

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water. The disinfection rates of a number of different relevant viruses would need to be considered and the design of the system should be such that the disinfection of the most resistant virus is optimized. Trials must also be undertaken under a variety of meteorological conditions, as the variation in solar intensity was found to have a significant impact on disinfection kinetics.

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Figure 1 (a) Laboratory scale CPC; (b) Full-scale CPC during mixing of TiO_2

Figure 2 Schematic of reactor set-ups for (a) laboratory scale CPC and (b) full-scale CPC.

Figure 3 Plot of log disinfection vs cumulative UV dose for bench-scale experiments under UV light. ✳ MS2 0 mg/L TiO_2; ● MS2, 50 mg/L TiO_2; ♦ ΦX174, 0 mg/L TiO_2; ■ ΦX174, 0 mg/L TiO_2; ▲ PR772, 0 mg/L TiO_2; × PR772, 50 mg/L TiO_2. (Note: error bars plotted on just one line to avoid confusion).

Figure 4 Plots of bacterial inactivation vs cumulative dose comparing findings under artificial UV light and natural sunlight for (a) MS2; (b) ΦX174; and (c) PR772. ♦ 0 mg/L TiO_2, UV; ■ 50 mg/L TiO_2, UV; ♦ 0 mg/L TiO_2, sunlight, first trial; ■ 0 mg/L TiO_2, sunlight, second trial; ▲ 50 mg/L TiO_2, sunlight, first trial; ▲ 50 mg/L TiO_2, sunlight, second trial;

Figure 5 Plots of $\log_{10}$(disinfection) vs cumulative dose for experiments conducted under natural sunlight in the (a) absence and (b) presence of 50 mg/L TiO_2. ■ MS2, first trial; ■ MS2, second trial; ▲ ΦX174, first trial; ▲ ΦX174, second trial; ♦ PR772, first trial; ♦ PR772 second trial

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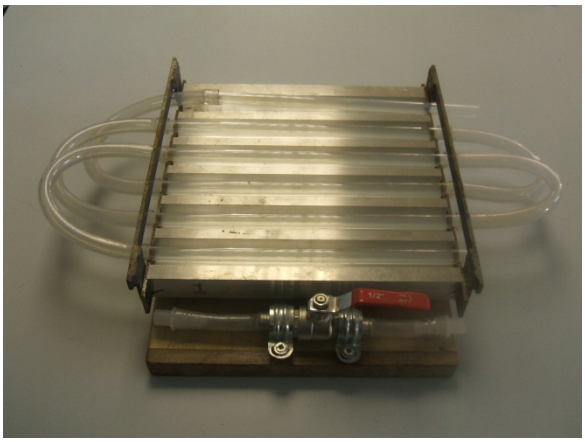

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Fig. (b)

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Fig. 2(a)

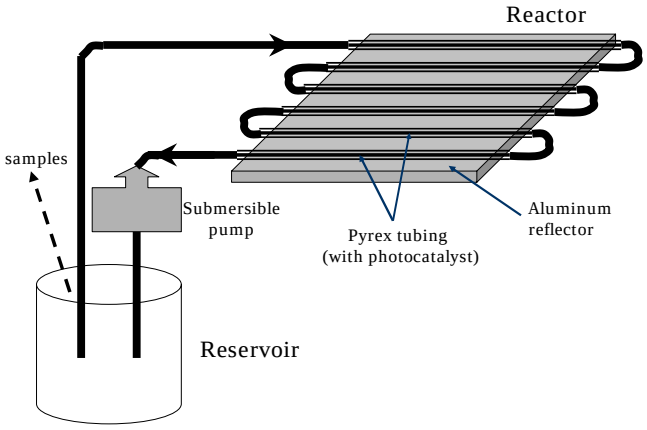

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Fig. 2(b)

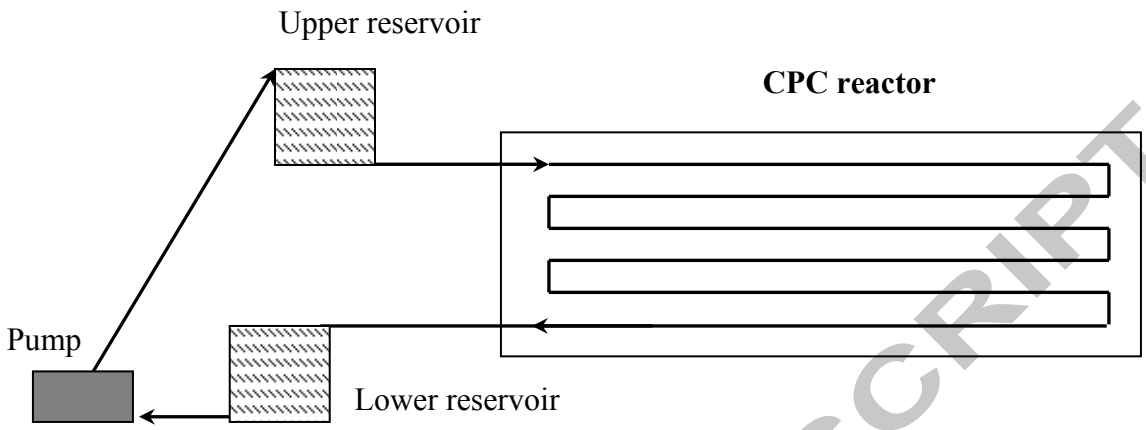

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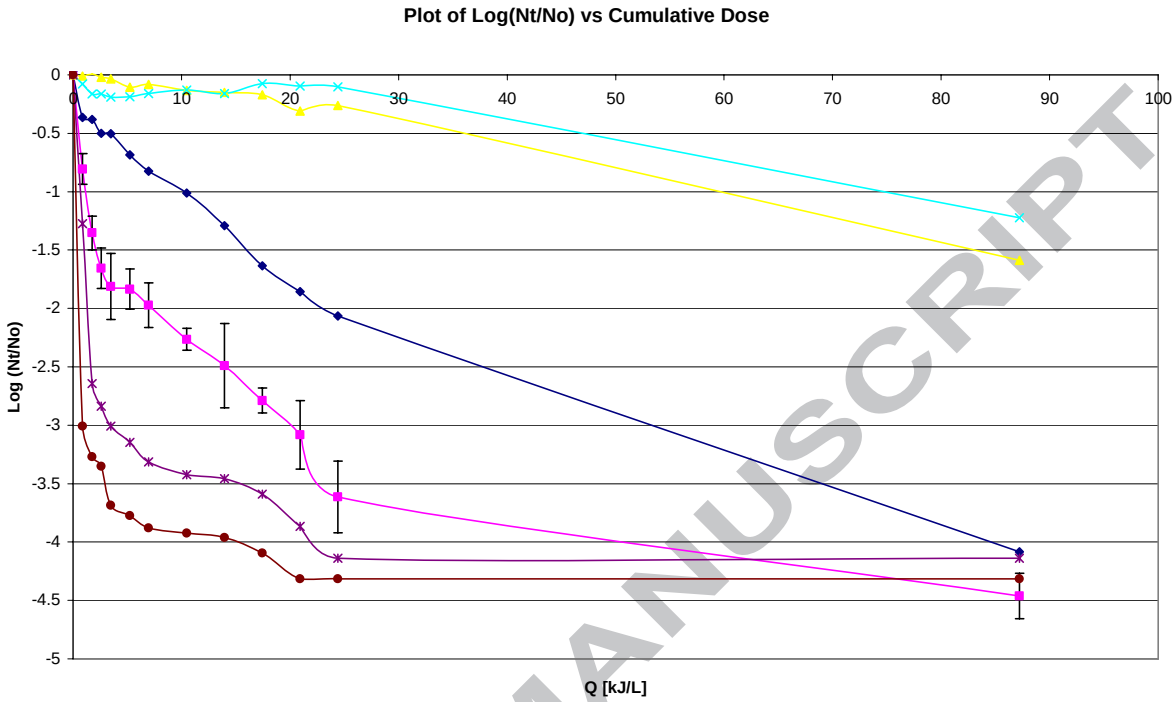
Fig. 3



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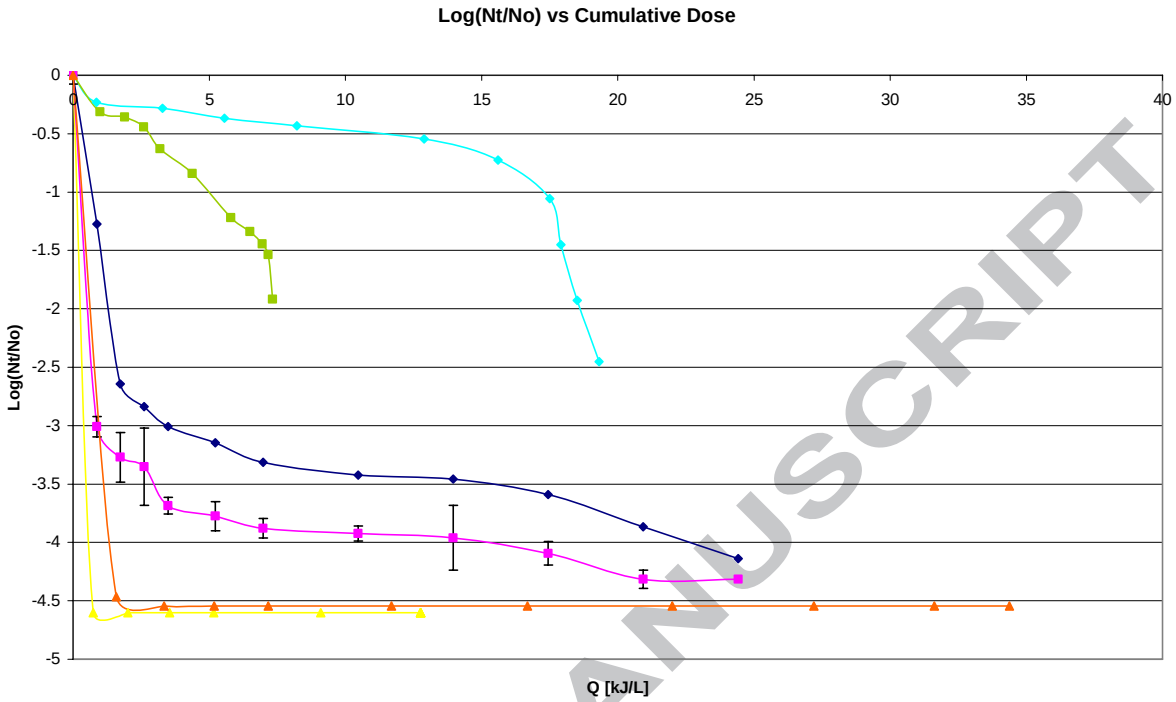

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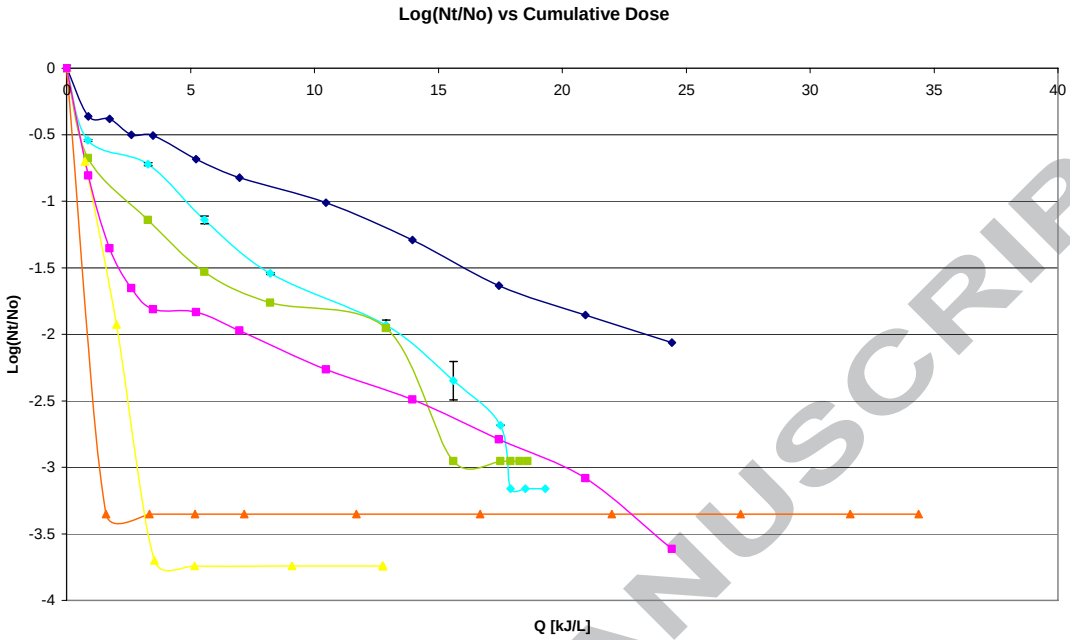

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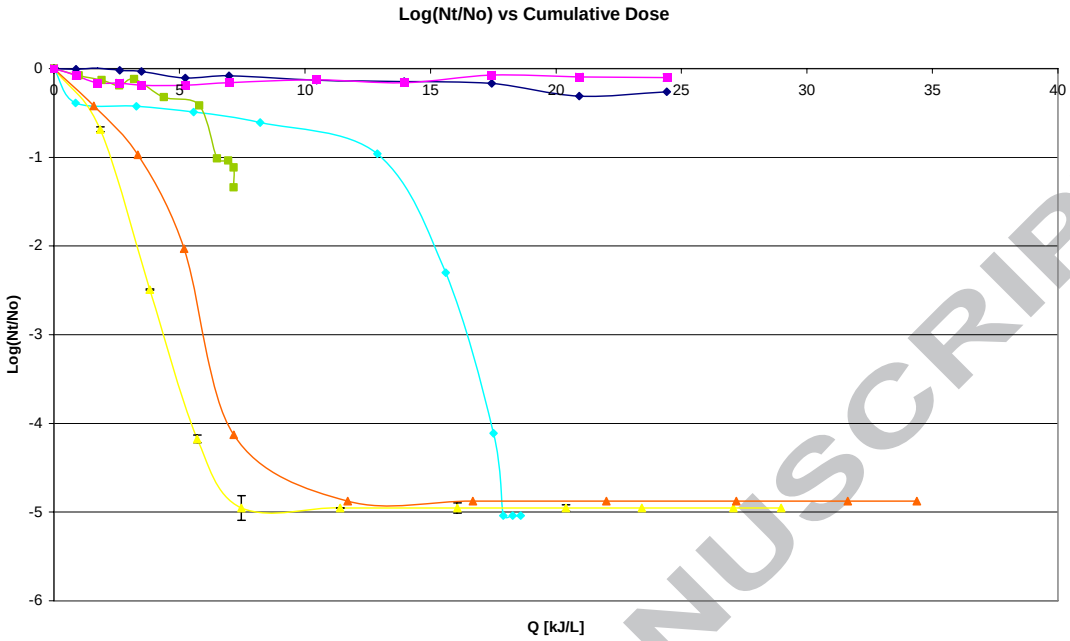

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Fig. 5(a)

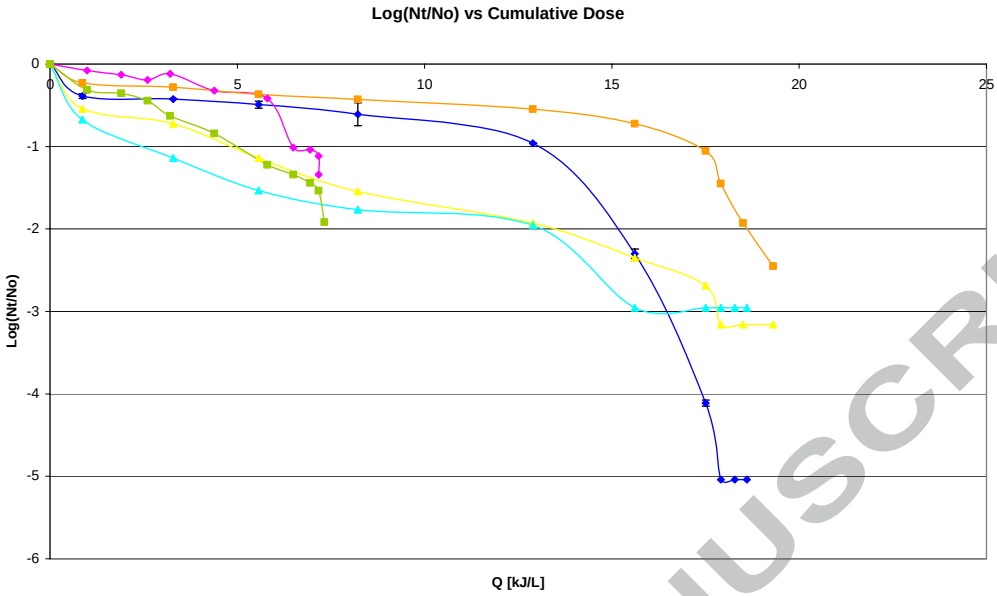

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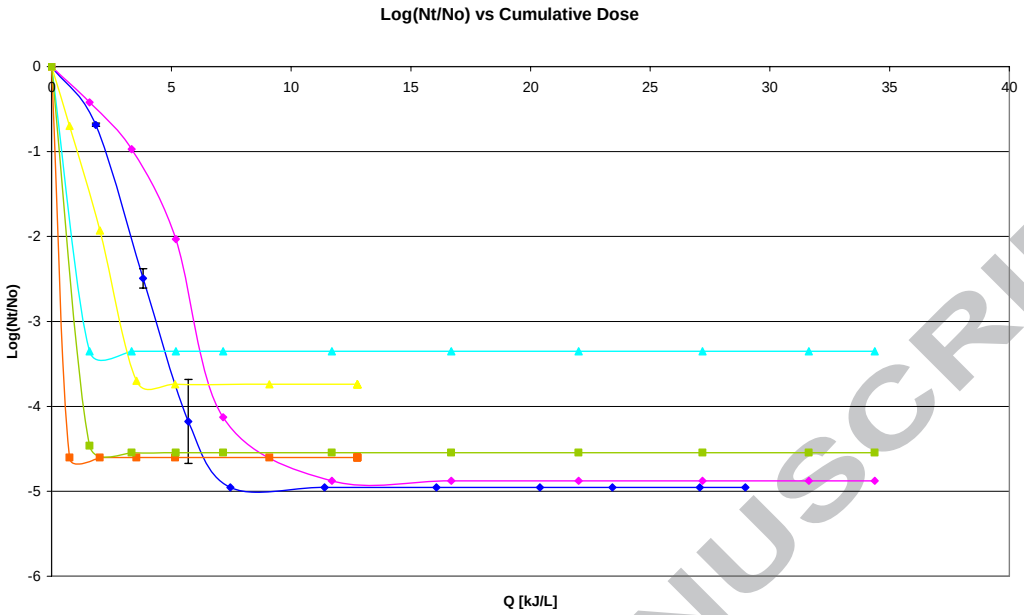
Fig. 5(b)



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Highlights

Inactivation characteristics of MS2, ΦX174 and PR772 studied under UV and solar treatment, with and without TiO_2 > Under UV light, PR772 more resistant to photolytic and photocatalytic disinfection than other two phages > PR772 inactivated more readily under sunlight than under UV light > Disinfection kinetics varied greatly with weather conditions > A wide range of organisms should be used as indicators in solar disinfection studies.

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