Is it all light? Mechanisms of virus removal in waste stabilisation ponds

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Introduction

Globally waste stabilisation ponds (WSP) offer a sustainable and economical method of treatment for wastewater. The actual removal mechanisms occurring within ponds are largely a black box and only a few studies have been conducted on virus removal (Hewitt et al., 2011; Maynard et al., 1999). Most studies predict virus removal using the indicator organisms *E. coli* and bacteriophages (Maïga et al., 2009; Sinton et al., 2002). While it is clear that sunlight (UV) and temperature play a major role in removal of pathogens in WSP's there are other mechanisms present in these complex systems that also play their part in removal of pathogens such as viruses such as pH, dissolved oxygen (DO) and enzymatic activity.

Objectives

Our research aims to gather more data on virus removal in WSP's and gain knowledge of light-dependent and light-independent mechanisms of removal of viruses e.g. pH and dissolved oxygen (DO), enzymatic activity, settlement and adsorption. Part of our research involved comparing holistic removal of indicator organisms with an enterovirus in the presence and absence of light over two consecutive summer seasons.

Methods

Large mesocosms were designed to hold WSP water (21.5 L), split into two discreet partitions by a permeable membrane, where the sunlight exposure and pH, DO could be adjusted (*Figure 1*). Each mesocosm was spiked with *E. coli*, R-RNA MS2 bacteriophage (MS2 phage) and Echovirus 7 at the same time (at dusk). At set time intervals over the experimental period samples were taken and analysed for *E. coli* (pour plating onto selective media (Oxoid, UK)), MS2 phage (overlay assay (American Public Health Association (APHA), 1998)), Echovirus 7 (tissue culture infectious dose fifty (TCID50) procedure (Karber, 1931)) and heterotrophic plate counts (HPC) (non-selective agar using pour plating (APHA, 2005)).

pH, DO and temperature were recorded during each experiment using in situ probes connected to dataloggers. Mesocosm experiments were set up to allow an exchange of pH and DO from the light mesocosm to the dark mesocosm (as seen in WSP's) (*Figure 1*) and a control was set up with no exchange of pH or DO. At the start and end of the experiment samples were taken and sent for algal identification and abundance (Cawthron Institute, Nelson, New Zealand).

The sunlight intensity (global radiation and UV) was obtained for the experimental periods from the NIWA CliFlo database (National Institute of Water and Air, New Zealand).

Results and discussion

By allowing pH and DO transfer between light and dark mesocosms biochemical parameters remained constant between light and dark (*Table 1*). In addition this system enabled the diurnal fluctuations in pH and DO seen in WSP's to be mimicked in the light and dark mesocosms. Previous research has demonstrated diurnal fluctuations of pH, DO and temperature

Figure 2: Mesocosms arrangement showing the pumping system used and the in-situ probes recording pH, DO and temperature during the experiment. Samples were taken using a sterile syringe from the top port seen in the figure on the right. The port had a right angled joint to ensure samples were taken from the middle of the mesocosm.

in WSP's in New Zealand (authors previous research, *Figure 1*) and overseas (Kayombo et al., 2002).

The results demonstrate that if the pH and DO levels in the WSP remain elevated similar levels of removal of indicator and virus can be achieved in the presence and absence of direct sunlight (*Tables 1 and 2*) ie throughout the pond.

The ability to equilibrate pH and DO between the dark and light mesocosms achieved similar removal rates in the light and dark mesocosms; 6 log reduction in *E. coli* during summer 2011 and 7 log during summer 2012, and 5 log reduction in MS2 phage during summer 2011 and 6 log during summer 2012, recorded in light and dark mesocosms (*Table 2*). Echovirus 7 removal was slightly lower during both summers but still achieved high and comparable removal in light and dark mesocosms: 4 (dark) and 5 (light) log during summer 2011 and 5 log during summer 2012 (*Table 2*).

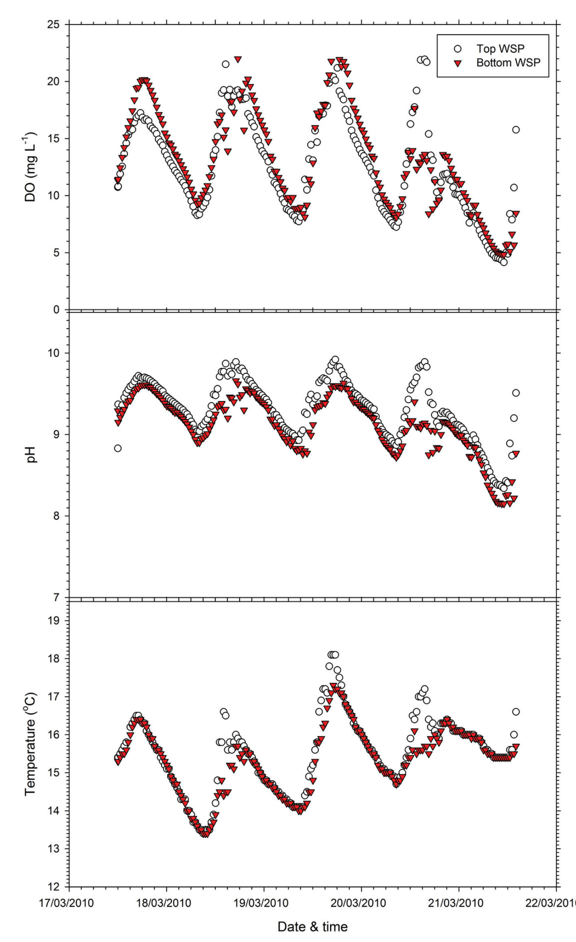
When the rate of removal between the summer experiments was studied a slower removal rate was seen during the summer 2012 experiment (*Figure 3*). This can be related to the lower temperatures occurring during this experiment (*Table 1*) and not related to the sunlight or UV radiation occurring (as there was no significant relationship seen).

During summer 2011 experiment a similar comparable removal was seen in static mesocosms (no exchange of pH and DO) compared to exchange mesocosms for all parameters (*Table 2*). This was most likely due to high temperatures occurring during the experiment enhancing removal in the dark static mesocosm (*Table 1*). A dark static mesocosm was set up at the same time as the exchange mesocosms during the summer 2012 experiment. A much lower removal of all *E. coli* compared to the exchange mesocosm was seen: 1 log removal in static compared to 7 log removal in exchange mesocosm (*Table 2*). MS2 phage and Echovirus 7 results were similar in the static and exchange mesocosms indicating these organisms were more sensitive to the temperatures reached (*Tables 1 and 2*).

Ranges of temperature, pH and DO recorded during each experiment are presented in *Table 1*. Temperatures were consistent between light and dark mesocosms during experiments. High temperatures were obtained during the summer 2011 experiment on one day but the average temperatures obtained were within the normal range seen in WSP during summer months in NZ (January – March). Average temperatures were lower during the summer 2012 experiment compared to summer 2011 (*Table 1*). Similar ranges and means for pH were recorded for static and exchange mesocosms as well as between the summer experiments. The exception being during the summer 2012 experiment the dark static mesocosm remained lower than the summer 2011 experiment: mean 7.9 during summer 2012 compared to 9.6 during summer 2011 (*Table 1*). In the exchange mesocosms for both summers the mean pH remained over 10 which in previous research has been shown to be an important removal mechanism in WSP (Curtis et al. 1992). Previously elevated pH and DO have been shown to increase efficacy of sunlight inactivation (Davies-Colley et al 1999) and in this experiment the same removal was observed in the dark mesocosms (*Tables 1 and 2*).

Comparing the amount of sunlight (global radiation) and amount of UV radiation occurring during the summer experiments higher global radiation occurring during summer 2011 compared to summer 2012 (*Figure 4*). The UV radiation showed the opposite trend however, and higher UV radiation occurred during summer 2012 (*Figure 4*). Although there was a slightly higher log removal observed for *E. coli*, MS2 phage and Echovirus 7 during the summer 2012 experiment there was only a significant (P = < 0.05) negative correlation seen between *E. coli* and UV radiation levels.

The algal abundance between the two experiments showed that during summer 2012 there was a higher abundance in terms of the number of species present (*Figure 5a*). Also a higher abundance of potentially toxic cyanobacteria was seen in summer 2012 (*Figure 5b*). If the cyanobacteria leave the WSP and enter the receiving water (river system) there is a potential for their spread downstream to recreational areas.



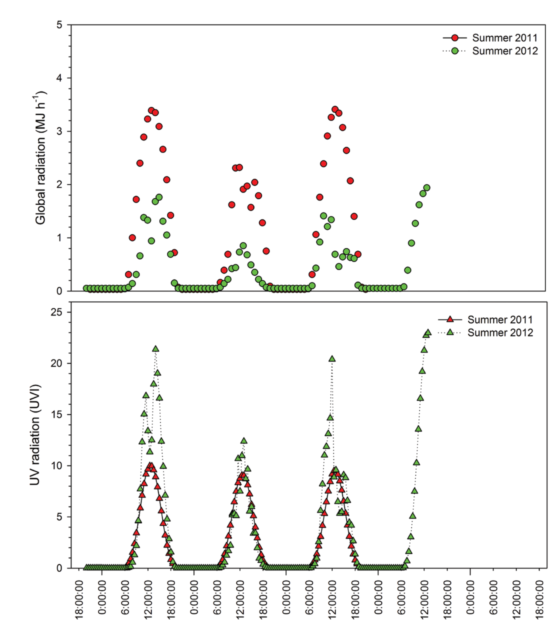


Figure 4 (above): Global radiation and UV radiation levels recorded during the summer experiments.

Figure 1 (left) : An example of typical DO, pH and temperature readings recorded at a local WSP.

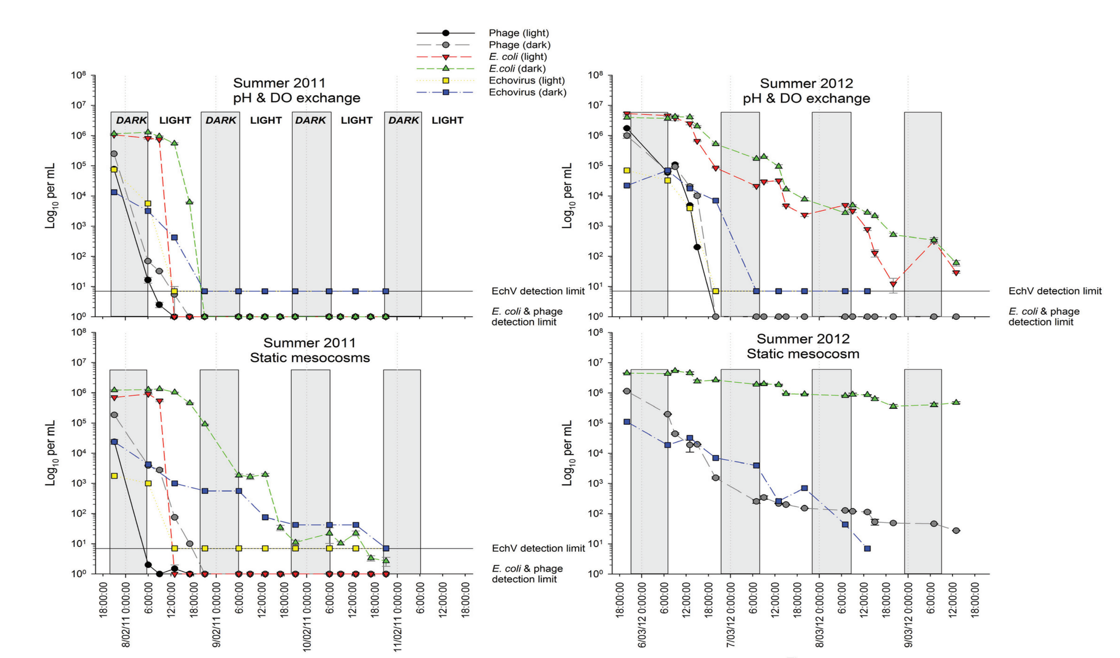


Figure 3: Removal rates of E. coli, MS2 phage and Echovirus during mesocosm experiments.

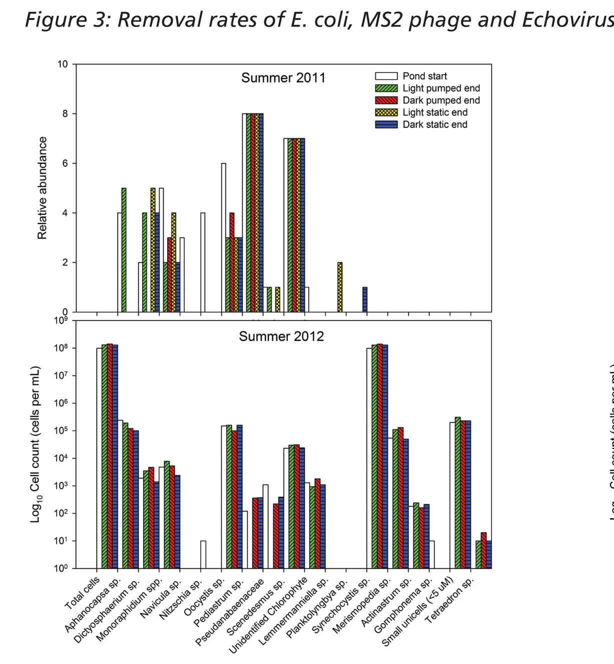


Figure 5a: Abundance of algal species during the experiments. Note the summer 2011 experiments only show relative abundance where 8 is the most abundant species and 1 is the least.

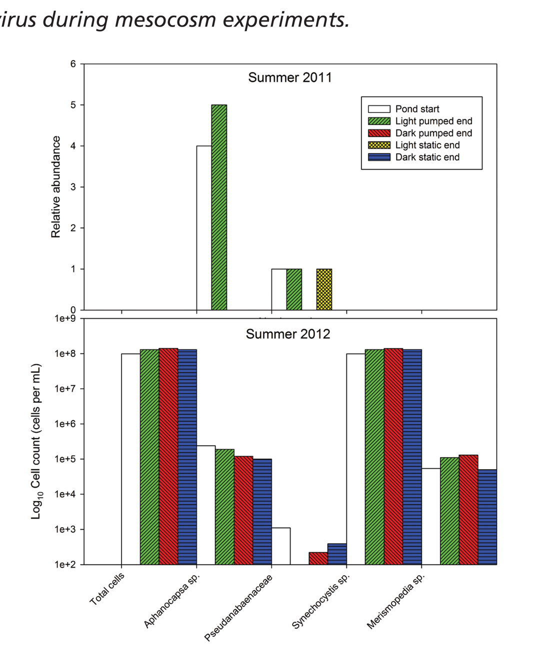


Figure 5b: Relative abundance of potentially toxic cyanobacteria species. Note the summer 2011 experiments only show relative abundance where 8 is the most abundant species and 1 is the least.

Table 1: Temperature, pH and DO measurements taken during mesocosm experiments.

Date	Experiment	Temperature (°C)		pH		DO (mg/L)	
		Light	Dark	Light	Dark	Light	Dark
Feb-11	pH DO exchange	10.0 - 30.2 (19.1)	13.4 - 27.6 (19.6)	10.6 - 11.3 (11.1)	10.2 - 10.7 (10.4)	8.7 - 22.0** (14.2)	6.9 - 11.1 (9.1)
Feb-11	static system	10.1 - 21.9 (18.7)	13.7 - 23.9 (18.2)	9.3 - 11.3 (10.5)	9.0 - 10.2 (9.6)	10.9 - 22.0** (15.4)	6.4 - 9.1 (7.7)
Feb-12	pH DO exchange	7.8 – 24.5 (14.9)	6.0 – 24.5 (15.4)	9.4 – 10.5 (10.1)	9.6 – 10.2 (9.9)	8.4 – 22.0** (14.3)	6.6 – 14.7 (11.5)
Feb-12	static system		10.6 – 24.5 (16.4)		7.5 – 9.4 (7.9)		4.6 – 12.9 (5.8)

** Values in brackets denote mean values. ** DO value of 22.0 is the maximum reading possible for the probe used.*

Table 2: Log_{10} removal of E. coli, MS2 phage, Echovirus 7 and HPC.

Date	Experiment	Parameter	Light	Dark
Feb-11	pH DO exchange	*E. coli*	6.02	6.06
		MS2 phage	4.89	5.4
		Echovirus 7	4.88	4.13
		HPC	5.98	6.09
Feb-11	static system	*E. coli*	5.85	6.08
		MS2 phage	4.38	5.27
		Echovirus 7	3.25	4.38
		HPC	6.1	6.1
Feb-12	pH DO exchange	*E. coli*	6.73	6.60
		MS2 phage	6.24	6.00
		Echovirus 7	4.85	4.64
		HPC	3.02	2.83
Feb-12	static system	*E. coli*	nd	0.98
		MS2 phage	nd	4.62
		Echovirus 7	nd	5.05
		HPC	nd	0.95

nd denotes no data as a light static mesocosm was not set up for this experiment.

Conclusions

- **Although sunlight is an important factor in virus removal in WSP other mechanisms are playing a role in removal.**
- **High levels of pH and to a lesser extent DO, which generate photoxidative by-products, are also a mechanism of removal in the dark sections of WSPs.**
- **Removal of *E. coli* is negatively correlated with UV radiation but MS2 phage and Echovirus 7 are not.**
- **Potentially toxic cyanobacteria are found in WSP systems, on occasions at high levels.**

Acknowledgements

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Further information

Please contact louise.weaver@esr.cri.nz for further information on this project.

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