A Review of the Factors Affecting Sunlight Inactivation of Micro-organisms in Waste Stabilisation Ponds: Preliminary Results for Enterococci

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Outline

- Significance
- Background
  - Factors affecting pathogen removal in WSPs
  - Summary of previous work
- Light experiments with enterococci
  - Aims
  - Methods
  - Results
  - Conclusions

Significance

Greater knowledge of factors affecting pathogen removal

↓

Improved pond design

↓

Higher quality effluent

↓

Higher value applications (crop irrigation, toilet flushing, potable use)/reduced risk to public health

Background

- WSPs typically achieve high rates of pathogen removal
- Maturation ponds are shallow (1-1.5m)
  - Maximises pond volume exposure to light
- Sunlight considered main factor in pond disinfection
  - High attenuation (sum of absorption and scattering effects)
    - UVB 1% penetrates to 12cm
    - UVA 1% penetrates to 27cm
    - PAR 1% penetrates to 30cm (Sweeney *et al*. 2007)
- How do other factors influence disinfection?
  - DO, pH, sensitisers

Factors Affecting Pathogen Removal

- Sunlight
  - UVB (280-320nm), UVA (320-400nm) & PAR (400-700nm)
  - Considered most important factor in disinfection
  - Disinfection mechanism dependant on wavelength
  - Disinfection by
    - Direct damage
    - Indirect damage (photo-oxidation)
      - ROS
- Photosensitisers
  - Light absorbing substances
    - Humic substances (suspended solids)
  - Produce reactive oxygen species
    - May react with and damage micro-organisms (photo-oxidation)
  - Decrease light penetration

Factors Affecting Pathogen Removal

- Dissolved Oxygen (DO)
  - Controlled by algal photosynthesis
  - Can reach very high levels
    - >30mg/L (Sweeney *et al*. 2007)
  - Identified as a significant contributor to disinfection in presence of light
    - Photo-oxidative processes
- pH
  - Controlled by algal photosynthesis
  - Diurnal fluctuations 7-9.4 (Kayombo *et al*. 2002; Sweeney *et al*. 2007)
  - Interacts with light to contribute to disinfection

Summary of Previous Work

	Organism	Faecal Coliforms	E. Coli				Enterococci	
Affect of inactivation rates by	increasing DO	↑*	↑	↑	↑	↑#	↑	↑
	Increasing pH	↑*	↑	↑	–	–	X	–
	Presence of Exogenous Sensitisers	↑	↑	X	–	↑	↑	–
	Matrix	WSP Effluent & distilled water	HRAP effluent & saline solution	WSP Effluent & phosphate buffer	Distilled water	River water & saline solution	WSP Effluent	Distilled water
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*Measured only in the presence of exogenous sensitisers (WSP effluent)

#Only in the absence of exogenous sensitisers (saline solution)

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Summary of Previous Work

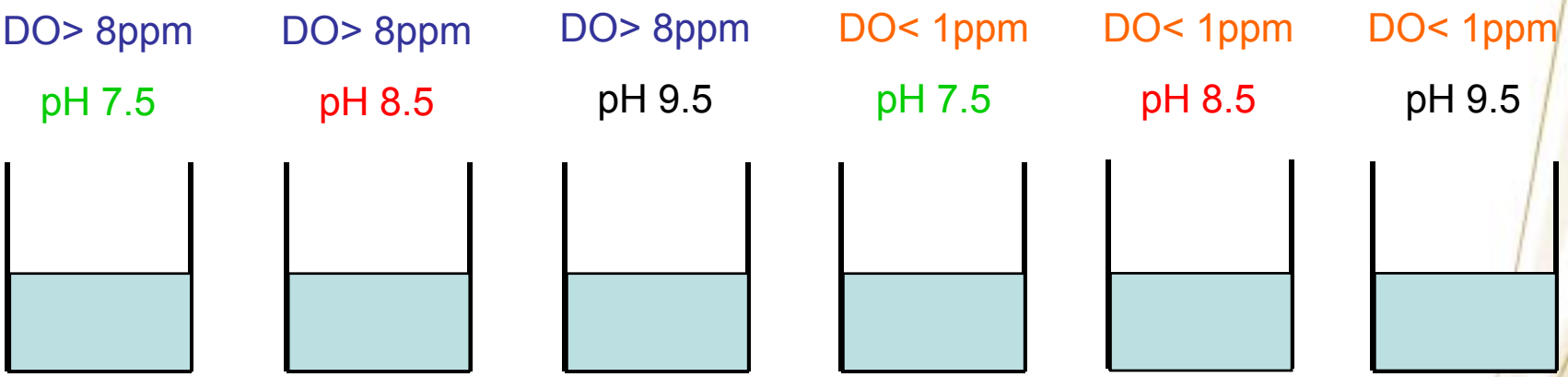
- Previous studies contributed greatly to knowledge
- Difficult to form comparisons
- Some confusion as to importance of factors
- Main aim of our research to investigate affect of these factors on inactivation rates
  - Enterococci, E. coli, phiX-174, MS2, adenovirus, enterovirus
  - Limit confounding factors

Aim

- To investigate the influence of light, DO, and pH on the removal of enterococci from buffered distilled water
  - Indicator organism for recreational waters
  - Initial part of a larger study
  - Subsequent studies using effluent

Methods

- 300mL buffered sterile distilled water inoculated with 300uL enterococci (10^9 CFU/mL)

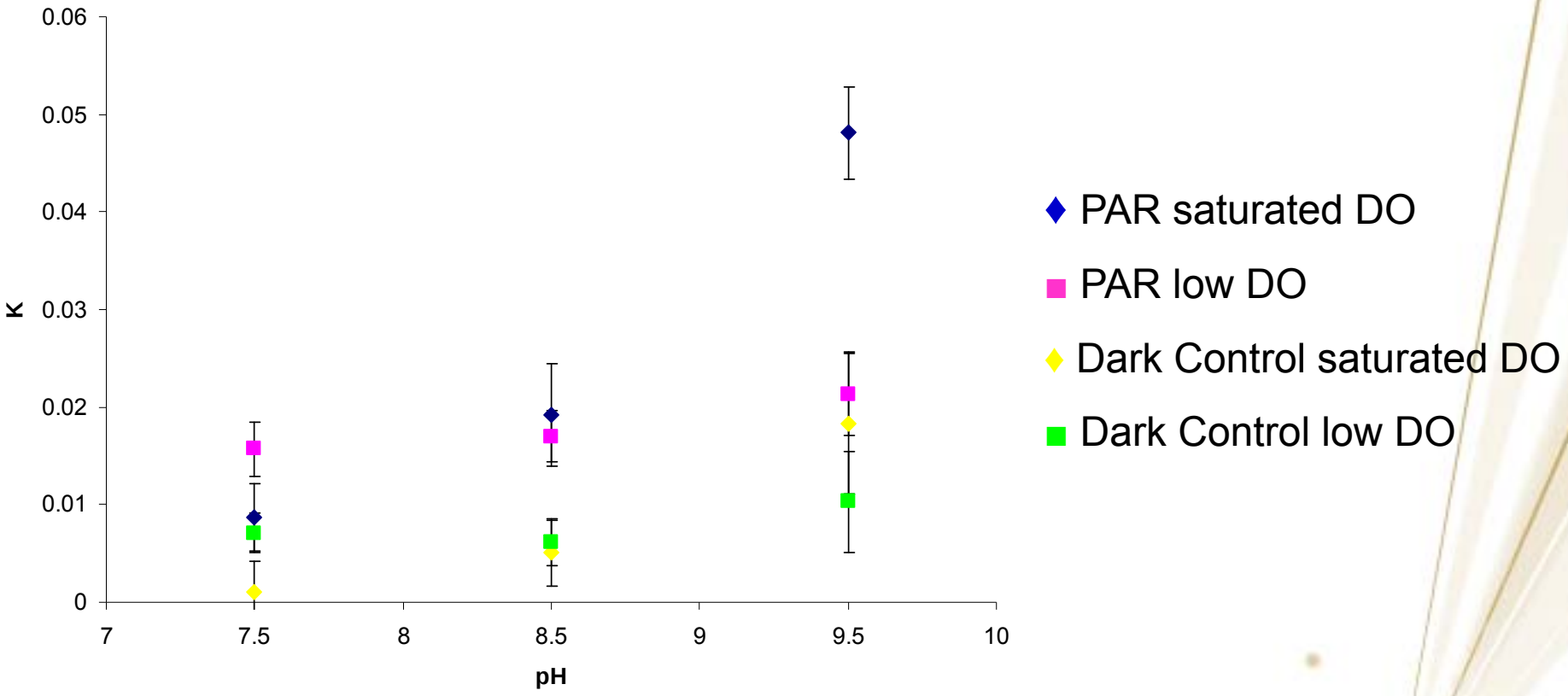


Methods

- Vessels irradiated with environmentally relevant levels UVB, UVA, photosynthetically active radiation (PAR) or incubated in the dark at 20ºC
  - Values obtained by integrating measured daylight
- Numbers quantified over 48hrs or until undetectable with Enterolert®
- Die off rates calculated $\log(n_t/n_0)$ vs time

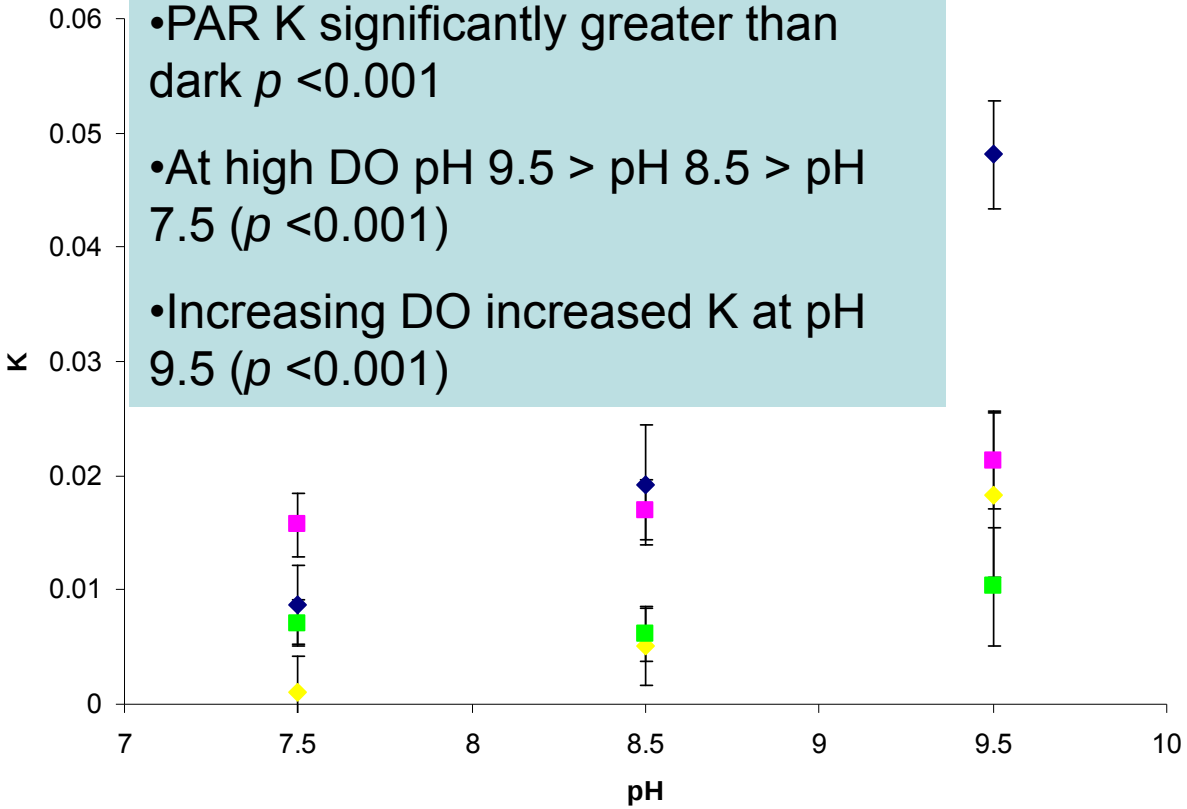
Results

Die-off rate (K) vs pH for 48hr Incubations with PAR at 141Js^{-1}m^{-2}



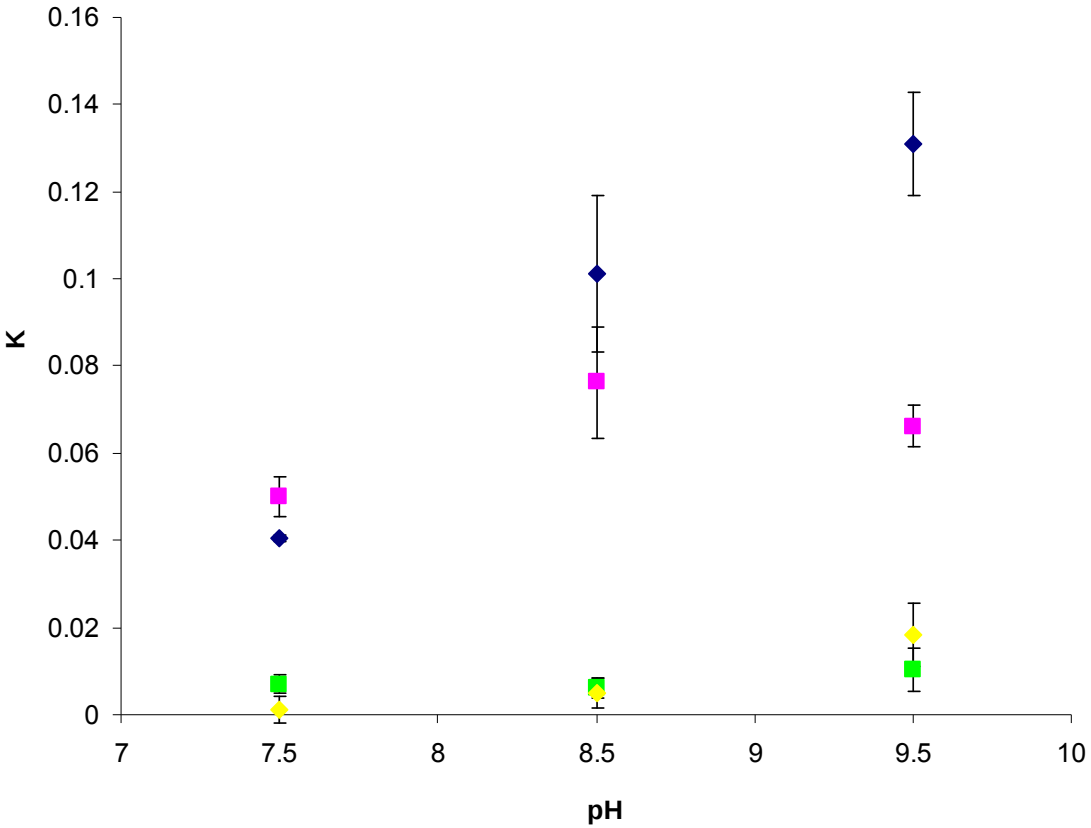
Die-off rate (K) vs pH for 48hr Incubations with PAR at 141Js^{-1}m^{-2}

- PAR K significantly greater than dark p <0.001
- At high DO pH 9.5 > pH 8.5 > pH 7.5 (p <0.001)
- Increasing DO increased K at pH 9.5 (p <0.001)



- PAR saturated DO
- PAR low DO
- Dark Control saturated DO
- Dark Control low DO

Die-off rate (K) vs pH for 48hr Incubations with UVA at 23Js^{-1}m^{-2}



- UVA saturated DO
- UVA low DO
- Dark Control saturated DO
- Dark Control low DO

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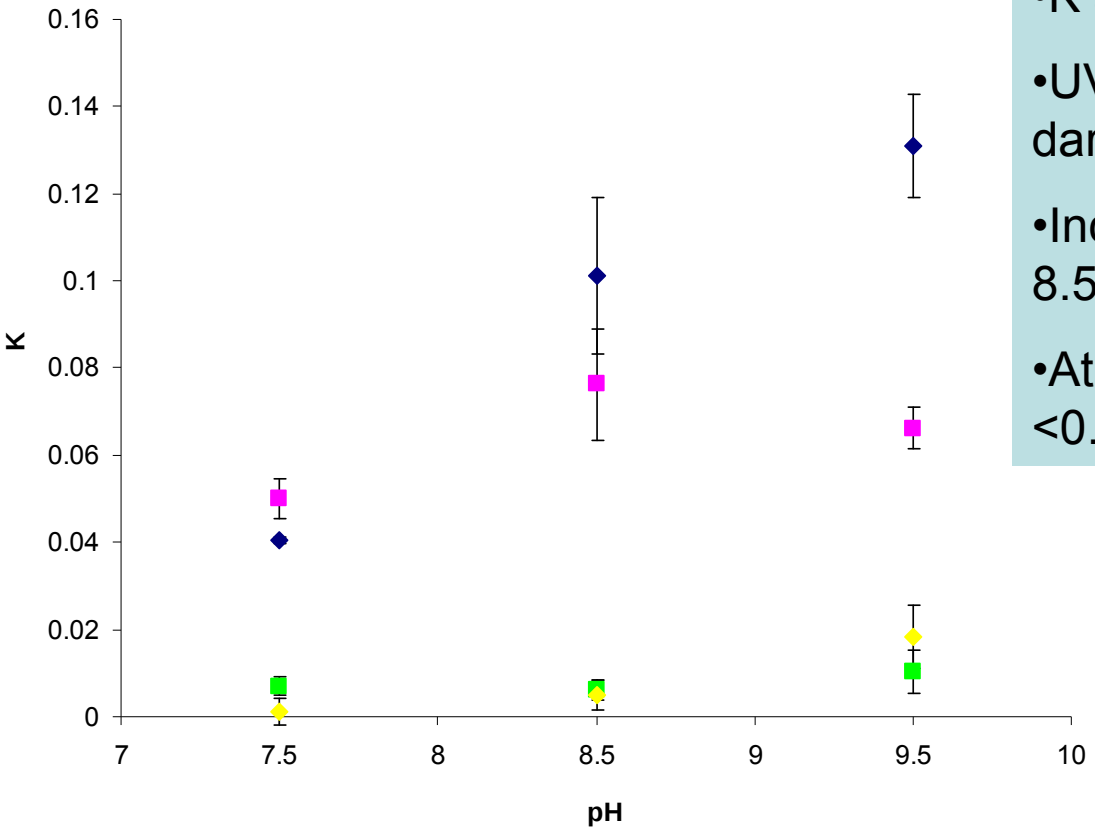
Die-off rate (K) vs pH for 48hr Incubations with UVA at 23Js^{-1}m^{-2}



- K approx 2-3x greater than PAR
- UVA K significantly greater than dark p <0.001
- Increasing DO increased K at pH 8.5 & 9.5 (p <0.001)
- At high DO K pH 9.5>8.5>7.5 p <0.001

- UVA saturated DO
- UVA low DO
- Dark Control saturated DO
- Dark Control low DO

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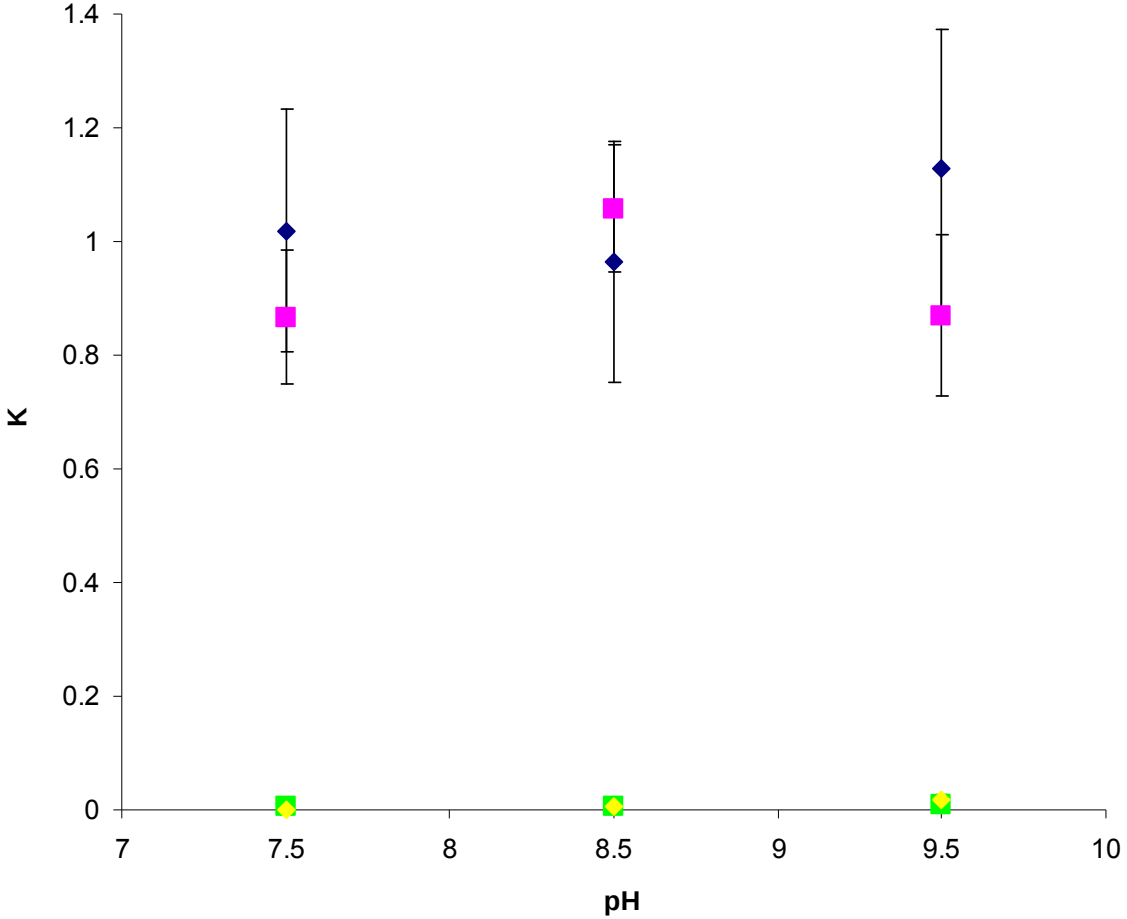
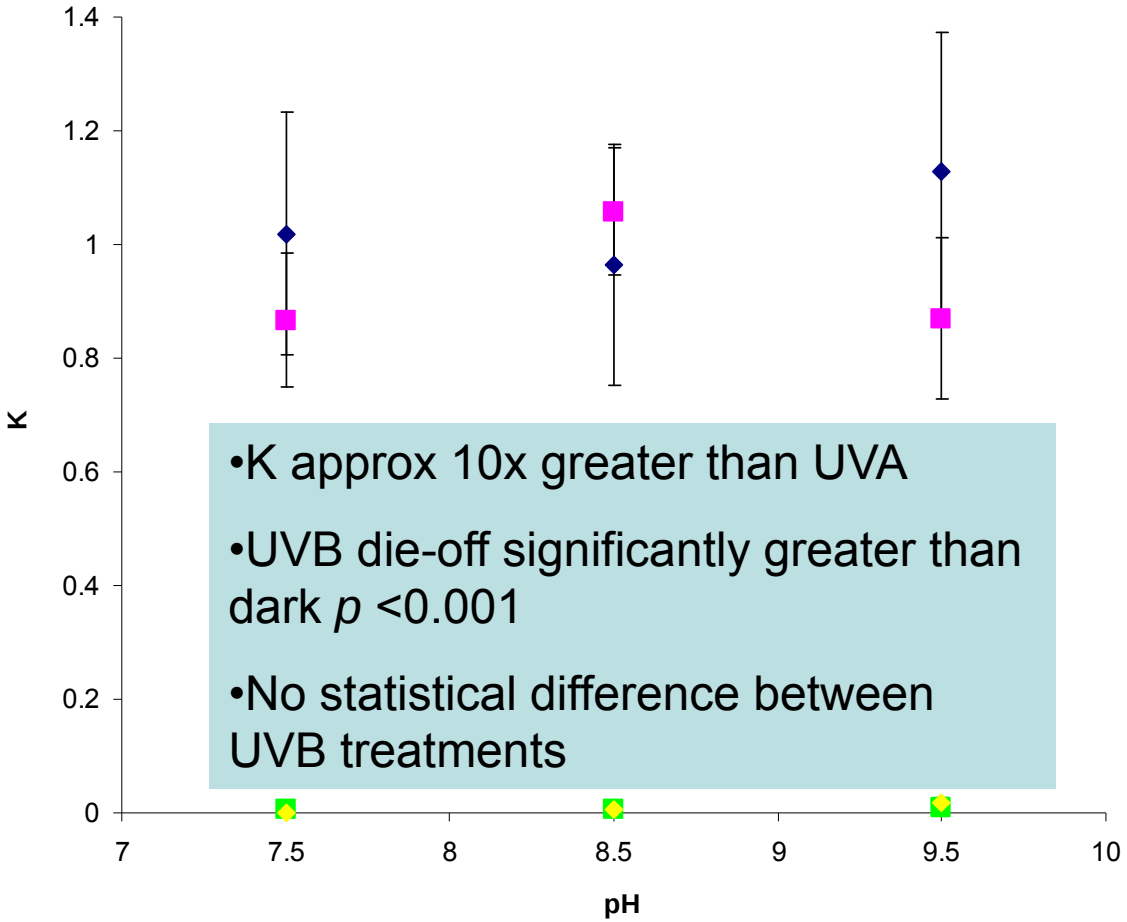
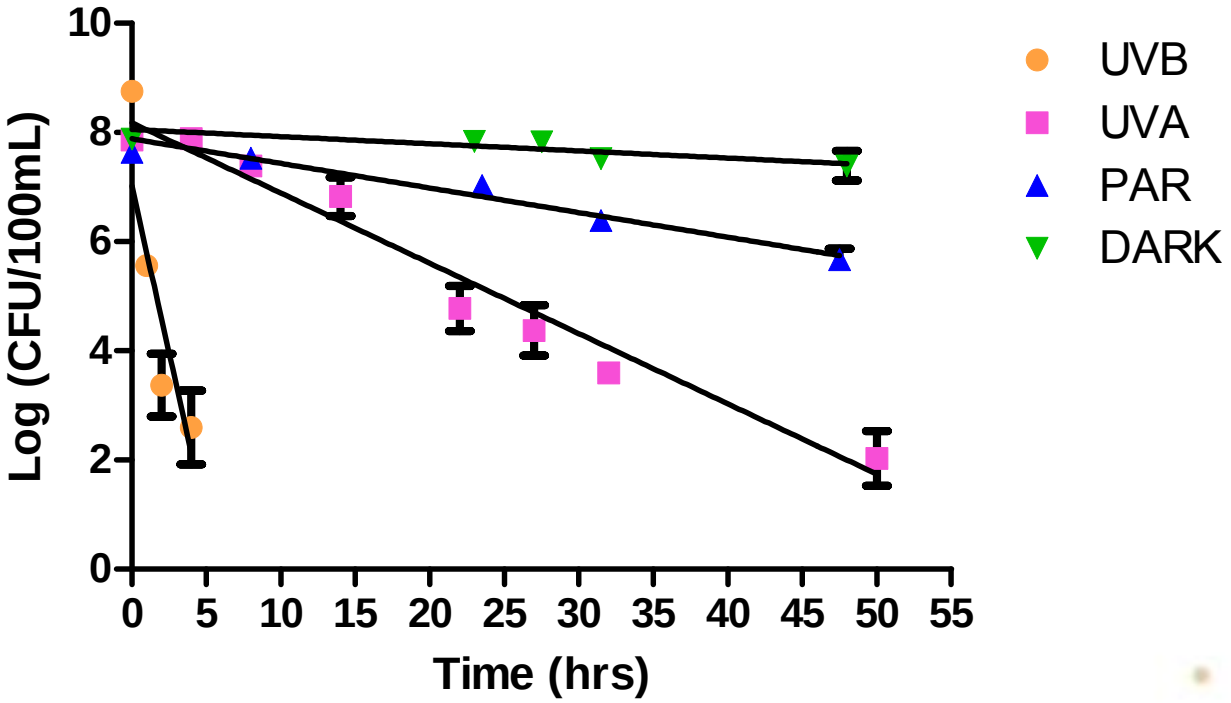
Die-off rate (K) vs pH for 4hr Incubations with UVB at 1.1Js^{-1}m^{-2}



Die-off rate (K) vs pH for 4hr Incubations with UVB at 1.1Js^{-1}m^{-2}



Log (CFU/100mL) vs Time For Light Incubations at pH 9.5 and Near Saturation DO



Results

	Organism	Enterococci				
Affect of inactivation rates by	increasing DO	↑	↑	X	↑	↑*
	Increasing pH	X	–	X	↑	↑
	Presence of Exogenous Sensitisers	↑	–	–	–	–
	Matrix	WSP Effluent	Distilled water	Distilled water	Distilled water	Distilled water
	Irradiated by	sunlight	sunlight	UVB	UVA	PAR
	Reference	Davies-Colley *et al*. (1999)	Reed (1997)	Current work		

*at high pH

Conclusions

- High degree of confusion and contradiction within literature
- All light significantly increased die-off when compared with dark control
- Die-off rates when irradiated with UVB more than 10 times greater than for UVA and PAR
- Mechanisms
  - Direct inactivation appears to be the most important process
  - Photo-oxidation (endogenous) and pH/light interactions appear to play small roles in enterococci removal

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