UVB AND DARK DISINFECTION IN WASTEWATER

A thesis submitted for the degree of Doctor of Philosophy

YU LIAN

B. Eng. (Environmental Engineering)

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School of the Environment

Faculty of Science and Engineering

Flinders University of South Australia

DECLARATION

I certify that the whole thesis does not incorporate without acknowledgment any material previously submitted for any degree or diploma in any university; and that, to the best of my knowledge and belief, it does not contain any material previously published or written by another person except where due reference is made in the text.

Yu Lian

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LIST OF ABBREVIATIONS

µm	micrometre
$^1O_2$	singlet oxygen
3NTU-W	wastewater with a turbidity of 3 NTU (low turbidity)
8NTU-W	wastewater with a turbidity of 8 NTU (high turbidity)
ADF	average daily flow
ANOVA	analysis of variance
ASCE	American Society of Civil Engineers
AWT	advanced wastewater treatment (unit)
BOD	biochemical oxygen demand
CDOM	chromophoric dissolved organic matter
CI	confidence interval
CMF	continuous microfiltration (unit)
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAF	dissolved air flotation (unit)
DALYs	disability-adjusted life years
DO	dissolved oxygen
DOC	dissolved organic carbon
DOM	dissolved organic matter
E. coli	*Escherichia coli*
ESS	endonuclease sensitive site
EU	European Union
FHA	Fluka humic acid
FLB	fluorescently-labelled bacteria
GDP	gross domestic product
GHG	greenhouse gas
GInaFiT	Geeraerd and Van Impe's Inactivation Model Fitting Tool
H_2O_2	hydrogen peroxide
HRAP	high-rate algal pond
HRT	hydraulic residence time
HTFW	high turbidity filtered wastewater
IC	inorganic carbon
Jm^{-2}	UVB dose

K_i	inhibition constant (inactivation rate)
K_{inact}	rate of inactivation
KoM	Kingston-on-Murray
LRV	log_{10} reduction value
LSD	least significant difference
LTFW	low turbidity filtered wastewater
MEP	Ministry of Environmental Protection (People's Republic of China)
ML	megalitre
mmol	millimol
MPN	most probable number
N/n	number
nm	nanometre
NTU	nephelometric turbidity unit
O_2^-	superoxide
OH^{-1}	hydroxyl radical
PFU	plaque-forming unit
pH	acidity
RMSE	root-mean-square error
RO	reverse osmosis
ROS	reactive oxygen species
RO_2^-	peroxyl radical
SD	standard deviation
SEPA	State Environmental Protection Administration (China)
SOD	superoxide dismutase
SODIS	solar water disinfection
SPSS	Statistical Package for the Social Sciences
SRHA	Suwannee River humic acid
SS	suspended solids
TOC	total organic carbon
TSA	tryptone soya agar (solution)
TSS	total suspended solids
UK	United Kingdom
UN	United Nations
UNESCO	United Nations Educational, Scientific and Cultural

	Organization
USA/US	United States of America
USEPA	US Environmental Protection Agency
UV	ultraviolet
UVA	ultraviolet A
UVB	ultraviolet B
UVC	ultraviolet C
UVR	ultraviolet radiation
Vis	visible light
WHO	World Health Organization
Wm^{-2}	UVB dose rate
WSP	wastewater stabilisation pond
WWTP	wastewater treatment plant
Xe	xenon
YLD	years lived with a disability or illness
YLL	years of life lost

SUMMARY

Natural wastewater treatment systems, such as wastewater stabilisation ponds (WSPs), provide a promising way to cope with fresh water shortages in the future via the production of reclaimed water. This applies especially in rural communities and developing countries: even in South Australia, over 110,000 people are served by natural wastewater treatment systems. Optimising utilisation of natural self-treatment processes in these systems decreases wastewater treatment cost, energy consumption, chemical by-product production and greenhouse gas (GHG) emissions.

High-rate algal ponds (HRAPs), with their shallower depth, meandering channel and paddle wheel mixing, are one of the outstanding members of the WSP family. Compared to traditional WSPs, HRAPs achieve the same disinfection performance in less time and with less capital costs. Sunlight, particularly UVB, the most damaging wavelength, plays a significant role in the whole disinfection process, with the dark disinfection also contributing. However, there has been a lack of knowledge about the effect of environmental factors on sunlight and on the dark disinfection mechanisms involved in these ponds. The current research's aim was to model the effects of a series of environmental factors on UVB and dark disinfection in laboratory-based experiments.

Escherichia coli (E. coli) and MS2, being the most common indicators of bacterial and viral pathogens, were used in the experiments. Their comparative sensitivity within different water media was also explored. Several environmentally relevant factors, namely, temperature, dissolved organic matter (DOM), suspended solids (SS), turbidity, chlorophyll *a*, UVB dose (Jm^{-2}) and UVB dose rate (Wm^{-2}) were assessed for their influence on UVB and dark inactivation. The contribution to MS2 inactivation by reactive oxygen species (ROS), produced by the interaction of UVB with dissolved organic carbon (DOC), was determined by comparison of inactivation rates and $\log_{10}$ reduction values in wastewater in the presence and absence of the ROS quencher L-histidine.

The effect of temperature on *E. coli* and MS2 inactivation in dark raw wastewater was determined. The dark inactivation rates of MS2 and *E. coli* increased significantly with increases in temperature from 10ºC to 30ºC in HRAP wastewater. Two models, describing the relationship between temperature and the dark

inactivation rates of *E. coli* and MS2, were provided for further application, with MS2 found to be more sensitive than *E. coli* in dark incubated wastewater.

In addition, MS2 was also observed to survive longer under UVB irradiance than *E. coli.* The temperature increase from 10ºC to 30ºC had a significant positive effect on MS2 inactivation but not on that of *E. coli.* Furthermore, in reverse osmosis (RO) water under the same UVB dose, UVB dose rate had a strong influence on UVB disinfection for both *E. coli* and MS2. Two distinct regions of the inactivation curve were identified in RO water, where inactivation was UVB dose rate-limited or UVB dose rate-saturated.

MS2 inactivation increased with increasing UVB dose rate in both filtered and wastewater. A mathematical equation was developed to model the relationship, in each water medium, between the UVB dose rates and MS2 inactivation rates. The presence of DOC significantly influenced the MS2 inactivation rates by two mechanisms, firstly, decreasing the MS2 inactivation rate by UVB attenuation and secondly, via the production of ROS. The depression of the inactivation rate in wastewater in the presence of the ROS quencher L-histidine, when compared with those rates measured in its absence, is indicative of the influence of ROS. Uniquely, this study estimated that 10–20% of the MS2 $\log_{10}$ reduction value recorded in raw and 0.22 µm filter wastewater could be attributed to the generation of ROS.

The relevance of the laboratory-acquired MS2 UVB inactivation rate data was clearly demonstrated by comparison with data obtained from a demonstration HRAP treating the wastewater from a rural community in South Australia (SA). Significantly, the laboratory determined MS2 $\log_{10}$ reduction value at 20ºC, at a UVB dose equivalent to the mean, annual daily dose in SA (39,0373 Jm^{-2}), was 1.46 ± 0.23 compared with the annual mean value of 1.59 ± 0.82 recorded in the HRAP.

The findings of the current research make a significant contribution to understanding the effects of a variety of environmentally relevant factors on UVB and dark disinfection. All results achieved aims which will be utilised in further practical applications, such as improving the design and operation of HRAPs. To the best of the author's knowledge, this is the first time that the effect of the UVB dose rate on MS2 inactivation, which is fundamental for UV-related research, has been assessed systematically in a range of water media. More generally, the results suggest that both dose and dose rate should be recorded in related solar disinfection research.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Definitions of wastewater and wastewater treatment

1.1.1 Definition of wastewater

Every community produces liquid, solid and gaseous waste. Generally speaking, wastewater is defined as the liquid or water-carried waste discharged by domestic, agricultural and industrial sewage, surface flow and occasionally rainwater and groundwater (Tchobanoglous et al., 2003). Australian communities generate large volumes of wastewater with domestic water use alone producing about 70,000 litres per person per year. Based on most standards in the European Union (EU), the wastewater generated every day ranges from 0.1–0.15 m^3 per person (Rozkošný et al., 2014). In China, the wastewater discharged has increased steadily from around 35.2 billion tons in 1990 to 46 billion tons in 2003 (Liu & Diamond, 2005). The physical, chemical and biological composition of wastewater is variable according to the location and the period of time. The significant constituents of concern in wastewater treatment are elaborated in Table 1-1.

1.1.2 Wastewater property

Obviously, leaving wastewater untreated leads not only to environmental damage, such as the eutrophication of water bodies and the production of malodorous anaerobic gases, but also harms human health and increases the risk of infection from many diseases, such as diarrhoea and even cancer. Nevertheless, according to the latest water report from 94 countries and 23 external support agencies, currently 748 million people cannot get access to improved drinking water and 1.8 billion people use a source of drinking water that is faecally contaminated: the majority of these are poorer people living in rural areas (WHO & UN-Water, 2014). Even in the United States of America (USA), more than 3200 GL of raw or untreated wastewater are discharged to natural streams, rivers and lakes every year, and the situation is continuing to worsen (Rivers, 2015).

Table 1–1: Typical physical properties, chemical and biological constituents, and their application to wastewater (Adapted from Tchobanoglous et al., 2003, p. 30)

Constituents	Abbreviation/ Unit	Wastewater treatment application
Physical Characteristics		
Total solids	TS	To assess the possibility for reuse of wastewater and to choose the appropriate treatment methods
Turbidity	NTU[c]	To assess wastewater quality in terms of light penetration and an indirect assessment of suspended particles
Odour	TON[d]	To judge if odours cause a problem
Temperature	ºC or ºF	To improve understanding of biological processes in wastewater treatment
Chemical Characteristics		
Free ammonia	NH_4^+	To measure the nutrients in wastewater or the degree of oxidation
Nitrites	NO_2^-	To measure the nutrients in wastewater
Nitrates	NO_3^-	To measure the nutrients in wastewater
Total phosphorus	TP	To measure the nutrients in wastewater
pH		A measure of the acidity or basicity in wastewater
Metals	As, Cd, Ca, Cr, Co, Cu, Pb, Mg, Hg, Mo, Ni, Se, Na, Zn	To assess the possibility for reuse of wastewater and toxicity effects
Five-day carbonaceous biochemical oxygen demand (BOD)	$CBOD_5$	To measure the oxygen needed to stabilise a waste
Total organic carbon	TOC	A replacement for the BOD test

Table 1–1: Typical physical properties, chemical and biological constituents, and their application to wastewater (Adapted from Tchobanoglous et al., 2003, p. 30) (continued)

Constituents	Abbreviation/Unit	Wastewater treatment application
Biological Characteristics		
Coliform organisms	MPN (most probable number)	To quantify pathogenic bacteria and assess disinfection efficiency
Specific micro-organisms	Bacteria, protozoa, helminths, viruses	To assess the presence of specific organisms in wastewater for reuse

Bacterial, viral and protozoan pathogens cause most diseases that result from contact with wastewater: although these diseases like diarrhoea and fever generally only cause short-term unpleasant feelings with no lasting effects, in some extreme cases, long-term illnesses or even death can occur in developed countries, caused by all three categories of pathogens. For instance, in May 2000, Walkerton, Ontario, Canada, owing to failure to use and monitor the right dose of chlorine, the water supply became contaminated by a dangerous enterohaemorrhagic serotype of bacteria: *E. coli* 0157:H7: as a result, 2,300 people became seriously ill and seven died (Hrudey et al., 2003). Furthermore, a waterborne outbreak was reported in Wallace Lakes, New South Wales, Australia, 1997 with 467 hepatitis A patients reported within three months. After conducting a study, more than two-thirds of patients had consumed oysters in which the virus hepatitis A was identified substantially (Conaty et al., 2000). Hepatitis A is one category of virus able to survive long term in oysters and cause severe disease. Moreover, in 1993, in Milwaukee, United States, the drinking water supply became contaminated by the parasite *Cryptosporidium*. As a consequence, 403,000 people became sick and 70–100 people died (Mac Kenzie et al., 1994).

Besides of pathogens' risk, the high nutrient concentrations in untreated wastewater can cause eutrophication and proliferation, like the blooms of toxic cyanobacteria that occurred in the Patos Lagoon in South America in the 1990s (Yunes et al., 1996), resulting in the deaths of wild animals, birds and fish. Some algae are even toxic to humans when they eat shellfish or are engaging in recreational activities in contaminated water. Additional health risks are associated with heavy metals which attracted the most attention in China in 2008 as their concentrations in wastewater-

irrigated plants were beyond the permitted levels according to the State Environmental Protection Administration (SEPA) in China and the World Health Organization (WHO) (Khan et al., 2008). In addition, consuming heavy metal-contaminated food is a well-known risk factor for cancer (Järup, 2003).

1.1.3 Definition of wastewater treatment

Wastewater treatment is defined as the process used to remove contaminants from wastewater. One of the earliest recorded instances of the implementation of wastewater treatment was in approximately 1500 BC in Mohenjo-daro near Pakistan (Wiesmann et al., 2007). At that site, toilets were constructed within houses: at the same time, canals were built to transport wastewater into natural water systems. After thousands of years of development (see Table 1-2), the 20th century witnessed important developments as significant wastewater treatment technology and regulations, such as the WHO guidelines for agricultural and aquacultural reuse of wastewater (Mara & Cairncross, 1989) were established.

Based on the practical conditions and demands, various wastewater treatment levels are able to be achieved. When describing the principal wastewater treatment levels, they can be listed as: primary treatment, secondary treatment, tertiary treatment and advanced treatment (Tchobanoglous et al., 2003). The end disposal route of the treated wastewater determines the degree of treatment required; a notion of the “fit for purpose” approach was applied to design the validation of waterborne pathogenic concentration. “Fit for purpose” includes four iterative activities: pre-validation, exploratory validation, in-study validation and advanced validation to confirm that biomarker data are valuable and are useful for guidelines (Lee et al., 2006; NRMMC, 2006). The concept has been conventionally applied by clinicians to monitor how the patient feels, and then is applied to areas like normal biological processes and pharmacological response. The primary treatment is defined as the removal of suspended solids (SS) by physical screening or sedimentation, with this including grit, silts and organic matter; Secondary treatment normally includes removal of organic matter (BOD) followed by suspended solids (SS) removal. In addition to further removal of suspended solids (SS) and disinfection, tertiary treatment often includes nutrient removal. When treated wastewater is intended for reuse, advanced treatment such as filtration, reverse osmosis (RO) and chemical oxidation would be undertaken (Tchobanoglous et al., 2003).

Table 1–1: Timeline of wastewater and sanitation systems development (Buchanan, 2014)

Timeline	Wastewater and Sanitation Systems Development
97 AD	Foundation of Water Supply Commission in Rome
1770	Legal use of wastewater for human waste disposal in London
1800	Publication of “Sanitary Status of Considerable Britain”, the Chadwick Report
1910	Collation of disinfection kinetics by Chick (United Kingdom [UK])
1970	Publication of US Clean Water Act
2000	Publication of US Environmental Protection Authority (USEPA) Guidelines for Water Reuse

According to a US Environmental Protection Authority (USEPA) survey in 2003, in the approximately the next 20 years, most US wastewater treatment plants will be able to reach a level higher than secondary treatment (Tchobanoglous et al., 2003).

1.1.4 Microbiology in wastewater

The biological characteristics of wastewater play a vitally important role in the control of diseases caused by waterborne pathogens. Generally, a very complex ecosystem exists in wastewater which includes micro-organisms, such as bacteria, archaea, fungi/yeast, protozoa, rotifers, algae and viruses. Among them, only bacteria, protozoa, helminths and viruses are pathogenic, but even a very low concentration of pathogens exceeding the minimum infectious dose may cause serious diseases. As a result, the health risk if exposed to wastewater is very high. Diarrhoeal diseases related to waterborne pathogens were recorded as responsible for 2.46 million deaths in 2008, with most of those who died being weak children under the age of five (WHO, 2008). The micro-organism concentrations found in untreated wastewater and the corresponding infectious doses are listed below on Table 1-3.

Table 1–3: Micro-organism concentrations found in untreated wastewater and the corresponding infectious dose (Bolton, 2011)

Organism	Concentration in raw wastewater[1], MPN100 ml^{-1}	Infectious dose, number of organisms	Survival time in wastewater and freshwater (days)	Disease	Reference
Bacteria:					
E. coli	10^7–10^8	10^6–10^{10}		Gastroenteritis	Mandilara et al. (2006); Kothary & Babu (2001)
Shigella spp.	10^0–10^3	10–20	<30 (<10)	Shigellosis	Kothary & Babu (2001); Vigneswaran & Sundaravadivel (2004); Godfree (2003); Tchobanoglous et al. (2003)
Salmonella spp.	10^2–10^4	10–10^8	<60 (<30)	Salmonellosis/ Typhoid fever	Kothary & Babu (2001); Vigneswaran & Sundaravadivel (2004); Godfree (2003); Tchobanoglous et al. (2003)
E. coli O15:H7	10^2	< 10^2		Gastroenteritis	Bitton (2005)
Protozoa:					
Cryptosporidium Parvum oocysts	10^1–10^3	1–10		Cryptosporidiosis	Kothary & Babu (Kothary & Babu, 2001); (Tchobanoglous et al., 2003)
Entamoeba Histolytica cysts	10^{-1}–10^1	10–20	<30 (<15)	Amoebiasis	Vigneswaran & Sundaravadivel (2004);

Giardia lamblia cysts	10^3–10^4	< 20		Giardiasis	Bitton (2005)
Helminths:					
Ascaris lumbricoides	10^{-2}–10^0	1–10	Many months	Ascariasis	Vigneswaran & Sundaravadivel (2004)
Viruses:					
Enteroviruses	10^3–10^4	1–10	<120 (<50)	Gastroenteritis, heart	Vigneswaran & Sundaravadivel (2004); Krikelis, Spyrou, Markoulatos & Serie (1985)
Adenovirus	10^2–10^3	1*		Respiratory disease/acute conjunctivitis/ gastroenteritis	Krikelis et al. (1985)
Hepatitis A	10^4	1–10		Infectious hepatitis	Godfree (2003)
Norwalk agent	1–10^3	10–10^2		Gastroenteritis	Bitton (2005)
Rotavirus	1–10^4	1*		Gastroenteritis	Godfree (2003); NRMMC(2008)

*In the absence of data, the minimum infectious dose is assumed to be 1 (Percival et al., 2004).

[1] Please note that there are no typical concentrations since they are dependent on the health status of the population.

It is obvious from Table 1-3 that the survival and infection abilities of pathogens in water vary significantly for different categories of micro-organisms. In fact, only a relatively small number of pathogens are listed above; therefore, it is very important to recognise the different characteristics and risk levels of these pathogens. Firstly, only a small dose such as 1–10 units of a virus in water can infect humans but generally viruses cannot multiply in wastewater as they need susceptible living cells in which to multiply: in a wastewater environment, their number would decrease or remain practically the same (Krikelis et al., 1985). In addition, the infectious dose for enteric protozoa such as *Cryptosporidium parvum*, which are frequently found in surface water, depends on the strain's virulence (Okhuysen et al., 1999). They are highly resistant to many commonly used disinfection processes and can survive very long term in a water environment. The infectious dose for most enteric bacteria is very high, from 10^7–10^8, but some species, such as *enterohaemorrhagic E. coli* O157:H7 can infect humans with a very low dose (Bitton, 2005), causing serious consequences (Hrudey et al., 2003). The period during which enteric bacteria survive in water largely depends on nutrients and temperature. Normally, they are assumed to survive in an oxygen-starved situation, but some have been found growing in fresh water, even in drinking water, for example, *Legionella spp. Aeromonas spp*. (Szewzyk et al., 2000).

Unfortunately, when people face a considerable variety of pathogens, it is not practical to detect them one by one due to the high cost in terms of money and time (Girones et al., 2010); therefore, faecal indicator organisms that detect the presence of pathogens are often quantified instead. Currently, much research has been conducted on identifying a suitable indicator organism for waterborne pathogens. Firstly, a good indicator organism needs to maintain a reliable concentration in wastewater. Secondly, it needs to be able to be measured easily and cheaply, thus helping to improve the efficiency of water quality monitoring. In fact, seven desirable characteristics for a faecal indicator organism have been determined as follows (Cooper, 2001; Maier et al., 2000):

- Be present when faecal contamination is present.
- Be equal to or greater than the number of target pathogenic organisms.
- Exhibit at least the same survival ability in the environment as the pathogen.
- Not reproduce outside of the host organism (i.e.it does not multiply in the environment, thus best reflects the original concentration)
- Be detected and quantified easily, reproducibly using robust and cheap

methodologies.

- Belong to the intestinal microflora of warm-blooded animals.
- Does not pose a serious health threat to researchers.

In reality, the perfect indicator organism according to the above features has still not been found. Generally, bacteria are most widely used as indicator organisms, with the most popular ones being total coliforms; for example, bacteria often used in specific circumstances and for particular purposes by the WHO (Blumenthal et al., 2000) include *Escherichia coli (E. coli)* or enterococci. However, as viruses display a lower concentration and a stronger ability to survive in treatment processes including disinfection, bacteriophages, and especially the F+ specific bacteriophage, MS2, have been suggested as an example of good indicators for waterborne viruses (Duran, 2002; Havelaar, 1991; Lucena, 2004).

The main indicator organism selected for my research was F+ specific bacteriophage MS2. The bacteriophage MS2 is an icosahedral, positive-sense single-stranded RNA virus that infects the bacterium *Escherichia coli* (*E. coli*) and other members of the Enterobacteriaceae (van Duin, 1988). Many research studies have been conducted on MS2 due to its fundamental properties and features. It is the simplest model system of viral RNA replication; in addition, it is convenient and helpful for understanding infection cell physiology and fundamental processes such as translation (Fiers et al., 1976; Valegård et al., 1990). More recently, MS2 has received increasing attention as a valuable waterborne pathogen indicator (Havelaar et al., 1993; Havelaar, 1991): it has been widely used in various areas, such as membrane filtration (Otaki et al., 1998) and the wastewater treatment disinfection process (Gersberg et al., 1987; Schijven et al., 2003).

Compared to other indicators, MS2 has some obvious advantages. The most important advantage for my research is that it has proven to be more resistant under UV disinfection processes than other indicators, for example, *E. coli* and *Enterococcus spp.* (Bolton, 2011; Fisher et al., 2012; Liu & Zhang, 2006; Theitler et al., 2012). For modelling the pathogen die-off rate, the long-term survival of the indicator is necessary so enough data points can be obtained and to understand the disinfection effect curve.

Feng (2003) compared the survival of coliphages MS2 and Qß under a wide range of pH and temperature buffer solutions: this study concluded that MS2 was a better indicator then Qß. Havelaar et al. (1993) found that enteric virus concentrations can

be predicted from MS2 in fresh water. Their study found that numerical relationships were consistent between the concentrations of F-specific RNA bacteriophages and virus concentrations over a wide variety of environments, including raw sewage, secondary effluent, coagulated effluent, chlorinated and UV-irradiated effluent, river water, coagulated river water and lake water. Even in seawater and groundwater, it is suggested as a long survival time indicator or tracer (International Association for Water Pollution Research and Control [IAWPRC] (Microbiology, 1991).

A mature double agar layer MS2 quantification method has been developed by Debartolomeis and Cabelli (1991) and Noble et al. (2004) as being more practical for the laboratory: in addition, the quantification price is acceptable. With regard to space, facilities and equipment, MS2 is able to be cultivated easily and rapidly (Noble et al., 2004). At the same time, MS2 is of low risk to humans.

Escherichia coli (*E. coli*), a facultatively anaerobic, rod-shaped bacterium, commonly found in the human intestine,has been chosen as a water treatment indicator since the 1890s. It has been defined as “the best” biological indicator in drinking water quality assessment (Edberg et al., 2000) as *E. coli* is abundant in human and animal faeces that spread into all natural water systems. It is able to survive for a long time in different water environments such as in goundwater, recharged wells and drinking water distribution systems (Edberg et al., 2000; Filip et al., 1987; Rice et al., 1999). In addition, it is cost-effective and simple to analyse. In summary, it perfectly fits the requirement as cited by the WHO that: “*Water must be examined regularly and frequently because pollution is often intermittent and may not be detected if examination is limited to one or only a small number of samples. For this reason, it is better to examine drinking water frequently by means of a simple test rather than less often by several tests or a more complicated test”* (Edberg et al., 2000).

In addition to the above advantages, the main reason to choose *E. coli* in this study is that the *E. coli* inactivation data are able to be compared to other large amounts of data from related research studies (Avery et al., 2008; Bolton, 2011; Buchanan et al., 2011a, 2011b; Fallowfield et al., 1996; Sethi, 2009; Theitler et al., 2012).

Many different terms and units are used in the literature to describe the die-off rate of micro-organisms in an aquatic environment. The micro-organisms’ die-off rate is the most important factor by which to judge the disinfection ability of different methods. At times, the selection of different units for measuring the die-off rate causes misunderstandings and makes valuable results incomparable. It is therefore

interesting to provide a review of several different units used to measure micro-organisms' die-off rate.

The decay in the number of micro-organisms is generally observed as an exponential curve. One of the classic previous works that sought to define the disinfection rate was undertaken by Harriette Chick in England (1908) when studying mercuric chloride. This study found that the disinfection effect was influenced by contact time and temperature, and this equation was derived:

$$K = -\ln (N_t/N_0)/t \qquad \textbf{Equation 1–1}$$

where,

K = inactivation rate (s^{-1} or h^{-1})

N_t = number of micro-organisms at time t

N_0 = number of micro-organisms at time 0

t = time (s or h).

A similar equation, semi-$\log_{10}$, is also applied for K.

As K provides a direct view of how the number of micro-organisms reduces, it is considered to be a meaningful description. For nearly a century, K has been adopted by most studies in the literature to model the micro-organisms' inactivation rate, and it has proven to be an accurate way to describe the log-linear inactivation.

T50/T90/T99 is a concept derived from K, being the time that it takes 50/90/99% of micro-organisms to die off. It is obvious the T50/T90/T99 is able to be converted from K:

$$\text{If } K = -\log_{10} (n_t/n_0)/t \qquad \textbf{Equation 1–2}$$

K = inactivation rate (s^{-1} or h^{-1})

N_t = number of micro-organisms at time t

N_0 = number of micro-organisms at time 0

t = time (s or h).

T50 = 0.5/k

T90 = 1/k

T99 = 2/k

Generally, T50/T90/T99 is used for comparison, for the reason that it is a unique concept that is easy to understand.

However, neither Chick's Law nor T50/T90/T99 are able to satisfy the demand of modelling the complicated shapes of micro-organisms' inactivation curve. The solution is provided by Geeraerd and Van Impe's Inactivation Model Fitting Tool (GInaFiT) which is used in this thesis to calculate the maximum inactivation rate K_i (Geeraerd et al., 2005). GInaFiT provides 10 different types of microbial survival models (Figure 1-1) and has been successfully used to fit *E. coli* solar disinfection inactivation results (Berney et al., 2006). The 10 different models involved are: log-linear regression; log-linear + shoulder; log-linear + tail; log-linear + shoulder + tail; Weibull; Weibull with fixed parameter *p*; Weibull + tail; double Weibull; biphasic model; and biphasic and shoulder.

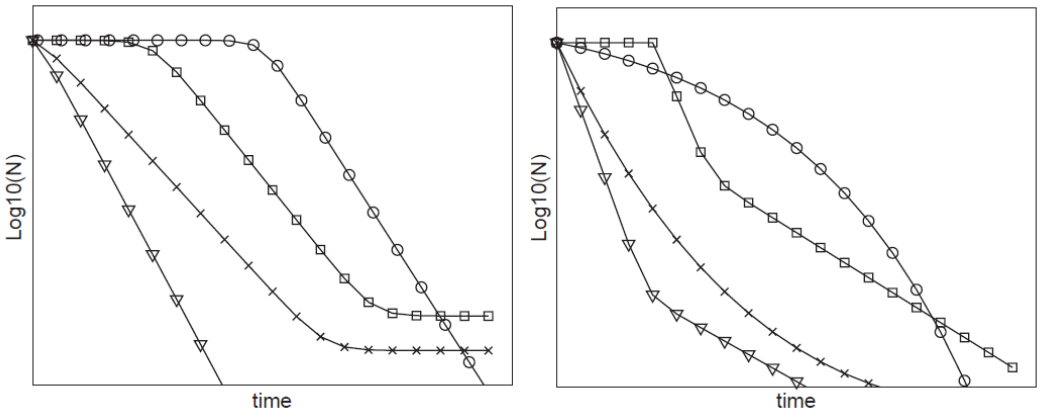


Figure 1-1: Eight (8) commonly observed types of inactivation curves

Notes: Left plot: linear (▽, shape I), linear with tailing (X), sigmoidal-like (□), linear with a preceding shoulder (o, shape IV). Right plot: biphasic(▽), concave (X), biphasic with a shoulder (□), and convex (o) (Geeraerd et al., 2005).

1.2 Comparison of wastewater treatment or discharge guidelines or standards between China and Australia

1.2.1 Why the guidelines matter

In order to prevent water pollution, protect important water resources and reduce the risk to human health and the ecosystem, detailed wastewater discharge standards or guidelines have generally been set up by each country's environmental protection department. Undertaking a comparison between two important countries, China and Australia, is of interest as the guidelines/regulations reflect the level of development of the wastewater treatment technology, the country's development level and the attitude of each country towards the protection of the water environment.

China has the world's largest population, the world's second largest economy by nominal gross domestic product (GDP) and perhaps the most serious environmental pollution (Liu & Diamond, 2005). The Ministry of Environmental Protection (MEP) of the People's Republic of China was established to replace the former State Environmental Protection Administration (SEPA) in Beijing in 2008. This momentous behaviour was an indication of considerable awareness among China's most senior government leaders. In terms of the ordinary people, a 2015 Chinese documentary "Under the Dome", directed by journalist, Chai Jing, attracted more than 150 million views within three days of its release (Chai, 2015, May 5). In "Under the Dome", Chai interviewed many top environmental scientists worldwide concerning air pollution. In China, every day a huge amount of discussion occurs on the topic of environment protection: it is therefore meaningful to have a detailed look at China's wastewater discharge regulations.

Australia has comparable value when taking into consideration its wastewater status and treatment guidelines. On one hand, Australia is a developed country which places great emphasis on its sustainable development strategy, for both the economy and the environment. On the other hand, Australia is the driest continent worldwide with a rapidly growing population: even modern cities like Sydney and Melbourne at times face water shortages (Bell, 2009). With other reasons such as boosting its population and its increasing urbanisation, Australia has to be very good at wastewater management, for example, treatment and reuse (Mitchell et al., 2004).

1.2.2 Detailed description of the two countries' guidelines

Many guidelines related to wastewater management were established in both China and Australia, for example, "Australian guidelines for water recycling: managing health and environmental risks", "Guidelines for sewerage systems – effluent management", "Environmental quality standards for surface water (GB3838-2002)" and "Integrated Wastewater Discharge Standard (GB8978-1996)", Australia has

relatively more guidelines in quantity. For this comparison, it is important to find two guidelines that are significant and comparable. Based on the “Environmental Protection Law of the People’s Republic of China”, the “Guideline for municipal wastewater reclamation and reuse technology (Trial)” (China, 2012) formulated by the Ministry of Housing and Urban-Rural Development of the People’s Republic of China in 2012 was chosen for this comparison. For Australia, benchmarking guidelines “Australian guidelines for water recycling: managing health and environmental risks” (NRMMC, 2006; NRMMC, 2008) as discussed below, was generated by the Australian Health Ministers Conference, Environment Protection and Heritage Council and Natural Resource Management Ministerial Council. Another important reason for why these two documents are comparable is that they laid a solid foundation for wastewater recycling regulations at a similar period; thus, most data within these two guidelines are still up to date. Furthermore, because the wastewater treatment system related to my research, the high-rate algal pond (HRAP), usually is the only facility treating rural raw wastewater before it is discharged for reuse for irrigation (Shilton, 2005a), the recycling and reuse topics in these two guidelines are the most applicable.

The “Guideline for municipal wastewater reclamation and reuse technology (Trial)” was developed based on “The Urban-Rural Planning Law of China”, “The Water Pollution Prevention and Control Law of China” and other such laws and regulations. It aims to improve municipal wastewater reclamation and reuse, promoting municipal water conservation to reduce emissions. Some environmental research facilities like the Ecological Environment Research Center of the Chinese Academy of Sciences, QsingHua University and Tianjing University participated in the drafting of this guideline.

The “Guideline for municipal wastewater reclamation and reuse technology (Trial)” consists of six chapters, comprising ‘General Introduction’, ‘Municipal wastewater recycling technology route’, ‘Municipal wastewater recycling technology’, ‘Municipal wastewater recycling treatment process’, ’Municipal wastewater recycling engineering construction and facilities maintenance’ and ‘Municipal wastewater reclamation and reuse risk management’.

For Australia, the “Australian guidelines for water recycling: managing health and environmental risks” was developed under the support of the National Water Quality Management Strategy (NWQMS). According to these guidelines, promoting recycling of water not only provides an additional resource for a variety of purposes,

but also reduces the discharge of wastewater to natural water systems. These guidelines were developed based on, and will replace. the NWQMS “Guidelines for sewerage systems, use of reclaimed water” (2000).

Table 1-4 compares the following key attributes of two guidelines.

1.2.2.1 Conclusion of comparison

The comparison of these two guidelines indicates that they share many aspects in common. Firstly, the motivation for drafting both guidelines was the realisation of the increasing pressure on freshwater supplies. In recent years, many research studies had been conducted to explore the health risks caused by heavy metals in both northern and southern China’s irrigation and drinking water systems (Bi et al., 2006; Cheng, 2003; Cui et al., 2004; Khan et al., 2008; Liu et al., 2005). Furthermore, China’s water shortage may pose a threat to world food security. In Australia, many cities are continuing to grow and the increasing demand for water is causing concern: long-term drought is also a regular occurrence (Rathjen et al., 2003). Therefore, if recycling wastewater enables it to become a valuable resource, this will release the pressure.

In addition, both countries have generated wastewater discharge guidelines according to their respective practical situations. The current water scarcity in China is caused by three main reasons: uneven distribution of freshwater resources, growing population and urbanisation and poor management of water resources (Jiang, 2009). The first of these is impossible to adjust, so publishing a plain, simple guideline like “Guideline for municipal wastewater reclamation and reuse technology (Trial)”, with an emphasis on the basic technology is a smart way to reach two targets. It provides an elementary enlightenment document for wastewater reclamation and reuse relevant to workers or anyone who is interested in this area. The plain concepts involved in the guideline are able to help the people in China to become acquainted with the fundamental and significant ideas: it is believed that more people in China will understand and remember these concepts after publication of the guideline. In addition, this guideline builds a solid foundation to improve poor water management; and the importance of municipal water recycling has been proven in many advanced countries around the world (Exall, 2004; Vigneswaran & Sundaravadivel, 2004). It is timely that publishing this guideline, to which the Chinese people can refer, with its advanced concepts regarding water recycling will definitely improve the level of water resource

management in China.

Table 1–4: Comparison of two wastewater recycling guidelines: *Guideline for municipal wastewater reclamation and reuse technology (Trial)* and *Australian guidelines for water recycling: managing health and environmental risks (Phase 1)*

Guideline/s Title	*Guideline for municipal wastewater reclamation and reuse technology (Trial)*	*Australian guidelines for water recycling: managing health and environmental risks (Phase 1)*
Date Released	December 2012	2006
Guideline Developer(s)	It was organised and edited by Ministry of Housing and Urban-Rural Development of the People's Republic of China. It was mainly drafted by the Ecological Environment Research Center of the Chinese Academy of Sciences, QsingHua University and Tianjing University.	Australian Health Ministers Conference, Environment Protection and Heritage Council and Natural Resource Management Ministerial Council.
Adaptation	Not applicable: The guideline was not adapted from another source.	The NWQMS *Guidelines for Sewerage Systems, Use of Reclaimed Water* (2000).
Principles	•Be systemic: the construction of wastewater treatment plants should undertake wastewater reclamation and reuse wastewater systemically. •Be integral: Wastewater reclamation and reuse should be	• Protection of public and environmental health is of paramount importance and should never be compromised. • Protection of public and environmental health depends on implementing a preventive risk management approach.

	taken into consideration as an integral strategy of wastewater treatment and disposal. •Be reasonable: All wastewater reclamation and reuse need to be planned in a reasonable manner. •Be prospective: Fully refer to domestic and foreign-related research. •Be safe all the time: Safety is the core part of wastewater reclamation and reuse.	• Application of preventive measures and requirements for water quality should be commensurate with the source of recycled water and the intended uses.
Guideline Objective(s)	To improve municipal wastewater reclamation and reuse, promoting municipal water conservation to reduce emissions.	To provide guidance on best practices for water recycling. They are not prescriptive and do not represent mandatory standards,
Target Application	To be used by urban centralised wastewater treatment recycling technology scheme selection, including the whole process management of municipal wastewater collection, treatment and reuse. Guide the planning of municipal wastewater reclamation and reuse, incorporate the construction, operation, maintenance and management of facilities.	To be used by anyone involved in the supply, use and regulation of recycled water schemes, including government and local government agencies, regulatory agencies, health and environment agencies, operators of water and wastewater schemes, water suppliers, consultants, industry, private developers, body corporates and property managers.

Major contents	38 pages, six chapters, including •General Introduction •Municipal wastewater recycling technology route •Municipal wastewater recycling technology •Municipal wastewater recycling treatment process •Municipal wastewater recycling engineering construction and facilities maintenance •Municipal wastewater reclamation and reuse risk management.	389 pages, six chapters, including •Introduction •Framework for management of recycled water quality and use •Managing health risks in recycled water •Managing environmental risks in recycled water •Monitoring •Consultation and communication
Notes	Fundamental	Advanced

In the meantime, in view of the long-term development of the “Australian Wastewater Treatment and Recycling Guidelines” (Agriculture and Resource Management Council of Australia and New Zealand and the Australian and New Zealand Environment and Conservation Council, 2000; Council, 1997), “Australian guidelines for water recycling: managing health and environmental risks (Phase 1)” offers an elaborate, accurate and integrated framework document for those engaged in wastewater recycling. Besides a description of the framework for management of recycled water, and lists of recycled water system regulations and requirements, it is exploring a new and advanced tool for risk management. An impressive innovation in “Australian guidelines for water recycling: managing health and environmental risks (Phase 1)” is the application of a risk management tool: disability-adjusted life years (DALYs) (DALYs=YLL (years of life lost) + YLD (years lived with a disability or illness). Havelaar and Melse (2003) and Water Services Association of Australia (WSAA) (2004) have provided a detailed data description for DALYs. Unlike the conventional risk assessment method of setting water quality criteria with maximum levels of infection or disease, by adopting disability-adjusted life years, DALYs overcome the limitation of the traditional approach which was unable to reflect the varying severity of consequences. After the application of preventive measures, residual risk should be less than 10^{-6} DALYs per person per year (NRMMC, 2006). The concept has been applied in many health-related organisations, such as the World Health Organization (WHO, 2004).

From Table 1-4, the apparent dissimilarities between the sets of two guidelines can be observed. Firstly, the application scale of the Australian guidelines is wider than for those of China. The Australian guidelines are for water recycling: managing health and environmental risks (Phase 1) is able to be used by anyone who is engaged in the supply, use and regulation of recycled water schemes, including government, agencies, operators of water and wastewater schemes, water suppliers, consultants, industry and private developers. In addition, the central feature of these two sets of guidelines is quite different: in the Australian guidelines, the core part is a generic risk management framework. In this risk management approach, the first step systematically identifies all the hazards in the recycled water; the possibility and consequences of the hazards are then assessed, followed by the development of protective measures for the identified hazards and confirmation that these measures are effective. Conversely, more than half of contents of the other set of guidelines were related to wastewater recycling technology, incorporating the effect and features of wastewater recycling for industry, landscape, irrigation and municipal

entertainment.

Many countries have to face increasing water scarcity; thus, learning from each other's experience is important for effectively solving the water shortage problem. From the perspective of long-term development, the Australian guidelines obviously have placed much more emphasis on risk management, philosophies and sustainable development strategy which have some implications for China.

Besides the need to update the guideline, another problem associated with environment protection in China is non-compliance and weak enforcement (Van Rooij, 2006). An interesting argument about this problem is that, on one side the Chinese environmental legislators believe stricter laws lead to a better environment for China (Xie, 2004); however, on the other hand in the current situation, stricter laws would make it harder for law enforcement agencies to adopt it, with greater resistance against the law (Van Rooij, 2003).

1.3 Natural wastewater treatment systems and high-rate algal ponds

1.3.1 Natural wastewater treatment systems' concept

The natural waste treatment system is defined as the waste treatment that obtains the ideal treatment goal, utilising to the maximum possible, natural self-treatment processes (Crites et al., 2006). The publication of the *Clean Water Act 1972* in the USA was the first time in history that natural wastewater treatment systems drew considerable attention, with the term 'zero discharge' also mentioned. However, people noticed that the combined cost of money and energy to achieve 'zero discharge' would be incredibly high through an advanced wastewater treatment (AWT) unit. Natural wastewater treatment provides an alternative direction to achieve the goal.

The natural wastewater treatment systems can be simply classified into three principal categories: aquatic, terrestrial and wetland (Crites et al., 2006). More exactly, five specific natural technologies of treatment can be listed as soil (ground) filters, constructed treatment wetland, waste stabilisation ponds, aquatic plants systems and bioeliminators, and irrigation by wastewater (minimally mechanically treated) (Rozkošný et al., 2014). According to the composition of the wastewater, the practical treatment level demanded and the level of local treatment technology development, different methods of natural wastewater treatment are chosen. The

focus of my study was on wastewater treatment through the design features and performance of the three types of natural wastewater treatment systems which are listed below (Table 1-5). As discussed below, many guidelines for the design, construction and management of natural wastewater treatment systems have been published (Crites et al., 2006; Middlebrooks et al., 1983; Rozkošný et al., 2014; WHO, 1970).

The natural characteristics are the most valuable part of the natural wastewater treatment facility. Lower costs for design, construction and operation are the first impression that people have of natural wastewater treatment systems with specific comparisons having been performed in some studies (Reed et al., 1979). Natural characteristics bring with them lower energy consumption, potential reduction in greenhouse gas (GHG) emissions if fossil fuel is the prime energy source, and also the avoidance of chemical by-products to a considerable extent. More importantly, based on environmental ethics, one significant idea is to recycle and reuse resources as much as possible; therefore, from the long-term perspective, the natural wastewater treatment facility's advantages will become even stronger in the future.

Except for reasons such as poor design and management, the disadvantages of natural wastewater treatment systems are that a relatively large space maybe required and potentially they have low efficiency in removing nutrients.

Table 1–5: Design features and performance of three natural wastewater treatment systems (Crites et al., 2006)

Type		Treatment Goals	Climate Needs	Detention Times (Days)	Effluent Characteristics (mgL^{-1})
Aquatic Treatment Units (Waste Stabilisation Pond systems)	Oxidation pond	Secondary	Warm	10–40	BOD, 20–40 TSS, 80–140
	Facultative pond	Secondary	None	25–180	BOD, 30–40 TSS, 40–100
	Partial-mix aerated pond	Secondary, polishing	None	7–20	BOD, 30–40 TSS, 30–60
	Storage and controlled discharge ponds	Secondary, storage, polishing	None	100–200	BOD, 10–30 TSS, 10–40
Wetlands	Natural marshes	Polishing, advanced water treatment with secondary input	Warm	10	BOD, 5–10 TSS, 5–15

	Free water surface	Secondary to advanced water treatment	None	7–15	BOD, 5–10 TSS, 5–15
	Subsurface flow	Secondary to advanced water treatment	None	3–14	BOD, 5–40 TSS, 5–20
Terrestrial Treatment Units	Slow rate	Secondary or advanced water treatment	Warmer seasons		BOD < 2 TSS < 2
	Soil aquifer treatment	Secondary, advanced water treatment or groundwater recharge	None		BOD, 5 TSS, 2
	Overland flow	Secondary, nitrogen removal	Warmer seasons		BOD, 10 TSS, 10[e]

1.3.2 Wastewater stabilisation ponds

As the obvious advantage of wastewater stabilisation ponds (WSPs) is their simplicity to build and operate (Shilton, 2005b), they are believed to be a significant additional direction for the future development of water treatment technology. In fact, pond technology has been under development for more than 100 years since 1901 with the construction of an impoundment with a mean depth of 1.4 m in the city of San Antonio, which is now known as Mitchell Lake (WHO, 1970). Unlike the conventional wastewater treatment process, wastewater stabilisation ponds (WSPs) mainly take advantage of solar irradiation to reach a high level of disinfection and have relatively minimal labour requirements; in addition, it is possible to recover protein or fertiliser resources by algae harvesting in the future.

A variety of pond systems is available for different applications. The 'standard' pond system generally consists of anaerobic ponds which are designed to receive organic loading; facultative ponds which provide aerobic and anaerobic zones for basic disinfection; and maturation ponds that follow the removal of pathogens and are mainly for nutrient removal. There are other advanced pond types for specific targets, for example, high-rate algal ponds, cold climate ponds and agricultural wastewater ponds (Shilton, 2005b, p. 7).

A worldwide survey was conducted by the World Health Organization (WHO) (Gloyna & Organization, 1971) about the extent of pond categories in at least 39 countries. This report's findings indicated that organic and volumetric loadings varied considerably, receiving several hundreds or even thousands of kilograms of BOD_5 per hectare per day. The removal efficiencies for comparable areas and loadings seemed to be fairly uniform throughout the world with it being not uncommon to obtain higher than 90% BOD_5 removal in wastewater stabilisation ponds (WSPs). The populations served by the ponds varied in number from less than 1,000 to several hundred thousand.

1.3.3 High-rate algal ponds (HRAPs)

High-rate algal ponds (HRAPs) are one of the outstanding members of the family of WSPs. The key differences in identifying high-rate algal ponds from other WSPs are that they have a shallower depth and a meandering channel with paddle wheels. The paddle wheel in association with baffles in a high-rate algal pond (HRAP) is the most distinguishing feature that enables differentiation of HRAPs from normal

wastewater stabilisation ponds (WSPs). The mean surface water velocity in an HRAP can be varied between 0.15 ms^{-1} and 0.5 ms^{-1} (Shilton, 2005a). The first advantages of mixing the wastewater is to maintain the algal-bacteria flocs in suspension to maximise light exposure and photosynthetic oxygen production (Buchanan, 2014). These excellent characteristics ensure a more uniform hydrodynamic environment namely no thermal, DO, pH stratification, and more uniform exposure.

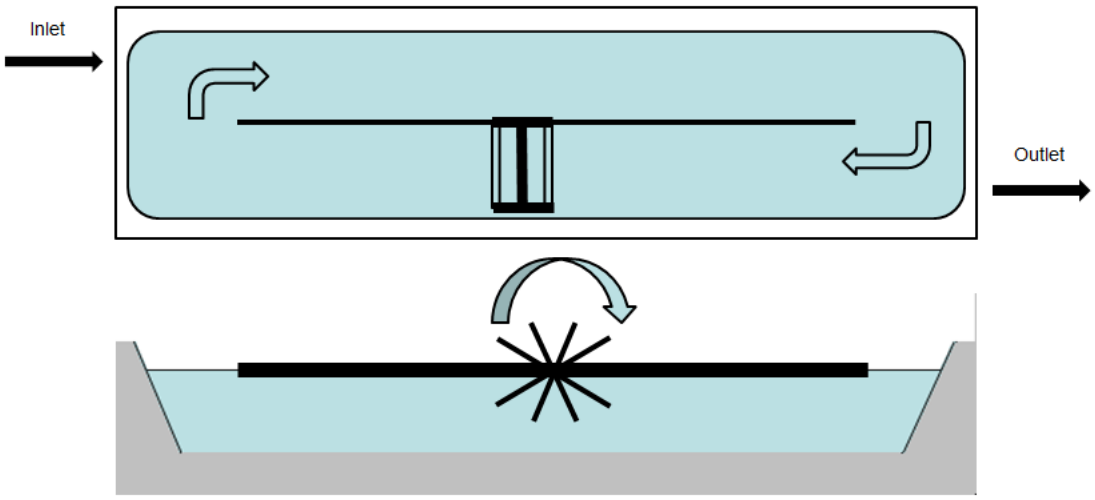


Figure 1-2: Diagram of HRAP and the HRAP located at Kingston-on-Murray, South Australia

of the pond volume to disinfecting wavelengths of sunlight (Fallowfield, 1985a) and, thus, they achieve better performance in disinfection compared to WSPs (Buchanan, 2014; Fallowfield et al., 1996; Oswald, 1988).

A variety of algae suspended within HRAP play significant roles. In the first place they provide photosynthetic oxygen to heterotrophic bacteria to mineralise the dissolved organic matter, meanwhile sufficient CO_2 from bacteria respiration support algal growth (Fallowfield, 1985b; Oswald, 1996). Nutrients like nitrogen and phosphorus are removed by assimilation into algae and by ammonia volatilisation and phosphorus precipitation within the high pH environment. The efficacy of HRAP nutrients removal is well documented (Cromar et al., 1996; Cromar et al., 1992; Oswald, 1988). A high level of disinfection level is achieved, presumed due to the high exposure to solar radiation (Oswald & ASCE, 1990).

Disinfection—the critical function of the high-rate algal pond (HRAP) when treated wastewater reuse is contemplated—has been investigated over a long period of time. A large number of factors may influence 'natural' disinfection in wastewater stabilisation ponds (WSPs) (Maynard et al., 1999). Some examples include: sunlight, temperature, hydraulic residence time (HRT), algal toxins, sedimentation, ingestion by antagonistic microbes, etc. (Manage, 2002; Maynard et al., 1999; Mayo & Noike, 1996; Oufdou et al., 2001). Sunlight is considered as the most important factor which is supported by a significant amount of evidence (Craggs, 2004; Maynard et al., 1999; Mayo, 1995).

1.4 Mechanisms of sunlight inactivation and affecting factors

1.4.1 Mechanisms of sunlight inactivation

The natural spectrum consists of the whole electromagnetic spectrum, for example, gamma rays, ultraviolet (UV) light, visible light and radio waves. Among them, the disinfection effect of UV light has been known for more than 70 years (Hollaender, 1943). Ultraviolet radiation (UVR) is defined as being divided into four bands: UVA (315–400 nm), UVB (280–315 nm), UVC (200–280 nm) and vacuum UV (100–200 nm) (WHO, 2002). The optimum UV light wavelength for bactericidal effect ranges from 250 nm to 270 nm, namely, UVC, although UVC is absorbed by the Earth's atmosphere and consequently does not impinge on its surface. However, light-emitting UVC wavelengths are used for disinfection within the water industry. Although vacuum UV light can disinfect micro-organisms (USEPA, 2006) in water, it

is not practical for application due to rapid attenuation. UV light and even visible light can damage DNA, RNA or other cell constituents, the mechanisms associated with the different bands of UV for killing micro-organisms are dissimilar.

UVB and UVC are able to directly damage nucleic acid within micro-organisms. Jagger (1967) provided evidence that light in the wavelength of 240–280 nm was within the maximum peak absorbance by nucleic acids. Nucleic acids like DNA and RNA are necessary for the reproduction of micro-organisms: it is believed that direct inactivation (Figure 1-2) is often due to the dimerisation of pyrimidine molecules (USEPA, 1999). Once the double bond occurs between pyrimidine molecules, it is hard for nucleic acid to replicate as DNA's helical structure has been distorted (Snider et al., 1991). Even if replication happens, the mutant cells remain unable to replicate (USEPA, 1996). However, since the atmosphere prevents all the wavelengths encompassing UVC in sunlight from reaching the Earth's surface it is not significant for wastewater disinfection in WSP systems. Evidence has shown that the mechanism of UVB is quite similar to that of UVC, forming pyrimidine dimers and causing direct damage (Jagger, 1985). There are two phenomena that influence the inactivation rate of this mechanism, namely, dark repair mechanisms and photoreactivation(Harm, 1968; Hill, 1965).

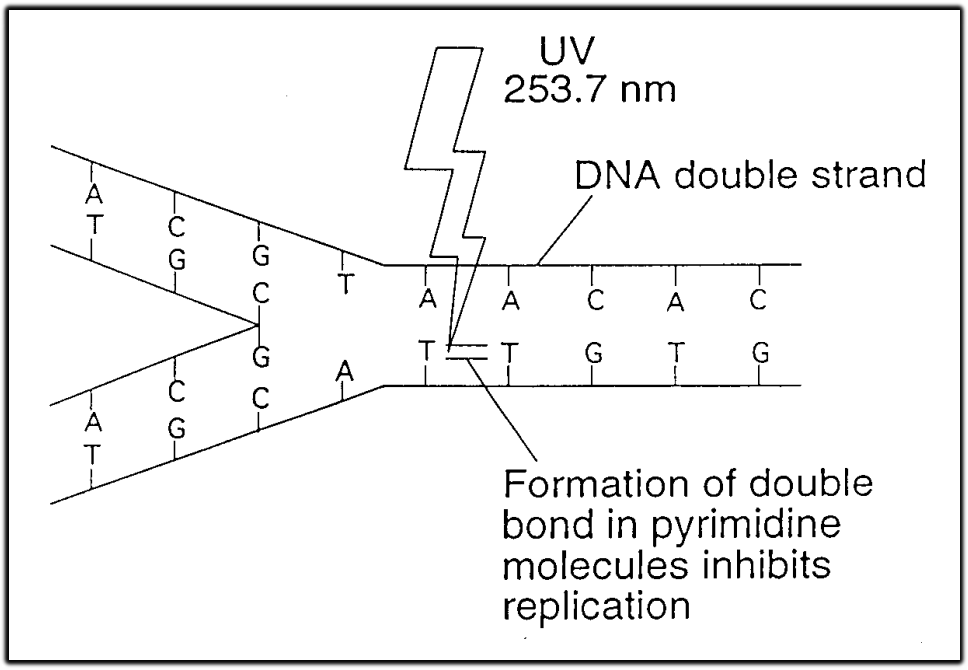


Figure 1–3: Direct germicidal inactivation by UV radiation (Tchobanoglous, 1997)

Davies-Colley et al. (2000) concluded that two photo-oxidation mechanisms related to UVA and visible light (Vis) were involved in inactivation, both of which could

contribute to the formation of reactive oxygen species (ROS; Fig 1-3). The first produces ROS within cells by interaction with endogenous photosensitisers, the second produces ROS outside cells via interaction with exogenous photosensitisers. Endogenous photosensitisers, such as flavins and porphyrin derivatives, absorb short solar UV wavelengths (UVB and some shorter wavelength UVA) to react with oxygen to form ROS: an 'inside' cell ROS attack would lead to membrane failure and finally cell inactivation (Bosshard, 2010). Exogenous photosensitisers outside of cells, for example, humic substances, photosynthetic pigments and dissolved organic matter, absorb a wide range of sunlight, especially UVA and visible light (Vis) (Curtis, 1992; Kohn & Nelson, 2006). Once the formation of ROS has occurred, it will also attack membrane.

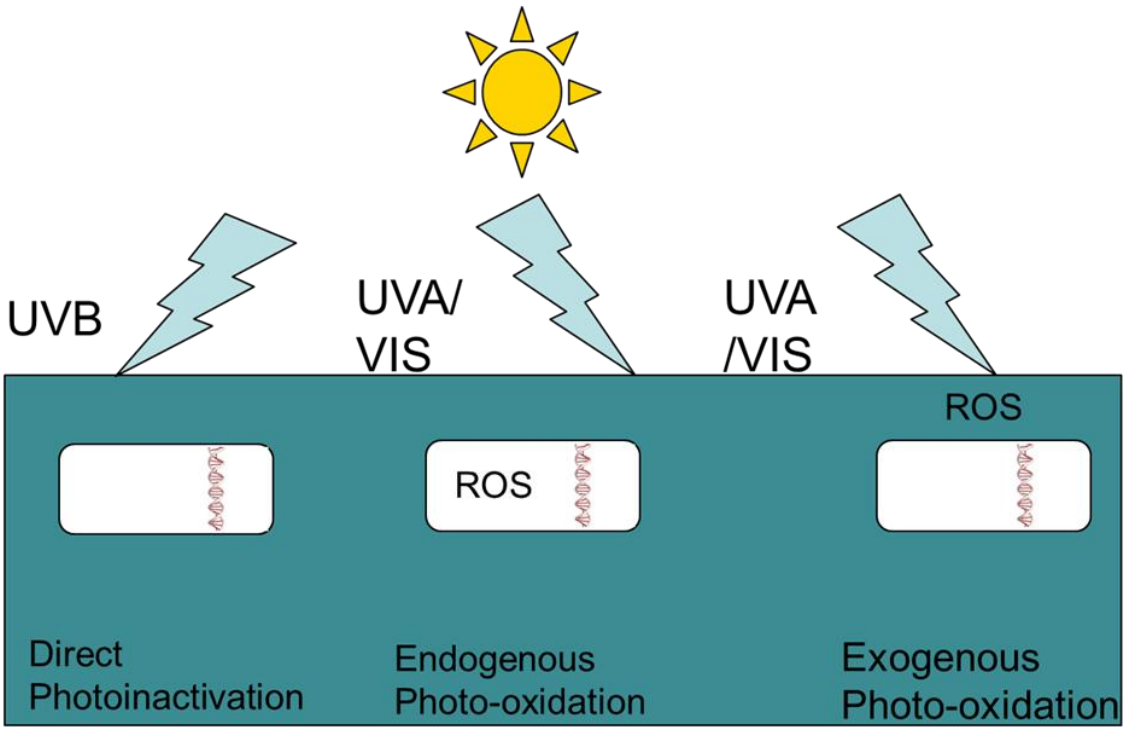


Figure 1–4: Simple introduction of sunlight disinfection mechanisms of micro-organisms in water

Note: ROS = Reactive oxygen species

Many different sunlight terms and units have been recorded which may cause inconvenience when undertaking comparisons (Table 1-6). In this thesis, UVB is investigated as it is the primary germicidal part of natural sunlight: the UV dose is defined as the dose rate multiplied by time (Equation 1-3):

$$\text{UV dose } (Jm^{-2}) = \text{UV dose rate } (Wm^{-2}) \times \text{Time (s)} \qquad \textbf{Equation 1-3}$$

A ccording to recommendations from the International System of Units and Thimijan and Heins (1983), the units 'Joule' and 'Watt' were elected as they are the appropriate units for reporting energy sums and are rated separately, further, Jm^{-2} is the dose whereas a W is a Js^{-1}, so Wm^{-2} incorporates a rate term.

Table 1–6: Terms and units appearing in sunlight disinfection-related literature

Irradiated by	Irradiance terms and units	Pathogen indicators	Method of reporting indicators' die-off rate	Reference
Artificial UVB	Irradiance (Wm^{-2})	*E. coli*, MS2, etc.	$K = Log_{10}(N_t/N_0)/t$	(Bolton, 2011)
UVC	Dose rate (Wm^{-2}); Dose (Jm^{-2})	*E. coli*, MS2, etc.	Log_{10} reduction value	(Sommer et al., 1998)
Artificial UVB	Intensity (Wm^{-2})	Marine bacterial	Survival percentage	(Matallana-Surget et al., 2012)
Natural sunlight	Irradiance (Wm^{-2}); Insolation (MJm^{-2})	*E. coli*	$K = Log_{10}(N_t/N_0)/t$	(Craggs, 2004)
UVC	Dose ($mWscm^{-2}$)	*E. coli*, etc.	1 log, 2 log inactivation time	(USEPA, 1996)
Natural sunlight	Insolation (MJm^{-2})	Faecal indicators	Log_{10} reduction value against insolation graph	(Davies-Colley et al., 1999)
Natural sunlight	Irradiance (Wm^{-2})	*E. faecalis* and *E. coli*	$K = Log_{10}(N_t/N_0)/t$	(Kadir & Nelson, 2014)

1.4.2 Influence of dose and dose rate on UV disinfection

Many research results have demonstrated that sunlight plays the most important role in the disinfection process of wastewater stabilisation ponds (WSPs) (Maynard et al., 1999; Mayo, 1995). In HRAPs, it has even been concluded that about 75% of the total *E. coli* inactivation was attributable to sunlight action (Craggs, 2004). However, the sunlight inactivation mechanism of pathogenic indicators in HRAPs is

not yet clear and needs further research. The focus of my research is to gain more understanding about UVB inactivation and thus to enhance the disinfection performance of high-rate algal ponds (HRAPs).

Sethi (2009) has compared the die-off rate of *E. coli* and MS2 in buffered distilled water (pH 7.5 and 9.5 group) under the 3.77 $Jm^{-2}s^{-1}$ UVB for 2.33 h (dose 30 KJm^{-2}) and 9.05 $Jm^{-2}s^{-1}$ for 0.972 h (dose 30 KJm^{-2}): through the significant differences shown by ANOVA analysis, it was concluded that the die-off rate of *E. coli* and MS2 decreased in the order of 9.05 $Jm^{-2}s^{-1}$ > 3.77 $Jm^{-2}s^{-1}$ > dark (Table 1-7). Sethi (2009) explained the phenomenon by stating that the higher UVB dose rate was able to achieve better penetration in distilled water, due to the absence of particles and solids in the water, thus decreasing attenuation to a minimum. Similar work has been done by Sommer et al. (1998): in their research, UVC at 253.7 nm was used to irradiate *E. coli* isolated from sewage at 0.02 $Jm^{-2}s^{-1}$, 0.2 $Jm^{-2}s^{-1}$ and 2 $Jm^{-2}s^{-1}$, respectively. They also concluded that at the same dose of UV light, a higher dose rate meant a higher decay rate. In their opinion, the reason could be that the repair enzymes of the cell are more influenced by high UV intensities.

However, according to the UVB data that Bolton (2011) measured by using spectroradiometers placed on the roof of Flinders Medical Centre (35° 01`S and 18° 34`E), the environmentally relevant UVB dose rate ranges from 0.5 $Jm^{-2}s^{-1}$ to 4.5 $Jm^{-2}s^{-1}$. But the research conducted above was far higher than this range. In addition, it did not focus on finding the accurate relative significance and boundary conditions of the dose rate and dose.

Table 1–7: Environmentally relevant (approx.) dose and dose rate of UVB, and two different dose rates and incubation times used in experiments to yield the same dose (Sethi, 2009)

Dose rate (Wm^{-2})	Time (hours)	Dose (KJm^{-2})
	Environmentally relevant	
1.1	8	30
	Doses used in experiments	
3.77	2.33	30
9.05	0.972	30

1.4.3 Influence of temperature on UV disinfection

Temperature is obviously a significant physical factor for ponds and is even a determining factor for pond retention time and categories of pond (Crites et al., 2006; Shilton, 2005a). Temperature is assumed to have an effect on the UVB disinfection process because it affects the rate of biological and chemical processes. However, currently little research has been conducted to systematically explore the influence of temperature on UV disinfection. The research described below is related but the temperature range chosen was beyond that of ponds.

Theitler et al. (2012) explored the synergistic effect of heat and solar UV on *E. coli* and MS2 under natural sunlight irradiances. They observed a significant synergistic impact on *E. coli* inactivation by heating: similarly, a slightly synergistic effect was found for solar dose rate and heating on MS2 inactivation at 60ºC in deionised water. Similar synergistic results have been found at 50ºC with UVB and visible light radiance for inactivation of *E. coli* and enteroviruses (Wegelin et al., 1994). The possible explanation given was that high temperature promotes the transport of reactive oxygen species (ROS) which are lethal (Heaselgrave et al., 2006).

1.4.4 Influence of turbidity on UV disinfection

Turbidity is a significant parameter with regard to pond technology as it is correlated with light attenuation. Light attenuation describes the ability by which sunlight is attenuated in water due to absorption and scattering. Different water environments, have large variation in the attenuation (Bolton et al., 2011). Turbidity has a negative influence on sunlight disinfection via two mechanisms, firstly, it is able to directly decrease the UV transmittance and dose delivery and secondly, concurrently it provides a shield protecting pathogens from UV light (Passantino et al., 2004).

Liu and Zhang (2006) tested the effect of water turbidity on the inactivation rates of the indicators of several pathogens under UVC light. In their study, UVC light was delivered by low pressure mercury vapour germicidal lamps at 253.7 nm, with turbidity offered by kaolin clay which was believed to be representative of many natural particles in water, three turbidity levels, 0.5, 4 and 12 were selected for the mixed suspension. They concluded that turbidity had a negative impact on bacteria inactivation under UVC light; however, under the same UVC dose, a higher UVC dose rate resulted in greater disinfection, which means a higher UVC dose rate might be able to overcome the influence of turbidity to some extent. However, further study conducted by Passantino et al. (2004) indicated that if the level of

turbidity was low, there would not be enough particles to interfere with the UVC light.

1.4.5 Influence of exogenous and endogenous photosensitisers

The exact constituents of endogenous photosensitisers are still unclear. Researchers have found they have characteristics such as being likely to spread throughout the whole micro-organism (Hollaender, 1943; Vermeulen et al., 2008) and playing a significant role in the entire photo-inactivation (Bosshard et al., 2010). However, the MS2 coliphage was found to not be influenced by endogenous photo-oxidation due to its lack of chromophores (Kohn & Nelson, 2006).

Bolton's (2011) research focused on an initial determination of the effect of exogenous photosensitisers, pH and dissolved oxygen on sunlight inactivation of MS2. As shown on Table 1-8, she concluded that dissolved organic matter (DOM), namely, dissolved photosensitisers in her experiments decreased photo-inactivation by UVB and photo-inactivation processes by UVA due to increasing attenuation. However, the DOM enhanced the exogenous photo-oxidation of MS2 by UVA and visible light (Vis).

Table 1–8: Apparent inactivation mechanisms of UVB, UVA and Vis and corresponding inactivation rates (Kh^{-1}) in RO water (no exogenous photosensitisers) and Mt Barker wastewater (exogenous photosensitisers) at pH 7.5 and DO < 1 mgL^{-1} (Bolton, 2011)

		No Photosensitisers			**Photosensitisers**		
		Mechanism	K	Target	Mechanism	K	Target
MS2	UVB	Direct photoinactivation	0.34	RNA	Direct photoinactivation	0.28	RNA
	UVA	--	--	--	Direct photoinactivation	0.06	capsid
	Vis	--	--	--	Exogenous photo-oxidation	0.04	capsid
		Total direct photo-inactivation (100%) Total photo-oxidation (0%)			Total direct photo-inactivation (73%) Total photo-oxidation (27%)		

1.5 Dark disinfection mechanism and environmental factors

The factors considered to influence disinfection in WSPs are presented in Table1-9. In wastewater stabilisation ponds (WSPs), light attenuation (decay through

absorption and scattering), as a physico-chemical characteristic, is very strong (Curtis & Curtis, 1994). Determination of the euphotic depth, at which 99% of incident surface light has been attenuated, in HRAPs, WSPs and environmental waters, (Bolton et al., 2011) found, in all waters, light attenuation was ranked UVB > UVA > Vis, the average euphotic depths were 0.09m, 0.15m and 0.5m respectively in wastewater. Typically, this meant that almost 70% of the depth of a WSP was in the dark. The mechanism of 'dark' die-off, that is, the inactivation of pathogenic viruses and potential bacterial and bacteriophage indicators that happens due to the absence of light under different temperatures requires more investigation since it is likely a significant process in WSPs.

Table 1–9: Factors that have been proposed to cause or influence disinfection in WSPs (Shilton, 2005, p. 106)

Factor	Likely mechanism(s)	Micro-organisms affected[1]	Ponds where active[2]
Temperature	Affects rates of removal processes	B, V, P, H	A, F, M
Hydraulic residence time (HRT)	Affects extent of removal (time for operation)	B, V, P, H	A, F, M
Algal toxins	Algal exudates are toxic to certain bacteria	Mainly B?	F, M
Sedimentation	Settlement of infectious agent (e.g. ova, cysts) OR Settlement of aggregated solids including the infectious agent	H P, H, (B, V?)	A, F, M A, F, M
Biological disinfection	Ingestion by higher organisms (protozoans)	B, V, (P?)	F, M
Sunlight	DNA damage by solar UVB radiation OR photo-oxidation (DO-sensitive) (range of wavelengths)	B[3], V, P B, (P?)	F, M F, M

1. Micro-organisms: B = bacteria, V = viruses, P = protozoan parasite, H = helminth worms.
2. Ponds: A = anaerobic, F = facultative, M = maturation.
3. Most of the DNA damage to bacteria by UVB radiation is repaired and the lethal effect is related mainly to the overwhelming of the repair capacity.

1.5.1 Temperature

It is unclear within the literature how the temperature mechanism influences dark die-off. Firstly, temperature is lethal to sensitive micro-organisms only when it is above 45ºC (Shilton, 2005). It is then known that temperature influences the biomass composition, nutrient requirement (Pirt, 1971) and metabolic reaction rate (Novak, 1974). It appears to have been widely demonstrated that the die-off rate of faecal coliforms increases with increasing temperature regardless of whether the faecal coliform bacteria is in river water (Flint, 1987) or in recreational coastal water (Craig et al., 2001, 2004). The same trend applies to coliphages: the lowest inactivation rate was observed at 5ºC for MS2 and Qβ, and then increased as the temperature increased (Feng, 2003). In contrast, Auer and Niehaus (1993) concluded that no important relationship was observed between the dark die-off rate and temperatures in the range of 5ºC–35ºC in raw sewage diluted with filtered lake water. A similar phenomenon was also found in wastewater lagoons by Moeller and Calkins (1980), over five temperature ranges, < 10ºC, 10–14ºC, 15–19ºC, 20—24 ºC and > 24ºC. In addition to temperature, dissolved oxygen (DO) and pH appeared to have little influence on inactivation rate (Craggs, 2004) , and were believed to interact with other factors.

1.5.2 Other environmental factors

Algal toxins are a controversial factor in dark die-off in ponds. Oufdou et al. (2001) reported in their study that both extracellular and intracellular products released by the blue-green alga (cyanobacteria) reduced the survival of some bacteria, for example, *E. coli* and *Salmonella*. After adding the supernatants (containing extracellular or intracellular products), the bacterial strain was shaken and incubated in darkness at 22˚C for 24 hours. According to their results, at the stationary growth phase, the growth of *E. coli* reduced by 62.48%, while intracellular products produced by *Pseudanabaena* isolated from a waste stabilisation pond reduced the growth of *E. coli* by 84.92% compared to the control group. Nevertheless, Toms et al. (1975) found that more algae did not result in the lower survival rate of bacteria in lagoon water. They concluded there was no direct evidence that showed there were specific toxins released by algae, and nor was there any correlation between the biomass of algae and the survival rate.

Sedimentation seems like an important mechanism responsible for dark die-off. For virus removal, Ohgaki (1986) found that coliphages would be absorbed by microbial particulates under aerobic conditions. In relation to bacterial pathogen removal, in a research article by Auer and Niehaus (1993), the dark die-off rate of faecal coliform was 0.73 d^{-1}(In) at 20˚C, and they reported 90.5% of faecal coliforms were associated with small particles (0.45–10 μ m) while 9.5% were associated with large particles (> 10 μ m). Gannon et al.'s (1983) research supported these results that demonstrated that sedimentation was a significant factor in faecal coliform disappearance in an artificial impoundment.

Biological disinfection such as predation and competition could also be a significant factor in the inactivation of bacteria and viruses in the dark. Manage (2002), in comparing pond water, filtered through 0.2 μ m, 5 μ m and 0.8 μ m, respectively, found that the loss rate of the virus-like particles was attenuated by size-selective filtration: the highest rate happened in the 5 μ m group, especially when the growth rate of heterotrophic nanoflagellates was high. Furthermore, (Decamp & Warren, 1998) also demonstrated that bacterivorous activity of ciliates occurs in wetland by using fluorescently-labelled bacteria (FLB), specifically *E. coli*.

Overall, further research should be conducted to clarify the effect of temperature on the dark die-off rate. There are numerous benefits of understanding the dark die-off mechanism, and clarifying whether its correlation with temperature is significant for wastewater stabilisation ponds (WSPs). Firstly, more evidence would show that the processes happening in the dark are more important than was previously thought in WSPs (Craggs, 2004; Davies-Colley et al., 1999) therefore, developing a greater understanding would be helpful in maximising the efficiency of the entire disinfection process. In addition, finding the critical influencing factors in the dark would provide the possibility of widening the usage of wastewater stabilisation ponds (WSPs). Currently, most established ponds are located in tropical areas as they receive more sunshine and run in relatively warm temperatures. Understanding and improving the dark die-off rate would reduce geographic differences.

1.6 Research objectives and hypotheses

An enormous amount of wastewater is in urgent need of being treated in almost all countries on Earth, including Australia and China. Natural wastewater treatment systems offer a potential direction for humans to fix the problem at a lower price and with reduced resource consumption. My research places special emphasis on one specific type of natural wastewater treatment system—the high-rate algal pond (HRAP. However, as little is known about the mechanisms that occur in HRAPs, all my research objectives below aim to improve understanding about the disinfection process in HRAPs:

- To investigate the influence of a wide range of environmentally relevant UVB doses, UVB dose rates, temperatures, turbidity and total organic carbon (TOC) on pathogen indicators' inactivation rates, based on different water environments.
- To develop models for the prediction of MS2 disinfection performance in HRAPs by a combination of all experiment results and validating it with a real South Australian high-rate algal pond (HRAP).
- To assess the influence of a range of typical environmental temperatures on the dark die-off rate of MS2 and *Escherichia coli* (*E. coli*) in mixed, unfiltered wastewater.

The hypothesis is that all factors tested in above research objectives, including UVB dose, UVB dose rates, temperature, turbidity and total organic carbon, play a significant role in UVB-induced pathogens' inactivation, and their importance proportion is able to be calculated.

CHAPTER 2: GENERAL METHODS

The chapter introduces general methods in the following chapters, incorporating experimental pathogen indicators stock preparation, quantification, UVB incubation setting, physical and chemical water analyses and statistical analysis. The more specific methods are outlined in the relevant chapter.

2.1 Experimental pathogens' indicators

2.1.1 Pathogen indicators MS2 and *E. coli*

The pathogen indicators selected for this thesis was is F+ specific bacteriophage MS2 and bacterium *Escherichia coli* (*E. coli*). MS2, the positive-sense single-stranded RNA virus, has shown its outstanding characteristics as an indicator under UV experiment (Bolton, 2011; Fisher et al., 2012; Liu & Zhang, 2006; Theitler et al., 2012). Mature qualification method are important reasons for MS2 to be chosen (Debartolomeis & Cabelli, 1991; Noble et al., 2004). *E. coli* has been recognised an iconic waterborne pathogens for long time (Edberg et al., 2000), it survives for long periods of time in natural water system (Edberg et al., 2000; Filip et al., 1987; Rice et al., 1999).The experiment results generated from both indicators enables comparison with results in the literature.

2.1.2 Preparation of MS2 stock

In the current study, the MS2 stock (ATCC #15597-B1) was prepared by flooding a plate of host *E. coli* containing over 100 MS2 plaques with 5 mL half-strength tryptone water. The plate was incubated for 30 minutes at 37ºC with swirling carried out every 10 minutes; the suspension was then decanted and filtered (0.2 µm) to remove the host *E. coli.* The final MS2 stock solution was stored at 4ºC in 100 mL half-strength tryptone water with 10% sterile glycerol.

2.1.3 Preparation of *E. coli* stock

The *E. coli* stock (ATCC #19434) was inoculated into 10 mL sterile nutrient broth (Oxoid™) and grown at 37ºC overnight. The *E. coli* stock was used immediately after incubation.

2.1.4 Quantification of MS2

Quantification of MS2 was accomplished by the double layer method (Debartolomeis & Cabelli, 1991; Noble et al., 2004). In summary, before quantification, this method involves preparation of an antibiotic stock (ampicillin/streptomycin stock), an overnight 35ºC culture of *E. coli* Famp (ATCC #700891) in 10 mL of tryptone soya broth (Oxoid^{TM}), 1.5% tryptone soya agar (TSA) solution and base plates. Where required, samples were serially diluted in 9 ml 0.5% tryptone water in a sterile 10 mL disposable tube; 5 mL of 1.5% TSA antibiotic stock and an overnight *E. coli* mixture were added to 5 ml of the sample, inverted and poured over one TSA base plate. Plates were left to dry, inverted and placed in a 35ºC incubator for 18–24 hours. Plaques were counted (the preferred plaque number ranges from 10 to 200) and the number of MS2 was expressed as plaque-forming units (PFUs) 100 ml^{-1}.

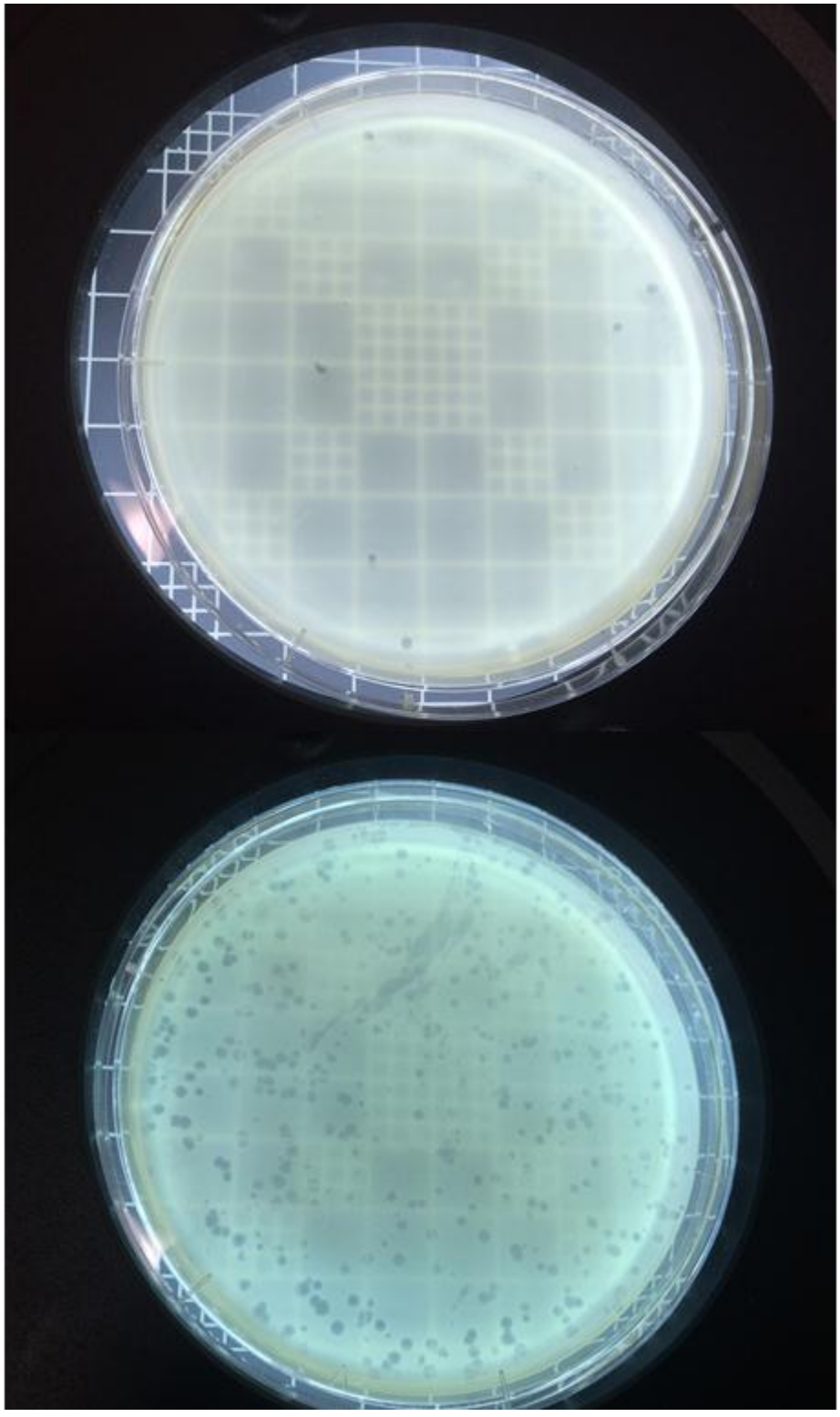


Plate 2–1: Two incubated MS2 quantification plates, the top one recorded about 10 plaques and the bottom one more than 150 plaques

2.1.5 Quantification of *E. coli*

The *E. coli* were enumerated by the defined substrate Colilert™ method (IDEXX Laboratories, Maine, USA). When the estimated sample was of a high concentration, the *E. coli* were diluted by 10-fold serial dilutions in 9 mL sterile 0.1% peptone water (Oxoid™). Appropriate concentration of samples were then added to a sterile vessel and made up to 100 mL with RO water. One pack of Colilert™ chromogenic substrate was added to 100 mL of solution and shaken until totally dissolved. The whole mixture was poured into a Quanti-tray®/2000 and sealed by IDEXX Quanti-tray® sealer. The tray was incubated at 35±0.5°C for 24 hours. The most probable number (MPN) was determined by counting the fluorescing wells on the tray under a UV lamp (365 nm) and by referring to the MPN table provided by Quanti-tray®/2000.

Plate 2–2: An incubated Quanti-tray® showed blue fluorescence under a UV lamp

2.2 UVB incubations

2.2.1 Ultraviolet (UV) cabinet

An ultraviolet (UV) cabinet (Figure 2-1) was designed to provide precise UVB irradiance for this research. The cabinet was constructed from medium-density fibreboard and, to inhibit UVB reflection, was painted black inside. An adjustable tray, with six, 15 W fluorescent light fittings able to be set at 13 different heights, was connected via the lamp and fan switches on top of the cabinet. On the floor of the cabinet was a shaking water bath (70 oscillations min^{-1}, Ratek Instruments, Vic, Australia) with nine experimental vessel positions. A cooler below the UV cabinet acted with the water bath to keep the sample vessel mixed at constant temperatures. To produce the UVB dose rate, 15 W fluorescent Hg lamps (Sankyo Denki, Japan) were used. The six UVB dose rate values at the vessel positions were achieved by varying the heights, number and positions of the six possible UVB lamps. A spectroradiometer was placed in each vessel position before starting the experiment to check the incident radiation at that point.

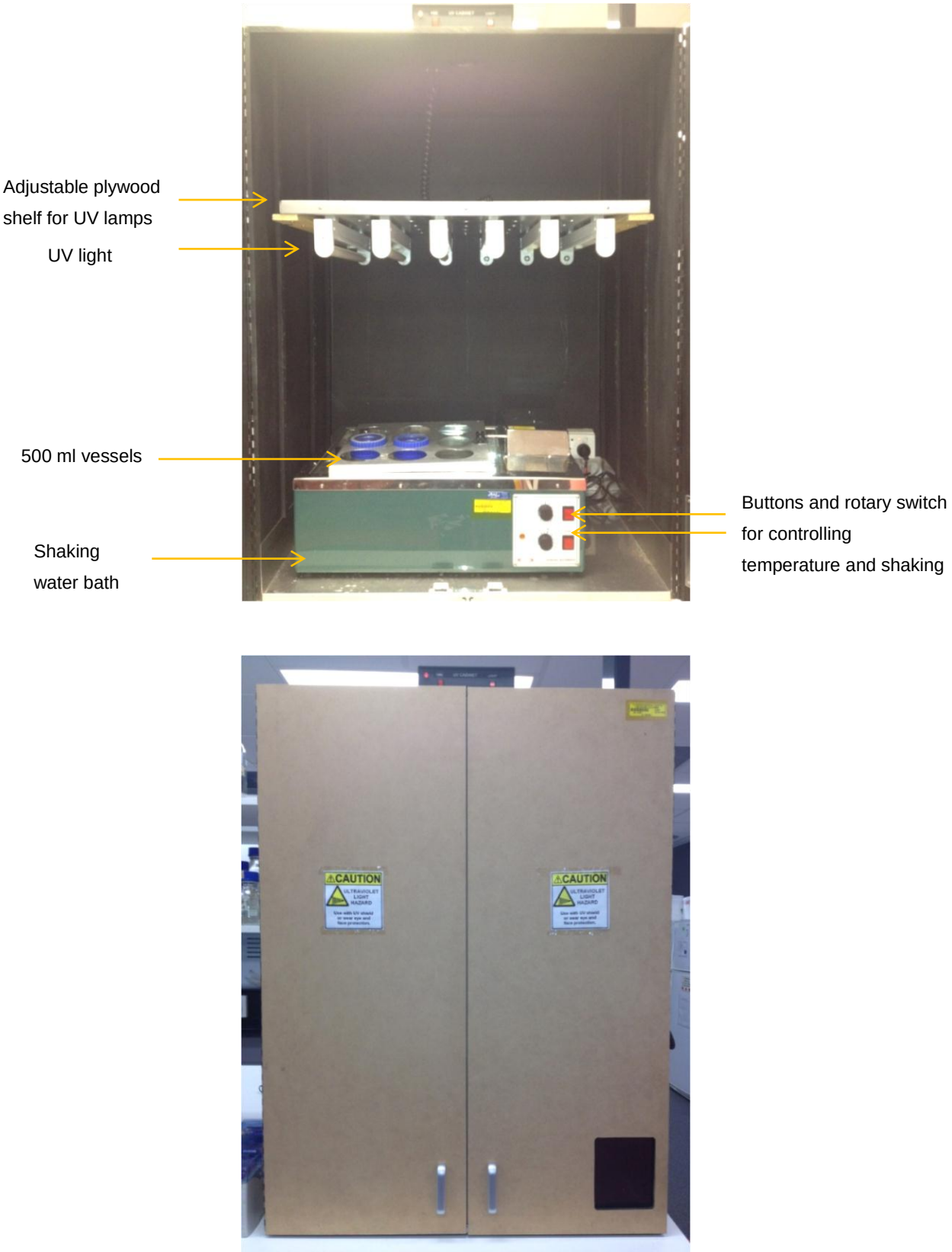


Figure 2–1: Inside and outside the ultraviolet (UV) cabinet

To reach the precise UVB dose rate needed, a database was built before starting the UVB experiment by varying the quantity and the position of the UVB lamps and the vessel positions (Appendix 2).

2.2.2 Design of UVB experiment sampling time

Environmental UVB data were collected over 28 randomly assigned days in 2009 by using spectroradiometers placed on the roof of Flinders Medical Centre (35° 01′ S and 38° 34′ E), Adelaide, South Australia (Bolton, 2011). The integrated UVB dose was logged every three minutes over a day. Data were collected for at least two 'different weather' days each month. The highest UVB dose of 86,490 Jm^{-2} was observed on 14 December 2009 (sunny summer): the lowest UVB dose of 6,126 Jm^{-2} was recorded on 28 July 2009 (cloudy winter). The average daily UVB dose on these 28 random days was 37,973 Jm^{-2}. Two more intermediate or mid-point values were added to allow further analysis. The highest UVB dose rate of 4.04 Wm^{-2} was recorded on 18 December 2009. For better assessment, six ordinal UVB dose rate values between 0 and 6 were chosen: 0.5, 1, 2, 3, 4.5 and 6 Wm^{-2}.

2.2.3 UVB lamps

As described above, six 15 W fluorescent Hg lamps (Sankyo Denki, Japan) were used for UVB incubation.

The sunlight and UVB lamp relative spectral irradiance against wavelength (nm) is shown on Figure 2-2 below. As shown on the figure, a small amount of UVA irradiance is produced by the UVB Hg lamp: this was ignored in the actual experiment as it was relatively very small in terms of both the environmentally relevant amount and its disinfection effect. The exact UVB dose rate radiated by the lamp decayed slowly with long working hours; therefore, to minimise the error, the real UVB dose rate was double-checked each time.

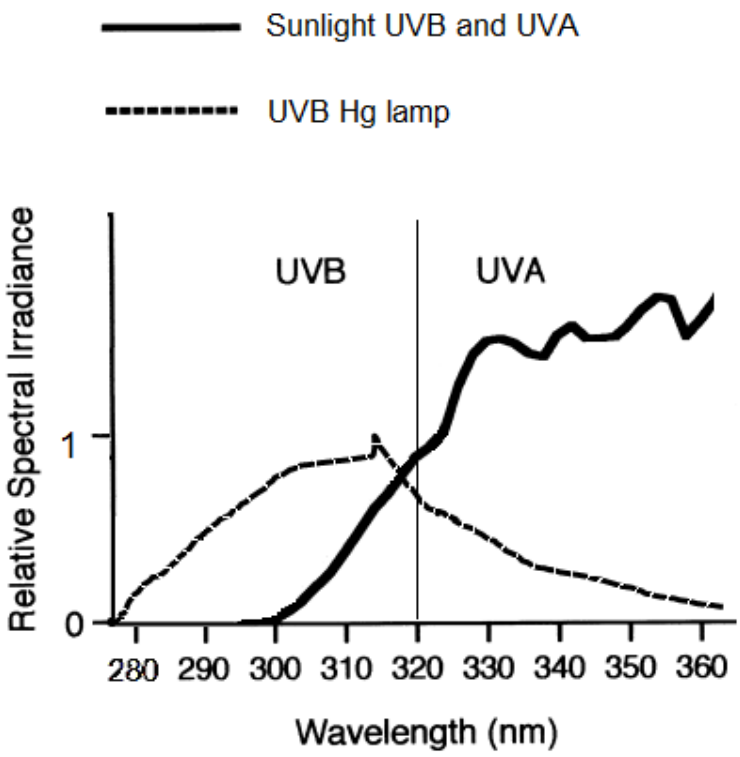


Figure 2–2: Relative spectral irradiance range against wavelength (nm) of sunlight and UVB fluorescent tube (15W, Hg; Sankyo Denki, Japan)

Note: The sunlight data were collected in Sydney, Australia 2001 (2003).

2.2.4 Incubation vessels

For the UVB experiment, nine 500 mL wide-necked vessels (Duran) were modified by inserting quartz windows into the plastic tops. Conducting a simple test indicated that the quartz tops only absorbed around 5% of incident UVB. Empty vessels were sterilised prior to conducting the experiment. For both the UVB and the dark incubation experiments, 300 mL of the suspension was decanted into each vessel.

2.3 Analysis of physical and chemical characteristics of wastewater

2.3.1 Turbidity

Turbidity was measured with a HACH 2100P Turbidimeter (HACH Company, Loveland, Colorado, USA).

2.3.2 Dissolved organic carbon (DOC)

The concentration of dissolved organic carbon (DOC) was measured as total organic carbon (TOC), as analysed using a DOC-5000A (Shimadzu) TOC analyser. For standards, 0–10 mg/L TOC with $KHC_8H_4O_4$ and 0–1 mg/L inorganic carbon (IC) with Na_2CO_3 and $NaHCO_3$ were prepared. This method was described as Test 2540 D by Greenberg et al. (1992).

2.3.3 Chlorophyll *a*

Triplicate 25 mL samples were filtered through glass-fibre filter paper discs (GF/C grade, 1.2 µm pore size, Whatman, UK). Filter papers with chlorophyll *a* were then placed in McCartney bottles with 10 mL of 90% acetone in water (v/v), and incubated in the dark for 48 h at 4°C: 1 mL of acetone extract was centrifuged for five minutes at 10.000g. After reading the optical density of the extract at three wavelengths of 664, 647 and 630 nm, the following equation(Jeffrey & Humphrey, 1975) was applied to obtain the concentration of chlorophyll *a*:

$$\text{Chl. a absorbance} = 11.8511.85\ (OD_{664}) - 1.54\ (OD_{647}) - 0.08\ (OD_{630})$$

Equation 2–1

$$\text{Chl. a}\ (\mu g\ L^{-1}) = \text{chl. a absorbance} \times (\text{volume of acetone (ml)/Sample volume (L)})$$

Equation 2–2

where,

OD_{664} = absorbance at 664 nm
OD_{647} = absorbance at 647 nm
OD_{630} = absorbance at 630 nm.

2.3.4 Suspended solids

Triplicate 200 mL samples were filtered through pre-weighed and dried (105°C,

overnight) glass fibre filter paper discs (GF/C grade, 1.2 µm pore size, Whatman, UK). Filter papers were then dried overnight at 105°C and weighed. Total suspended solids in mg L^{-1} were calculated by difference.

2.4 Statistical analysis

Many different terms and units are used in the literature to describe the die-off rate of micro-organisms in an aquatic environment. The micro-organisms' die-off rate is the most important factor by which to judge the disinfection ability of different methods. At times, the selection of different units for measuring the die-off rate causes misunderstandings and makes valuable results incomparable. The specific terms chosen was discussed in the respective chapters.

The inactivation rates of pathogen indicators were achieved with different models by GInaFiT (Geeraerd et al., 2005). Details about models of GInaFiT were discussed in each chapter. The influence of various factors was checked using two-way analysis of variance (ANOVA) conducted by SPSS: $p \leq 0.05$ was accepted for statistical significance.

CHAPTER 3: TEMPERATURE INFLUENCE ON DARK DIE-OFF RATE OF PATHOGEN INDICATORS IN RAW WASTEWATER

3.1 Introduction

The dark die-off of pathogens in natural wastewater treatment systems is defined as the microbial inactivation process without the participation of light. Due to the proven significant role of sunlight in the disinfection process of natural wastewater systems, the emphasis on sunlight in pond disinfection-related research is well-documented (Craggs, 2004; Curtis, 1992; Ohgaki, 1986). In contrast, little is reported in the literature about the dark die-off of pathogens in wastewater (Bolton et al., 2010; Curtis, 1992; Davies-Colley et al., 1999). Therefore, the factors which influence the dark die-off process remain unclear, although many hypotheses including the effect of temperature have been proposed.

The relative significance of dark disinfection in pond systems should not continue to be ignored. Bolton (2011) measured sunlight attenuation in a wide range of natural water environments in South Australia, including three waste stabilisation ponds (WSPs), three lakes, one wetland and one high-rate algal pond (HRAP). Higher concentrations of chlorophyll *a* and turbidity were found in the WSPs and HRAP, with these leading to more attenuation. In terms of different sunlight wavelength attenuation, UVB > UVA > Vis. For example, in a WSP (typical depth 1.5 m), UVB, the strongest germicidal sunlight wavelength, the euphotic depth (depth at which 1% of surface irradiance penetrates) was only 0.08 m ± 0.07 m. More than two-thirds of the depth of WSPs are kept dark all the time. According Bolton's observation, turbidity was suggested a better predictor of UVB, UVA and Vis attenuation in these water systems than chlorophyll a or SS. Turbidity will likely be a good predictor of attenuation and is a simple parameter to measure in the field. Assessment of its predictive capability with a larger data set is highly desirable

Temperature is regarded as one of the most important factors influencing the performance of high-rate algal ponds (HRAPs). The Kingston on Murray HRAP was constructed in 2008 and has been used to serve a local community of approximately 140 people. 12000 litres of wastewater is pumped from a central pumping station to HRAP six times a day. According to the Bureau of Meteorology climate data recorded in 2012 at Loxton Research Centre weather station close to the HRAP (Buchanan, 2014), the average of daily sunshine hours, as measured by a

Campbell-Stokes recorder for the whole year was 8.2 hours; the shortest average daily sunshine hours of only 5.2 hours occurred in June. Air temperature ranged from 0ºC to 40ºC; at the same time, the HRAP water temperature varied to a lesser extent ranging from approximately 5ºC to 30ºC.

Almost all the equations relating to the WSP design involved temperature, for example, the pond retention time equation presented by Gloyna and Espino (1969) and the BOD_5 loading rates equation reported by Mara and Pearson (1987). More specifically, temperature not surprisingly plays an important role in ponds for several reasons: firstly, it directly affects the chemical and biological processes and, secondly, temperature is generally positively correlated with other important weather factors such as sunlight and wind. However, in some of the literature, the independence of pathogens' dark inactivation rates from temperature was also observed (Auer & Niehaus, 1993; Moeller & Calkins, 1980).

The aim of the current research was:

- To assess the influence of a range of typical environmental temperatures on the dark die-off rates of MS2 and *E. coli* in mixed, unfiltered wastewater.

3.2 Method

3.2.1 Wastewater sample collection

The raw wastewater (5L), containing native strains of *E. coli* and MS2, was collected during an inlet pumping cycle directly from the inlet splitter box of an HRAP located at Kingston-on-Murray (S 34 14', E 140 20'), South Australia (Plate 3.1).

Plate 3–1: High-rate algal pond located at Kingston-on-Murray (S 34 14', E 140 20'), South Australia

3.2.2 Incubation vessels

For the dark incubation experiment, 500 mL, wide-neck glass bottles (Plate 3-2) were selected as the incubation vessels. The vessels were autoclaved (121ºC; 15 min^{-1}) each time before being filled with 300 mL unfiltered wastewater. They were then sealed with quartz glass lids to minimise evaporation, wrapped completely in aluminium foil and incubated in the dark in a thermostatically controlled, shaking water bath at 10ºC, 20ºC or 30ºC for six days. Three replicate vessels were incubated for each temperature. The 80 mm-wide outer diameter of the neck of the bottle allowed the temperature, pH and DO (dissolved oxygen) to be easily measured with probes.

Plate 3–2: Empty 500 mL, wide-neck bottle (Duran)

3.2.3 Temperature, pH and DO

A 970 model portable DO_2 meter (Jenway) and a 370 model Enterprise Portable pH meter (Jenway) were used to measure the temperature, pH and DO of unfiltered wastewater two or three times a day. Both meters were calibrated regularly to maintain accuracy.

3.2.4 Quantification of *E. coli* and MS2 samples

Every 10 hours, *E. coli* samples were quantified; however, MS2 samples were quantified every 24 hours as MS2 was assumed to be more resistant in a dark environment. The quantification methods were described in Chapter 2.

3.2.5 Statistical analysis

After the raw data for *E. coli* and MS2 dark inactivation was collected, Chick's law (Equation 3.1) was initially applied.

$$K\ (h^{-1}) = -\log_{10}\ (N_t/N_0)/t \qquad \textbf{Equation 3-1}$$

Where, K is the inactivation rate constant, N_o is the initial number of organisims $100mL^{-1}$ and N_t, the number $100mL^{-1}$ at time t (h)

The semi $\log_{10}$ plot of $-\log_{10}\ (N_t/N_0)$ versus time did not yield statistically useful relationships from which K (the slope of the line) could be calculated. Consequently, the Gearaerd and Van Impe Inactivation Model Fitting Tool (GInaFiT) was tested and used to calculate the maximum inactivation rate K_i (Geeraerd et al., 2005). Ten different types of microbial survival models, including $\log_{10}$-linear regression, $\log_{10}$-linear + shoulder, $\log_{10}$-linear + tail, $\log_{10}$-linear + shoulder + tail, weibull, weibull, fixed p–parameter, weibull + tail, double weibull, biphasic model and biphasic + shoulder were applied to *E. coli* and MS2 dark inactivation calculation. Finally, $\log_{10}$-linear + shoulder + tail was shown to be the best fit model with the smallest root mean sum of squared errors (RSME).

3.3 Results

3.3.1 pH and DO variation

The pH and DO of mixed raw wastewater were observed to be significantly influenced by temperature over 144 hours' incubation in the dark. The initial unfiltered wastewater was pH = 7.68 and DO = 7.03 mgL^{-1}.

As shown in Figure 3-1, for approximately the first 45 hours, the pH curves of dark incubated, raw wastewater at all three temperatures initially experienced a similar upward trend. After the increase from the initial pH value of 7.68, the pH of incubations at 10ºC reached a peak of approximately 8.75 at 48 hours: it then remained stationary for the rest of the experiment. In contrast, at temperatures of 20ºC and 30ºC, the pH of the wastewater decreased rapidly; after 144 hours of incubation, the pH of the 20ºC and 30ºC groups dropped to the lowest points of 6.14 and 6.17, respectively, presumably due to microbial respiration.

The measurements of DO concentrations in the types of wastewater incubated at 10ºC, 20ºC and 30ºC are shown in Figure 3-2. Wastewater incubated at 10ºC

maintained a relatively constant DO of 10 mgL^{-1} over the duration of the incubation. The DO concentration of the wastewater incubated at 20ºC ranged between 7.0 and 9.0 mg L^{-1}. In marked contrast the 30ºC incubation decreased rapidly from an initial value of 7.0 to a minimum of 3.0 mgL^{-1}.

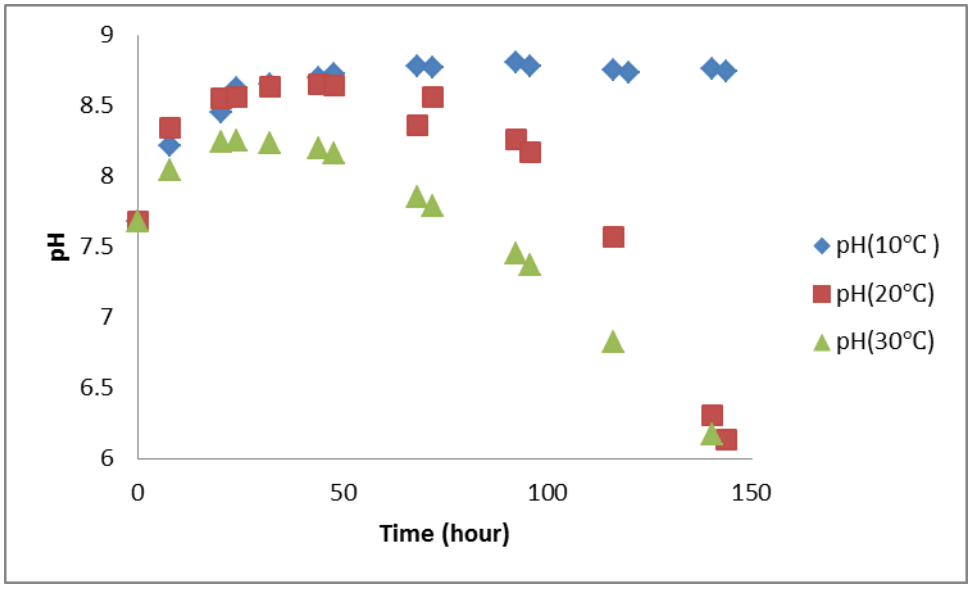


Figure 3-1: pH variations in mixed, dark incubated, raw wastewater at 10ºC, 20ºC and 30ºC (pH measured minimum of twice a day)

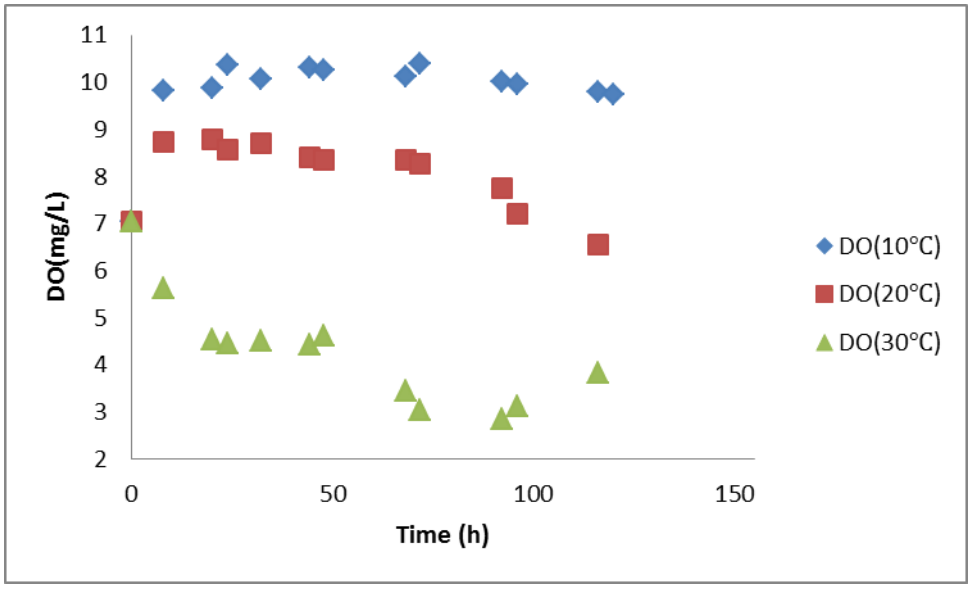


Figure 3-2: DO (mgL^{-1}) variation in mixed, dark incubated, raw wastewater at 10ºC, 20ºC and 30ºC (DO measured minimum twice a day)

3.3.2 Temperature influence on *E. coli* inactivation

The log_{10}-linear + shoulder + tail model (Geeraerd et al., 2005) was applied to the

E. coli concentrations recorded over the duration of the experiment to enable calculation of the inactivation rates at 10ºC, 20ºC and 30ºC (Figures 3-3, 3-4, 3-5). A shoulder (lag) phase in inactivation was observed over the first 70 hours for the 10ºC incubation. Similarly, for the first 30 hours at 20ºC, there was also little inactivation, however, there was no lag phase at 30ºC. The inactivation rate K_i at 20ºC was twice that at 10ºC. While from 20ºC to 30ºC the increase in K_i was less. The dark inactivation rates (K_i) of native strains of *E. coli* were in the order of 30ºC > 20ºC > 10ºC (Table 3-1).

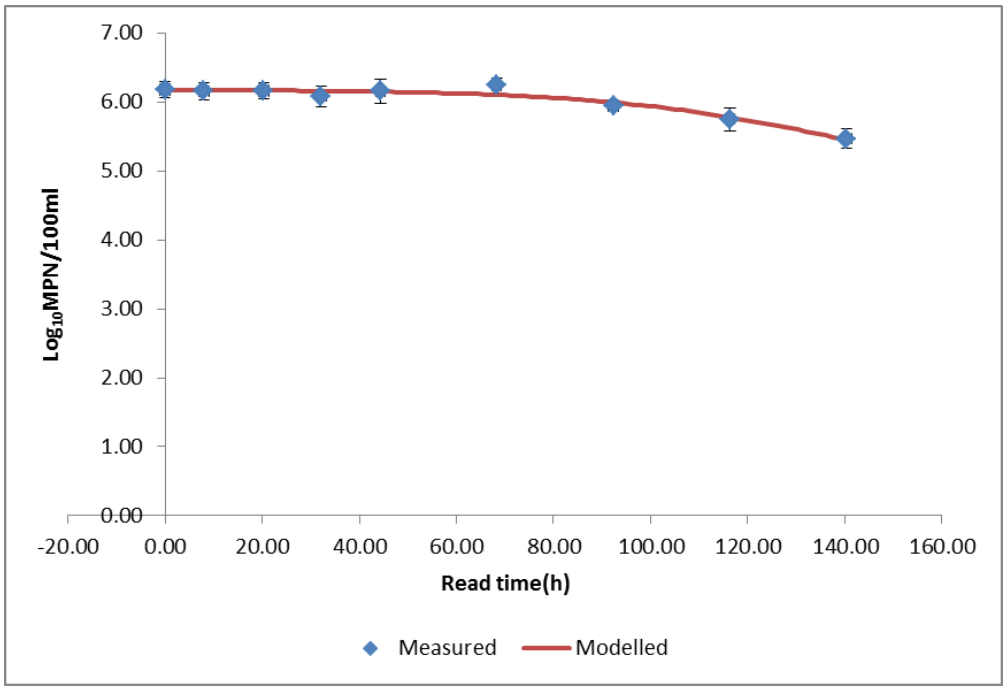


Figure 3-3: Inactivation curve of *E. coli* concentration (mean ± SD $log_{10}MPN100mL^{-1}$) and data modelled using log_{10}-linear + shoulder + tail (Geeraerd et al., 2005) in raw, mixed wastewater incubated in the dark at 10ºC for 144 hours, R^2=0.88

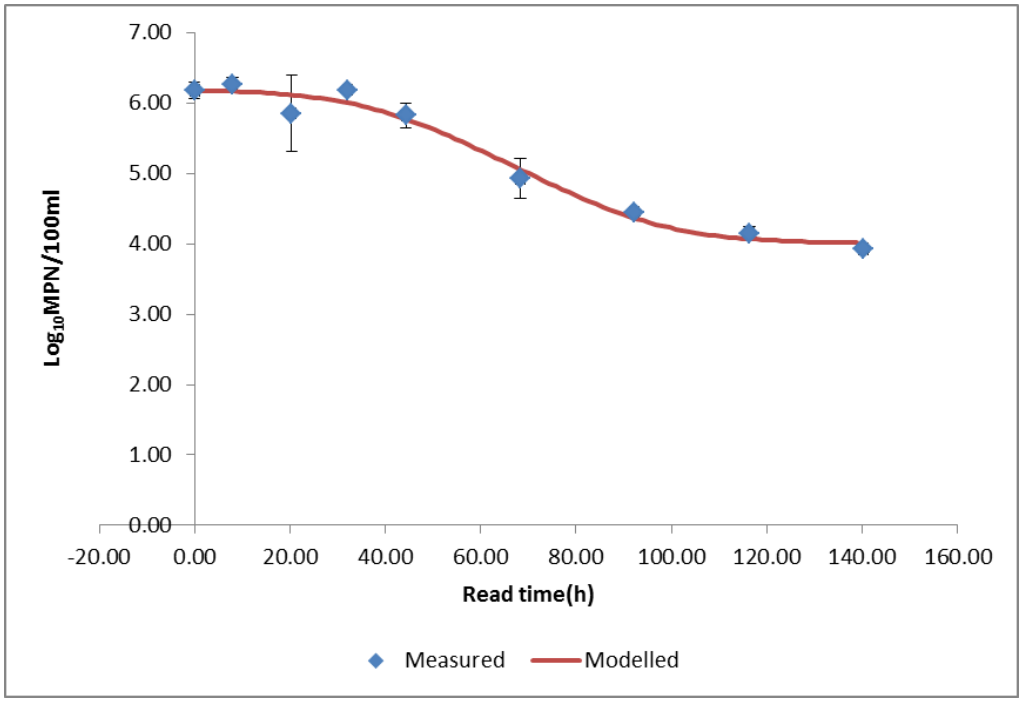


Figure 3-4: Inactivation curve of *E. coli* concentration (mean ± SD $log_{10}MPN100mL^{-1}$) and data modelled using log_{10}-linear + shoulder + tail (Geeraerd et al., 2005) in raw, mixed wastewater incubated in the dark at 20°C for 144 hours, R^2=0.99

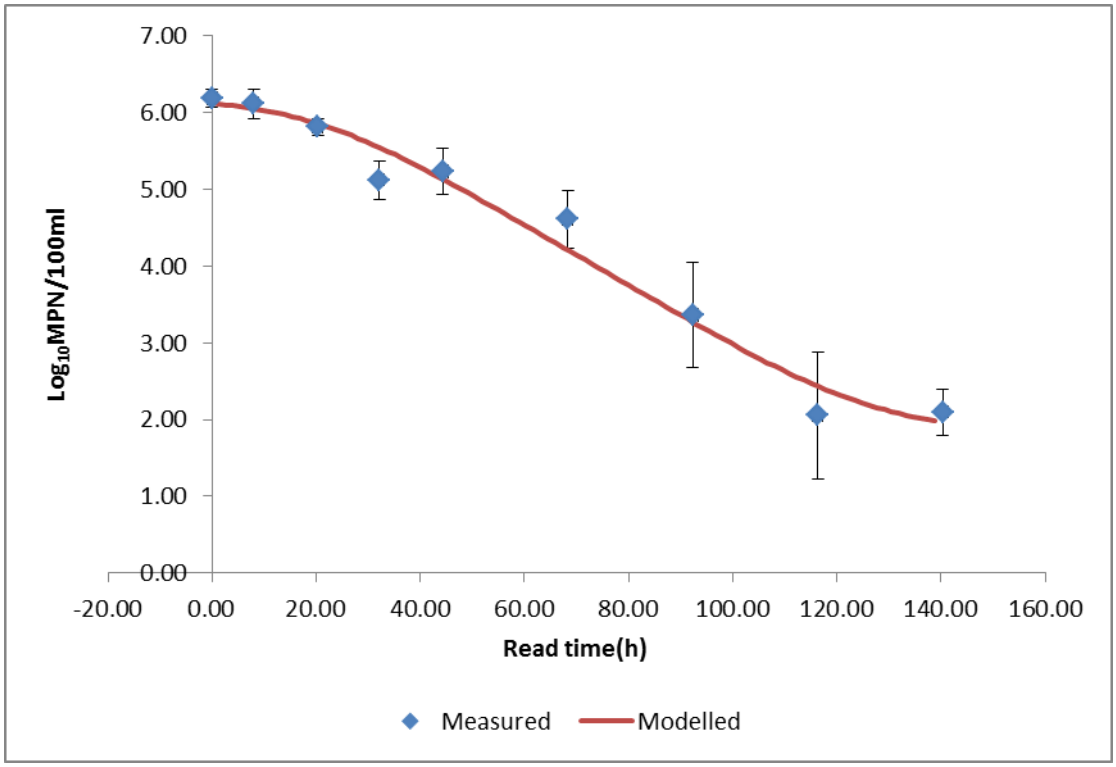


Figure 3-5: Inactivation curve of *E. coli* concentration (mean ± SD $log_{10}MPN100mL^{-1}$) and data modelled using log_{10}-linear + shoulder + tail (Geeraerd et al., 2005) in raw, mixed wastewater incubated in the dark at 30°C for 144 hours, R^2=0.99

Table 3-1: Inactivation rates K_i (h^{-1}; mean ± standard deviation (SD)), number of data points (N) and *p*-value of native strains of *E. coli* in dark incubated, raw mixed wastewater at 10ºC, 20ºC and 30ºC

Temperature	K(h^{-1})	SD	N	R^{2a}	RMSE[a]
10ºC	0.0441	0.0000	27	0.9454	0.0751
20ºC	0.0889	0.0207	27	0.9788	0.1741
30ºC	0.0918	0.0151	27	0.9752	0.3231

[a] The R^2 and RMSE represent the fit level; higher R^2 and low RMSE mean a model with a better fit.

3.3.3 Temperature influence on MS2 (F-RNA coliphage) inactivation

The $\log_{10}$-linear + shoulder + tail model (Geeraerd et al., 2005) was applied to MS2 concentration ($PFU100mL^{-1}$) to calculate dark inactivation rates at 10ºC, 20ºC and 30ºC (Figures 3-6, 3-7and 3-8).

The inactivation rate (K_i) at 20ºC was twice that at 10ºC and approximately 70% of the K_i at 30ºC. The native MS2 (F-RNA coliphage) in the wastewater was at relatively low concentration (~ 10^4 $PFU100mL^{-1}$). Consequently, at the higher temperatures the numbers rapidly decreased to zero, after 100h and 70h incubation in the dark at 20ºC and 30ºC, respectively. The dark inactivation rates (K_i) of native strains of MS2 was in the same order as it was for *E. coli*: 30ºC > 20ºC > 10ºC (Table 3-2).

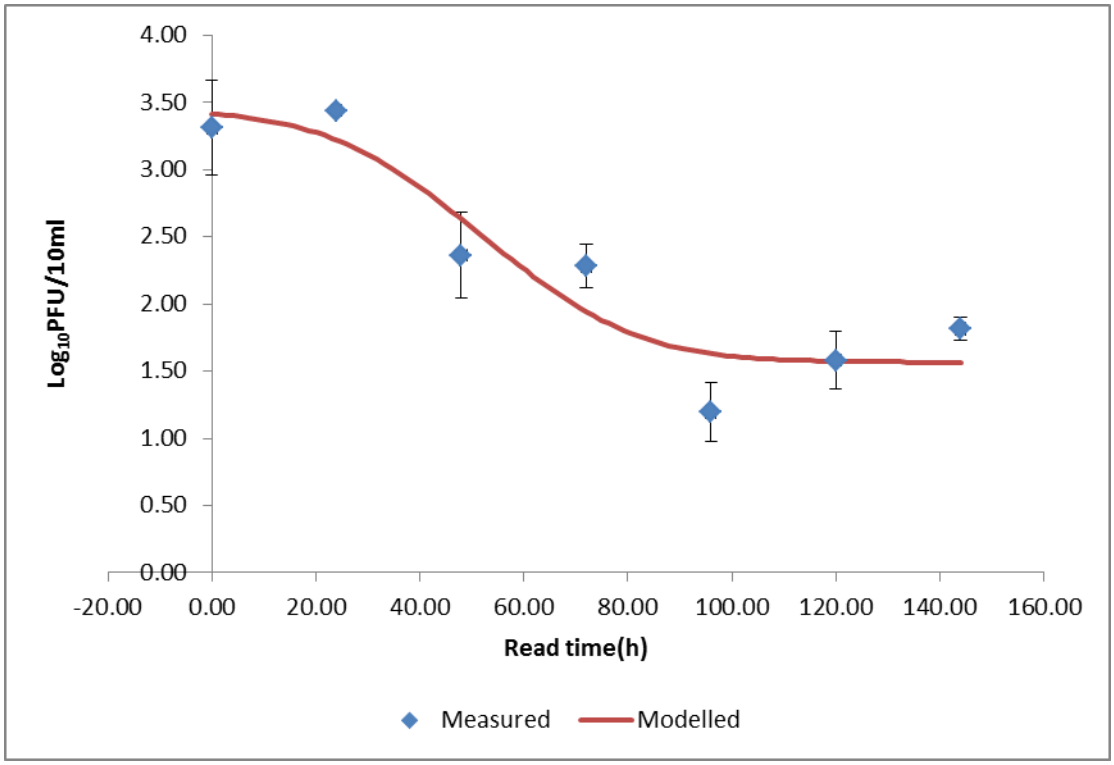


Figure 3-6: Inactivation curve of MS2 (F-RNA coliphage) concentration (mean ± SD $log_{10}PFU100mL^{-1}$) and data modelled using log_{10}-linear + shoulder + tail (Geeraerd et al., 2005) in raw, mixed wastewater incubated in the dark at 10ºC for 144 hours, R^2=0.88

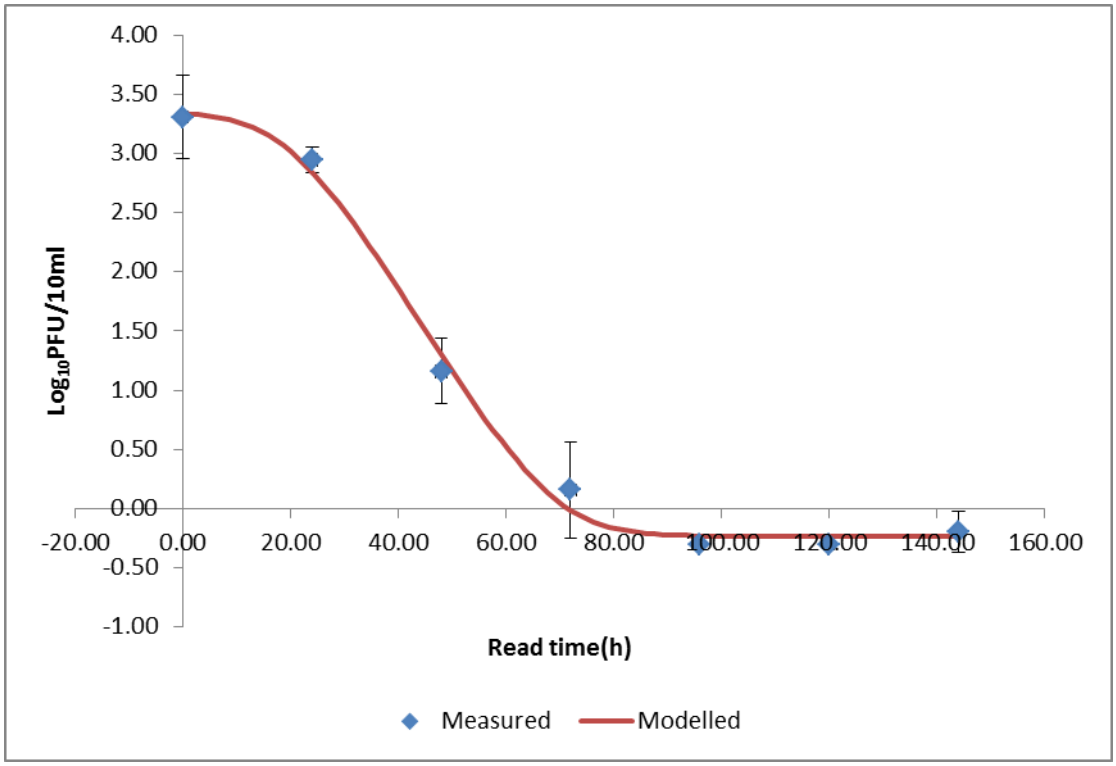


Figure 3-7: Inactivation curve of MS2 (F-RNA coliphage) concentration (mean ± SD $log_{10}PFU100mL^{-1}$); data modelled using log_{10}-linear + shoulder + tail (Geeraerd et al., 2005) in raw, mixed wastewater incubated in the dark at 30ºC for 144 hours, R^2=0.88

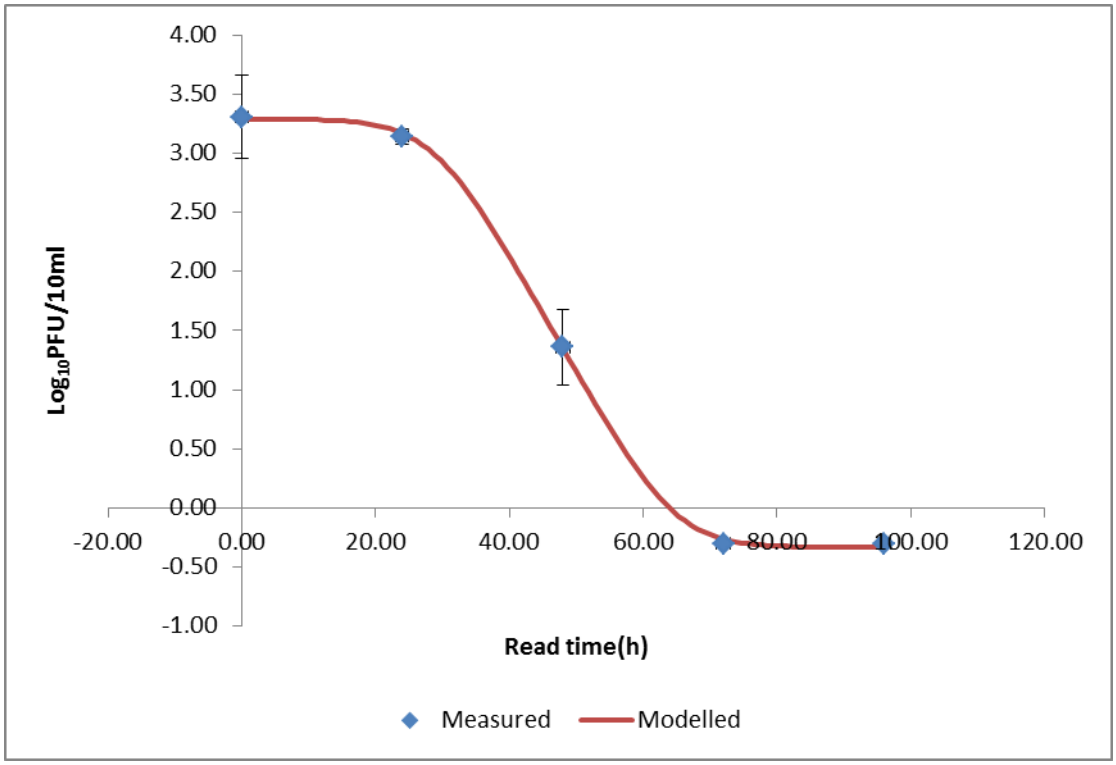


Figure 3-8: Inactivation curve of MS2 (F-RNA coliphage) concentration (mean ± SD $log_{10}PFU100mL^{-1}$) and data modelled using log_{10}-linear + shoulder + tail (Geeraerd et al., 2005) in raw, mixed wastewater incubated in the dark at 30ºC for 144 hours, R^2=0.88.

Table 3-2: Inactivation rates K_i (h^{-1}; mean ± standard deviation (SD)), number of data points (N) and RMSE of native strains of MS2 (F-RNA coliphage) in dark incubated, raw mixed wastewater at 10ºC, 20ºC and 30ºC

Temperature	K(h^{-1})	SD	N	R^{2a}	RMSE[a]
10ºC	0.0904	0.0693	21	0.8844	0.4066
20ºC	0.1639	0.0213	21	0.9950	0.1555
30ºC	0.2330	0.0251	15	0.9997	0.0591

[a] The R^2 and RMSE represent the fit level; higher R^2 and low RMSE mean a model with a better fit.

3.3.4 Comparison of MS2 and *E. coli* dark inactivation rates

As shown in Figure 3-9, the MS2 dark inactivation rates K_i (h^{-1}) in raw wastewater incubated at 10ºC, 20ºC and 30ºC, were all significantly higher than the dark inactivation rate for *E. coli* incubated at the equivalent temperature. The dark inactivation rate of both organisms increased with increasing temperature.

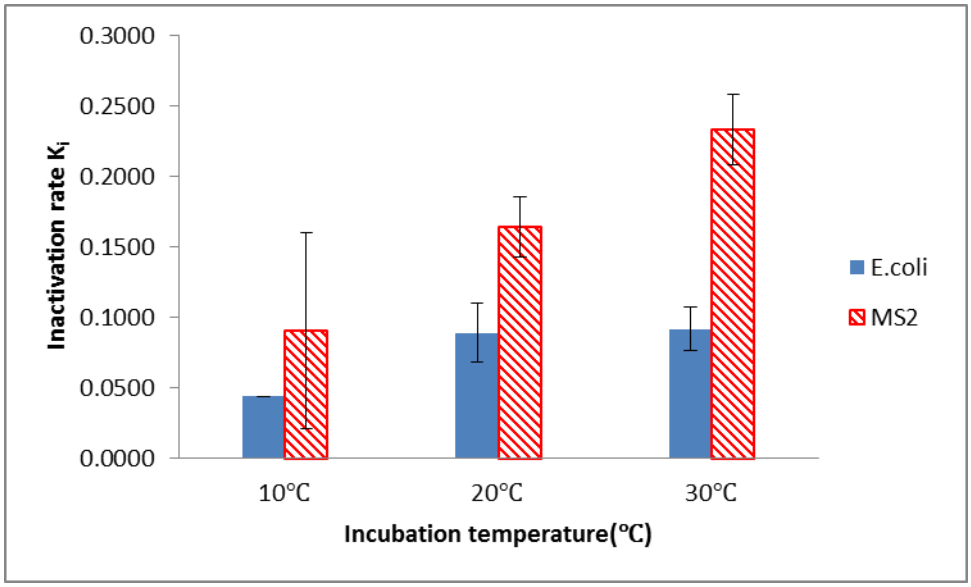


Figure 3-9: Inactivation rates K_i (h^{-1}; mean ± standard deviation) of native strains of MS2 (F-RNA coliphage) and *E. coli* in dark incubated, raw mixed wastewater at 10°C, 20°C and 30°C

3.3.5 Modelling the temperature effect on MS2 and *E. coli* inactivation rate K_i

In order to understand the temperature effect on MS2 and *E. coli* dark inactivation better in mixed raw wastewater, and for further practical application in HRAP as well. Two best-fitting mathematic equations were developed for modelling based on the data setting in current study (Figure 3-9).

Equation 3-2 was found to provide the best fit to describe the relationship between MS2 inactivation Ki and temperature from 10°C to 30°C (Figure 3-10). (R2=0.9997).

$$K_i = 0.0071\,\mathrm{T} + 0.0198 \qquad \text{Equation 3–2}$$

where, K_i is the MS2 inactivation rate constant (h^{-1}); and T is the raw mixed wastewater temperature(°C).

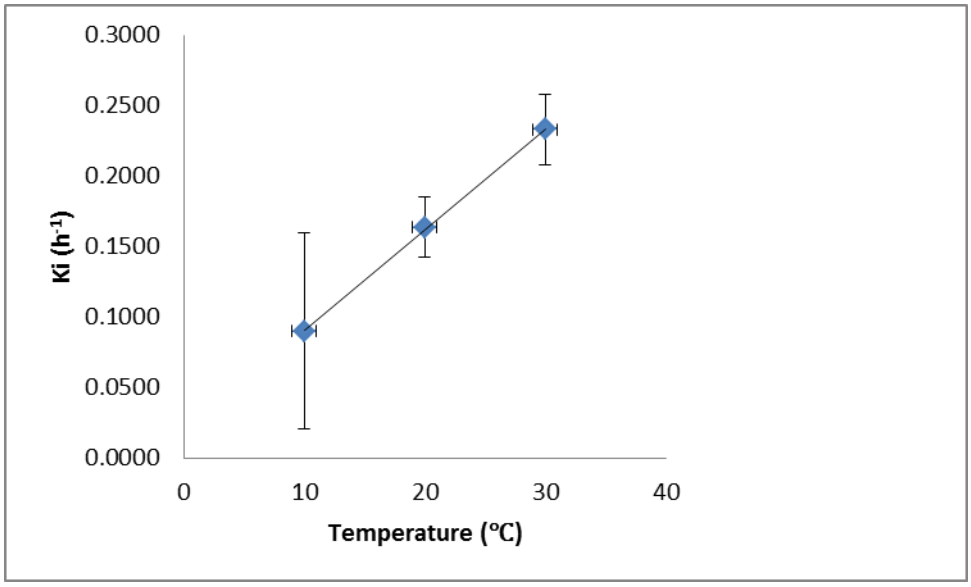


Figure 3-10: Modelling the inactivation rates K_i (h^{-1}; mean ± standard deviation) of native strains of MS2 (F-RNA coliphage) in dark incubated, raw mixed wastewater from 10ºC to 30ºC

In contrast, a $\log_{10}$-linear relationship did not fit the *E. coli* inactivation rate curve well, however, a squared polynomial was found to fit the K_i and temperature relationship (Equation 3-3) with high R^2 value (R^2=1).

$$K_i = -0.002\mathrm{T}^2 + 0.0108\mathrm{T} - 0.0429 \qquad \textbf{Equation 3–3}$$

Where, K_i is the *E. coli* inactivation rate constant (h^{-1}), T is the raw mixed wastewater temperature (ºC).

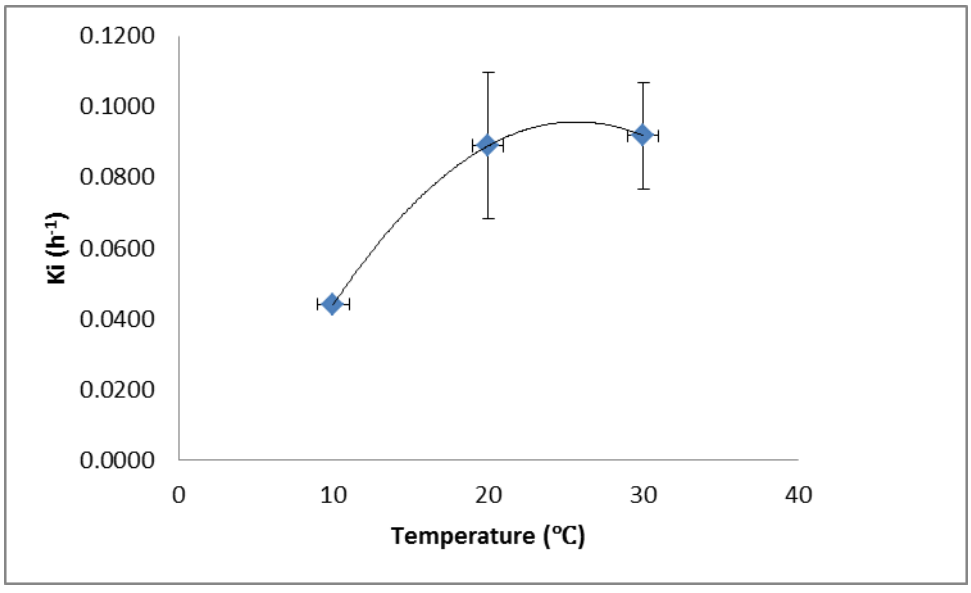


Figure 3-11: Modelling the inactivation rates K_i (h^{-1}; mean ± standard deviation) of native strains of *E. coli* in dark incubated, raw mixed wastewater from10ºC to 30ºC

3.4 Discussion

As can be seen from Figures 3-3 and 3-4, temperature had a significant effect on the pH and DO values of wastewater; during six days of incubation, the pH ranged from 6.14 to 8.81 while DO ranged from 2.85 mgL^{-1} to 10.38 mgL^{-1} over the three temperatures The variation of DO in current study was anticipated since higher temperature enhances the oxygen consumption of bacteria and degradation of organic matter. In WSP system pH is temporally and spatially variable (Shilton, 2005a). The pH in dark raw wastewater is influenced by the respiration of the biota, which as demonstrated here is temperature dependent, producing CO_2 which decreases the pH of the wastewater. The pH and DO concentrations experience diurnal variation in WSPs and HRAPs mediated by algal photosynthesis in the daytime and respiration at night.

However, the possibility that pH and DO directly influence micro-organisms' dark inactivation was not a focus of this study. Both these two parameters experienced a significant variation during the 6 day incubations; however, no statistical relationship was identified between the DO and pH changes and *E. coli* or MS2 inactivation. A similar conclusion was made for a high-rate algal pond (HRAP) by Craggs (2004), who recorded pH and DO changes (pH 8.0–9.2; DO 0–22 mgL^{-1}) over a four-day period in an HRAP in 2000, DO and pH appeared to have little impact on the inactivation rate of *E. coli*. Sethi (2009) and Bolton (2011) conducted a series of lab-based UV inactivation experiments with their control experiments (dark) of buffered water at three different pH levels (7.5, 8.5 and 9.5) and at two different DO levels (< 1 mgL^{-1} versus 8.5 mgL^{-1}). Both concluded that *E. coli* dark inactivation was independent of pH and DO. In contrast, Roslev et al. (2004) concluded that over 35 days' incubation of drinking water, DO (< 0.01% versus 48%) was a major factor influencing the survival of *E. coli.*

As indicated on Figure 3-9, an increase in temperature from 10ºC to 30ºC had a positive effect on *E. coli* and MS2 dark inactivation rates in mixed raw wastewater. A similar phenomenon was reported in the literature. For example, in experiments conducted by Craig et al. (2004), the temperatures were set at the same levels as in the current study. During an incubation period of 28 days, *E. coli* were able to survive longer in recreational coastal water at a lower temperature. Even in a 260 days' long-term experiment, the survival of *E. coli* was 4ºC > 15ºC > 25ºC > 37ºC in river water (Flint, 1987). Feng et al(2003) tested the MS2 inactivation at a range of temperatures (5-35ºC) in a range of buffered water, potassium hydrogen (KH)

phthalate/HCl for pH 3–4, KH phthalate / NaOH for pH 5, KH_2PO_4 / NaOH for pH 6–8, Tris (hydroxymethyl) – aminomethane / HCl for pH 9, and $NaHCO_3$ / NaOH for pH 10–11. They found generally when temperature increased, the MS2 inactivation rate rose, especially at the extreme pH value. The only exception happened at pH 6, when the MS2 was inactivated faster at 15ºC than 25ºC and 35ºC.

In contrast to MS2 (f-RNA coliphage), the *E. coli* inactivation curves had an obvious shoulder (lag) stage at the beginning of incubations. Nutrients and temperature are recognised as important factors for micro-organisms' survival in aquatic environments (Bissonnette et al., 1975; Mancini, 1978), the lag phase of *E. coli* inactivation reported here at 10ºC and 20ºC was considered mostly likely due to nutrient availability within the raw wastewater.

At temperatures of 10ºC, 20ºC and 30ºC, native strains of *E. coli* were found to survive better than MS2 during six days' incubation in the dark in mixed raw wastewater. In contrast, MS2 (F-RNA coliphage) has been shown to be more resistant than *E. coli* in other aquatic environments, regardless of temperature, such as in deionised (DI) water (Theitler et al., 2012); in bottled water under natural sunlight (Fisher et al., 2012; Theitler et al., 2012); and in buffered water under UV lamps (Bolton, 2011; Sethi, 2009). The better survival of *E. coli* compared with *MS2* (F-RNA coliphage) reported here may be nutrient availability in raw wastewater enabling *E. coli* survival and potentially re-growth. A further explanation maybe a difference in behaviour between wild-type strains used here compared with laboratory strain pathogens used in other studies (Roslev et al., 2004).

Two equations (Equations 3-2 and 3-3) were developed to understand and model the effect of temperature on dark inactivation rates of native strains of *E. coli* and MS2 in mixed, raw wastewater. Table 3-4 was generated to enable comparison of the results presented here with those published in the literature for a range of water matrices and incubation conditions. Whether the water medium was mixed is associated with the potential for sedimentation which has been shown to contribute to pathogen indicator's inactivation in dark experiments (Craig et al., 2001; Davies et al., 1995). Furthermore, the source of the indicator may influence the outcome (Fisher et al., 2012; Flint, 1987).

Table 3-3: Dark inactivation rates K_i (h^{-1}) of MS2 (F-RNA coliphage) and *E. coli* in the literature

Water resource	Mixed or not	Pathogen indicators	Temperature (ºC)	Inactivation rates (h^{-1}) & duration of incubation	Reference
HRAP water	Y	Native *E. coli*	10	0.0441* 6d	Yu Lian (2015, this study)
			20	0.0889* 6d	
			30	0.0918* 6d	
HRAP water	Y	Native MS2	10	0.0904* 6d	Yu Lian (2015, this study)
			20	0.1639* 6d	
			30	0.2330* 6d	
HRAP water	Y	Native *E. coli*	2	0.006[a] * 56d	(Buchanan et al., 2011b)
			22	0.052[a]	
Coastal water	N	Lab strain *E. coli*	10	0.043[b] * 28d	(Craig et al., 2004)
			20	0.043[b] * 28d	
			30	0.089[b] * 28d	
Unfiltered river water	N	Lab strain *E. coli*	4	0.040[c] * 6d	(Flint, 1987)
			15	0.048[c] * 6d	
			25	0.062[c] * 6d	
			37	0.139[c] * 6d	
Buffer solutions	N	Lab strain MS2	5	0.0025[d]	(Feng, 2003)
			15	0.005[d]	
			25	0.017[d]	
			35	0.029[d]	

Firstly, in Table 3-4, *E. coli* inactivation rate values at temperatures from 10 to 30ºC are almost all located within in the model (Eq 3-3). In literature, in HRAP wastewater at 22ºC *E. coli* the K_i was 0.052 h^{-1} (Buchanan (2011b) which was data obtained as

follows:

[a & c] The inactivation rates was transferred from K (d^{-1})

[b] The inactivation rates was transferred from T90, k=1/T90

[d] The inactivation rates was deduced from figure, pH=9

from wastewater from the same HRAP.

In addition, in coastal water from 10ºC to 30ºC, inactivation rates ranged from 0.043 to 0.089, the K_i values at 10ºC and 30ºC were extremely close to the squared polynomial model range. However, at 20ºC, in costal water (Craig et al., 2004) the *E. coli* inactivation rate was much slower than in HRAP wastewater. Similarly, in Flint's study, *E. coli* was incubated at four temperatures for 28 day, at 15ºC and 25ºC the *E. coli* inactivation rate was almost within the model range (Flint, 1987).

The MS2 dark inactivation rate from the only related paper was not close to current MS2 model. In Feng's data (Feng, 2003), when the temperature increased from 5 to 35ºC, the MS2 inactivation rates in buffered water were generally lower than rates reported here in HRAP wastewater.

3.5 Conclusions

The following conclusions can be drawn, in dark raw mixed wastewater:

- Log_{10}-linear+shoulder+tail was found to be the best model for determination of MS2 and *E. coli dark* inactivation using GInaFiT (Geeraerd et al., 2005).
- Incubated in the dark in sealed vessels, an increase in temperature from 10ºC to 30ºC significantly decreased the pH and DO concentration of the wastewater, however, no influence of pH and DO on *E. coli* and MS2 inactivation rate was identified.
- The inactivation rates of MS2 and *E. coli* increased significantly with increases in temperature from 10ºC to 30ºC ($p < 0.0001$).
- At temperatures of 10ºC, 20ºC and 30ºC, native strains of *E. coli* were found to survive better than MS2.
- Two equations were developed to model the temperature effect on MS2 and *E. coli* dark inactivation from 10ºC to 30ºC and the equations were partly validated by the related literature.

CHAPTER 4: UVB DOSE AND TEMPERATURE DEPENDENCE OF MS2 AND *E. coli* UVB INACTIVATION IN BUFFERED (pH 7.5) REVERSE OSMOSIS (RO) WATER

4.1 Introduction

Sunlight potentially provides a cost-effective, highly accessible and sustainable natural disinfection method for humanity. These unique advantages have attracted considerable research activity seeking to understand the mechanism and take better advantage of the resource, for example, drinking water purification, titanium dioxide, solar water disinfection (SODIS) (Fisher et al., 2012; Hashimoto et al., 2005; Mbonimpa et al., 2012; McGuigan et al., 1998) and wastewater treatment including wetlands, WSPs and HRAPs (Bolton et al., 2011; Bolton et al., 2010; Craggs, 2004; Diamond et al., 2005; Ohgaki, 1986). The high-rate algal pond (HRAP) is one of these popular wastewater treatment areas (Craggs, 2004).

The UVB (280–315 nm) wavelength was selected as a focus for this study as UVB has been recognised as the primary germicidal component in sunlight that reaches the Earth's surface.

In order to avoid confusing different terms used in the UV research literature, for example, irradiance, intensity, influence and insolation, the following explanations are provided. In this thesis, UV dose (Jm^{-2}) is defined as the dose rate (Wm^{-2}) multiplied by time as in the following equation:

$$\text{UV dose } (Jm^{-2}) = \text{UV dose rate } (Wm^{-2}) \times \text{Time (s)} \qquad \textbf{Equation 4–1}$$

The Bunsen–Roscoe (1857) reciprocity law states that a photo-biological effect is proportional to the total energy dose and is independent of how that dose is delivered, that is, the combination of intensity and exposure time. The outcome of UV irradiation on biological systems, however, also depends on other interactions, for example, the presence of photosensitisers which may modulate the response. However, it is recognised that the influence of how the total energy dose is delivered, the exposure–intensity relationship, on the biological effect—such as inactivation—needs to be determined in individual cases. Sommer et al. (1996), for instance, reported that under the same UVC energy dose (Jm^{-2}), short exposure times were less effective in killing micro-organisms. Many authors report the UV dose as the

sole descriptive parameter (Chang, 1985; Hijnen et al., 2006; Thompson et al., 2003; Thurston-Enriquez et al., 2003; USEPA, 1996). This appears to ignore the influence of how that UV dose is delivered. Furthermore, the influence of ambient temperature is also frequently ignored.

The UV dose may not be the only important parameter that affects the disinfection outcome: many studies have been conducted, especially for the UVC disinfection process for drinking water or the food industry, to explore the effect of how the dose is delivered (Murakami et al., 2006; Sommer et al., 1998). The concept may be considered similar to the concentration–contact time concept in chemical water disinfection. In this context, the outcome on the disinfection process is compared in relation to achieving the same UV dose using different combinations of UV dose rates and exposure time. This approach has not been studied with regard to the impact of natural UVB irradiation on disinfection of wastewater and is the subject of research reported in this chapter.

UV irradiances have been shown to interact with many environmental factors such as the environment, pH and DO (Kadir & Nelson, 2014): among them, temperature was recognised as one of the most important factors (Chan & Killick, 1995; Roos et al., 1998; Šolić & Krstulović, 1992; Theitler et al., 2012). However, there is a paucity of information regarding the interaction between UVB and temperature on the outcome of inactivation of *E. coli* and MS2 in different types of wastewater.

The objectives of this study were to:

- Determine the influence of UVB dose (Jm^{-2}), dose rate (Wm^{-2}) and exposure time and incubation temperature on the inactivation of *E. coli* and MS2 in optically clear water.
- Create a database for modelling disinfection effect of UVB irradiance in further practical application, like in HRAPs.

All these objectives offer the potential to better understand natural UVB disinfection and, consequently, to improve the design, operation and management of HRAPs.

4.2 Method

4.2.1 Preparation of buffered matrix

Buffered water (Appendix 1) (pH=7.5 tris (hydroxymethyl) aminomethane (Tris HCl)

was prepared for the liquid medium for all experiments conducted in this chapter. Briefly, 1 L pH 7.5 buffered reverse osmosis (RO) MilliQ water was made by adding 250 mL 0.2 M tris (hydroxymthyl) aminomethane (Sigma) and 200 mL 0.2 M HCl, making the volume up to 1 L with sterile RO water.

4.2.2 UVB irradiation

UVB irradiation was delivered using 15W fluorescent Hg lamps (Sankyo Denki, Japan) in the UV cabinet described in Chapter 2. The UVB dose rate (Wm^{-2}) was measured using a Photometer/Radiometer PMA2100 (Solar Light Company, Inc, California).

The UVB dose rates were achieved by varying both the number of the six available UVB lamps and the height of the lamps above the nine available positions for the incubation vessels in the temperature-controlled water bath. The combinations required to achieve the six specific UVB dose rates used in the study were predetermined (Appendix 2). As the intensity and spectral quality of the lamps decay over time, the experimental UVB dose rate was confirmed by measurement before the commencement of each incubation.

4.2.3 Stock preparation and quantification of MS2 and *E. coli*

Details of MS2 stock (ACTT#15597-B1) and *E. coli* stock (ATCC # 19434) preparation and quantification were described in Chapter 2. Briefly, MS2 stock was made by incubating 5 mL tryptone water over an agar plate containing numerous MS2 plaques. The double layer method was applied to quantify MS2. The *E. coli* stock was inoculated and incubated in nutrient broth overnight. The chromogenic substrate Colilert™ method was applied for quantification. When it was necessary, a serial, 10-fold dilution in 9 ml 0.5% tryptone water was applied for both *E. coli* and MS2.

4.2.4 Experimental design

As described in Chapter 2, according to (Bolton, 2011) one-year environmental UVB data record in South Australia, the UVB dose ranged from 6126 Jm^{-2} to 86490 Jm^{-2}. The average daily UVB dose was 37673 Jm^{-2}. The dose rates ranged from 0 Wm^{-2} to 4.04 Wm^{-2}.

For the MS2 inactivation experiments, six ordinal UVB dose rates of 0.5, 1, 2, 3, 4.5

and 6 Wm^{-2}, and UVB doses of 6126, 22049, 39973, 62232 and 86490 Jm^{-2} were chosen: for *E. coli* experiments, UVB dose rates of 0.5, 1, 2, 3 and 4.5 Wm^{-2} and UVB doses of 6126, 22049, 39973, 62232 and 86490 Jm^{-2} were chosen. The duration of the incubation to achieve the UVB dose at various dose rates was calculated (Table 4-2). Dark incubated controls at the respective incubation temperatures were also included in the study.

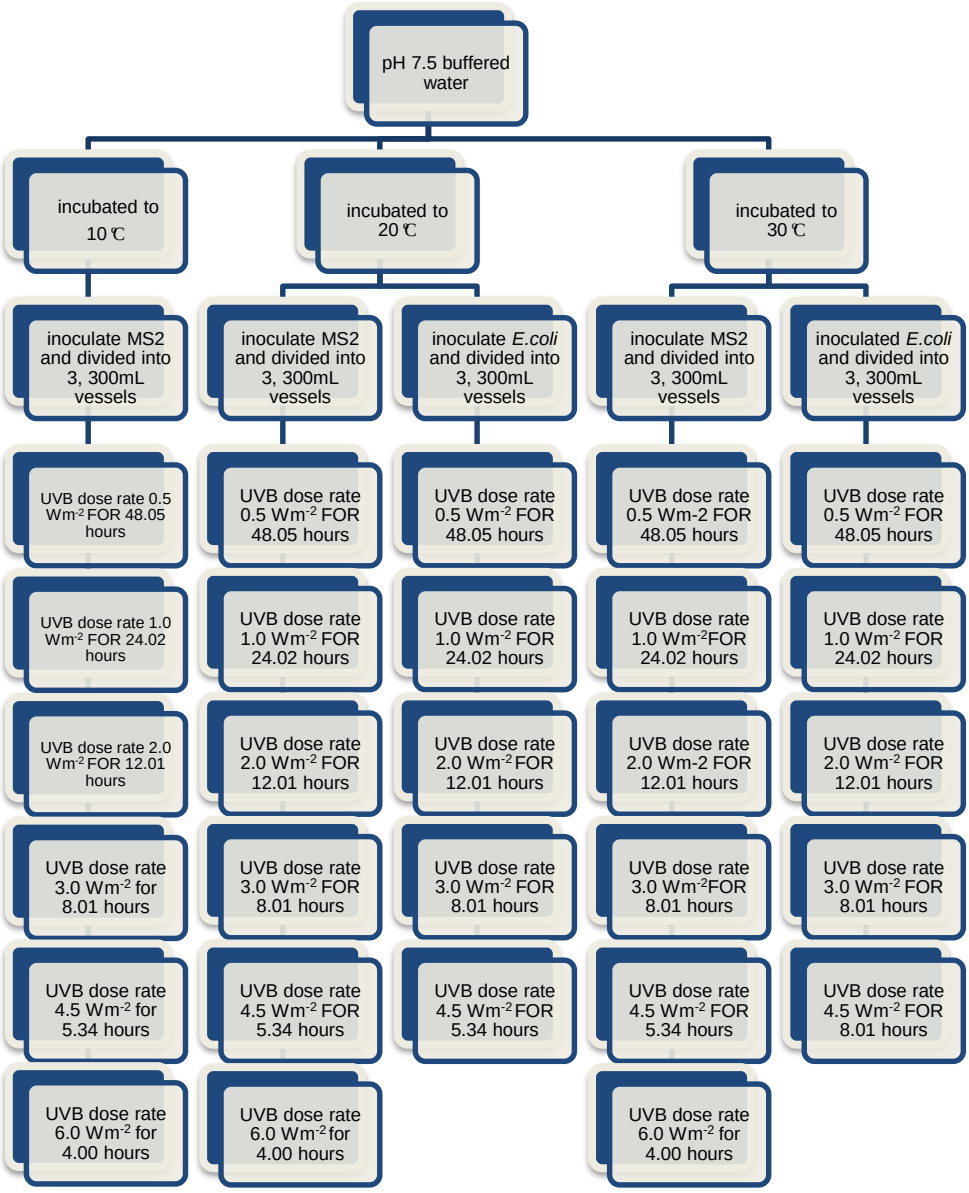


Figure 4-1: Flow chart showing the combination of temperature, dose rate (Wm^{-2}) and time (h) to achieve the required dose (Jm^{-2}) for the UVB inactivation experiments for both MS2 and *E. coli*

Table 4-1: Sampling timetable using six ordinal, environmentally relevant UVB dose rates (Wm^{-2}) to achieve five UVB doses (Jm^{-2}) to determine MS2–UVB inactivation Note: UVB inactivation of *E. coli* shared the same sampling timetable except for the 6.0 Wm^{-2} dose rate which was excluded from that study.

Dose rate (Wm^{-2})	1st sampling time (h)	Dose received (Jm^{-2})	2nd sampling time (h)	Dose received (Jm^{-2})	3rd sampling time (h)	Dose received (Jm^{-2})	4th sampling time (h)	Dose received (Jm^{-2})	5th sampling time (h)	Dose received (Jm^{-2})
0.5	3.40	6,126	12.25	22,049	21.10	39,973	34.57	62,232	48.05	86,490
1	1.70	6,126	6.12	22,049	10.55	37,973	17.29	62,232	24.02	86,490
2	0.85	6,126	3.06	22,049	5.27	37,973	8.64	62,232	12.01	86,490
3	0.57	6,126	2.04	22,049	3.52	37,973	5.76	62,232	8.01	86,490
4.5	0.38	6,126	1.36	22,049	2.34	37,973	3.84	62,232	5.34	86,490
6.0	0.28	6,126	1.02	22,049	1.76	37,973	2.88	62,232	4.00	86,490

4.2.5 Statistical analysis

As the application of Chick's Law to calculate the inactivation rate *K* was clearly not yielding the best statistical 'fit', the Geeraerd and Van Impe Inactivation Model Fitting Tool (GInaFiT) was examined and used to calculate the maximum inactivation rate K_i for the UVB inactivation experiment in this study (Geeraerd et al., 2005). Figure 4-1 shows the outcome of using both Chick's Law (first-order kinetics) and GInaFiT on the model (log_{10}-linear + tail) applied to the same data. GInaFiT provides 10 different types of microbial survival models and has been successfully used to fit *E. coli* solar disinfection (SODIS) inactivation results (Berney et al., 2006). Ten different models available within GinaFiT were applied to each experimentally derived inactivation curve, with the best fit model being the one with the smallest root mean sum of squared errors (RSME). Eventually, log_{10}-linear + tail (Appendix 3) was found to have the best fit model for most MS2 inactivation curves under UVB, while the log-linear model was the best fit for MS2 dark control groups, as they demonstrated neither a shoulder nor a tail. In both these cases, a comparable K_i value was obtained.

The log_{10} reduction value (LRV) was used in the analysis of the relationship between UVB dose (Jm^{-2}) and dose rate (Wm^{-2}). The LRV was calculated for each sampling time point to describe the reduction in numbers of either *E. coli* or MS2 with incubation time (Equation 4-2).

$$\mathrm{LRV} = log_{10}\frac{N_t}{N_0} \qquad \textbf{Equation 4–2}$$

where, N_t is the number of pathogen indicators at time t; and N_0 is the number of pathogen indicators at time 0.

Two-way analysis of variance (ANOVA) was applied to the data to determine the significance of dose, dose rate and temperature on MS2 and *E. coli* inactivation rates.

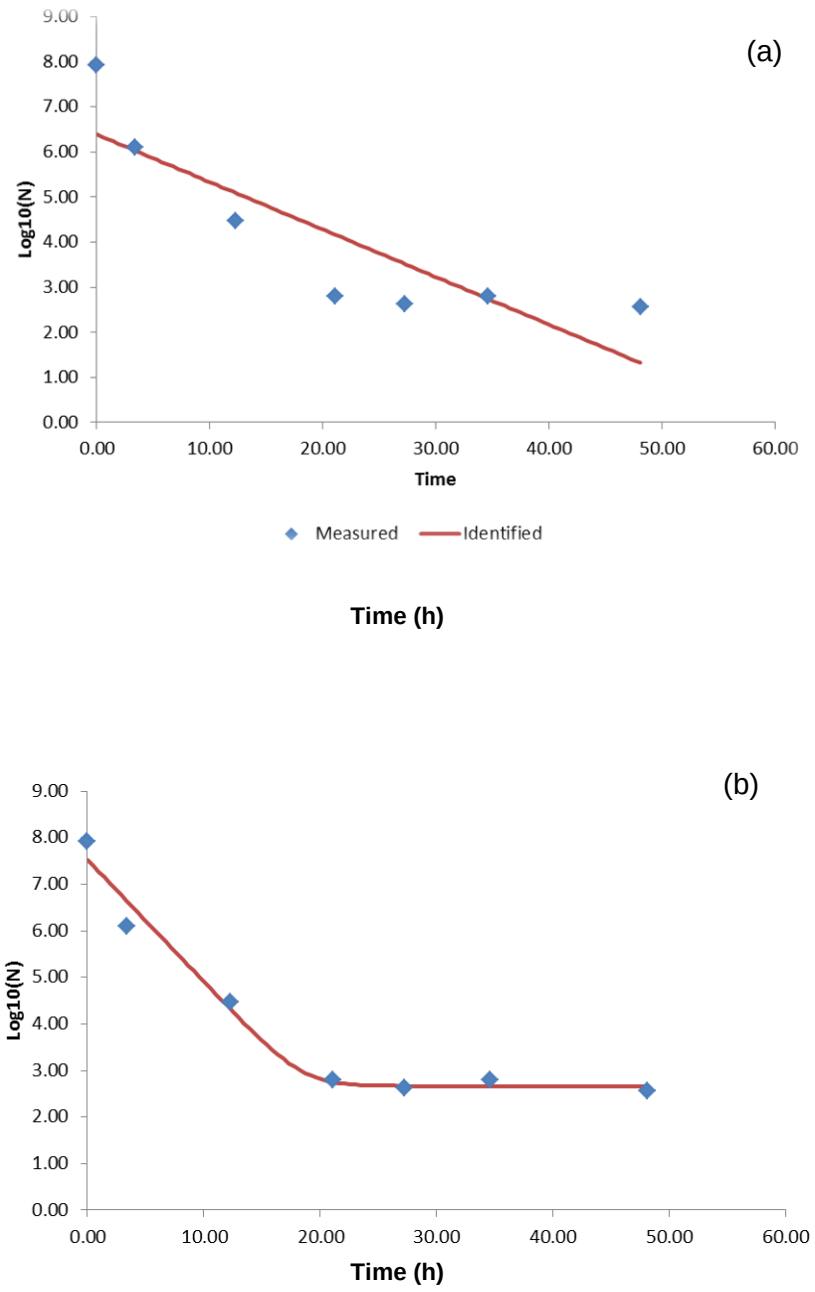


Figure 4-2: Same data set (*E. coli* incubated at 20°C at a UVB dose rate=0.5 Wm^{-2}) modelled using (a) first-order, log_{10}-linear model and (b) log_{10}-linear + tail using GInaFiT (Geeraerd et al., 2005)

4.3 Results

4.3.1 Temperature effects on MS2 UVB inactivation

This study explored the effect of three environmentally relevant temperatures on the MS2 inactivation rate in pH 7.5 buffered RO water under a range of UVB dose rates (Wm^{-2}). The exposure times at specific UVB dose rates were calculated to maintain a constant dose (Jm^{-2}) both at each sampling time and at the end of the incubation at each dose rate.

Dark inactivation of MS2 over the 48-hour incubation period was minimal at all three incubation temperatures (Table 4-4). There was no statistically significant difference (two-way ANOVA) in MS2 inactivation rate (K_i) at 10°C, 20°C and 30°C in the dark control incubations.

The MS2 UVB inactivation rate constants (K_i) for UVB dose rates ranging from 0.5–6.0 Wm^{-2} at 10°C, 20°C and 30°C are shown in Table 4-3. The highest K_i was 3.71 h^{-1} at 30°C, 6 Wm^{-2}, while the lowest K_i was 0.06 h^{-1} at 10°C, 0.5 Wm^{-2}. The MS2 inactivation rates K_i at 30°C were significantly higher than those recorded at 10°C ($p<0.001$). A similar statistically significant difference was also observed between K_i at 20°C and 30°C ($p<0.001$). However, no statistically significant difference in K_i was identified between 10°C and 20°C ($p=0.179>0.05$).

Preliminary analysis showed that a linear approximation gave a poor relationship between the measured and modelled values. Consequently, growth curve analysis (Mirman, 2014) was used to model the data on the effect of temperature on K_i on UVB-induced inactivation of MS2 at dose rates from 0.5 Wm^{-2} to 6 Wm^{-2}. The interaction between K_i and dose rate was modelled using a third-order (cubic) orthogonal polynomial for water temperature 10°C, 20°C and 30°C (Figure 4-3; Equations 4-3, 4-4 and 4-5).

Generally, at all incubation temperatures, the MS2 inactivation rates K_i increased significantly ($p<0.001$) as the UVB dose rates increased from 0.5 Wm^{-2} to 6 Wm^{-2}. However, the K_i appeared to plateau between dose rates of 4.5 Wm^{-2} to 6 Wm^{-2} at 10°C and 20°C: at 10°C, the K_i at 4.5 Wm^{-2} was higher than K_i at 6 Wm^{-2} ($p=0.375>0.05$).

Table 4-2: MS2 inactivation rates K_i (h^{-1}) in buffered pH 7.5 RO water at UVB dose rates of 0.5, 1, 2, 3, 4.5 and 6 Wm^{-2} at 10ºC, 20ºC and 30ºC

Notes: K_i(h^{-1}); mean ± SD) was obtained from a log_{10}-linear + tail model using the GInaFiT model (Geeraerd et al., 2005) for which the individual R^2 values are shown together with n, the number of data points included in the model.

Temperature (℃)	UVB dose rate (Wm^{-2})	Incubation time (h)	K_i (h^{-1})	SD	R^2	n
10	Dark Control	48	0.01	0.01	0.4146	18
	0.5	48.05	0.06	0.03	0.9056	18
	1	24.02	0.34	0.02	0.9971	18
	2	12.01	1.31	0.13	0.9853	18
	3	8.01	2.17	0.03	0.9998	18
	4.5	5.34	2.87	0.13	0.9973	18
	6	4.00	2.68	0.15	0.996	18
20	Dark Control	48	0.02	0.00	0.9725	18
	0.5	48.05	0.08	0	0.9976	18
	1	24.02	0.35	0.01	0.9994	18
	2	12.01	1.27	0.04	0.9982	18
	3	8.01	2.51	0.05	0.9993	18
	4.5	5.34	2.84	0.09	0.9988	18
	6	4.00	3.02	0.14	0.9971	18
30	Dark Control	48	0.00	0.01	0.01	18
	0.5	48.05	0.16	0.01	0.9978	18
	1	24.02	0.96	0.1	0.9894	18
	2	12.01	1.82	0.13	0.9952	18
	3	8.01	2.63	0.07	0.9987	18
	4.5	5.34	3.4	0.11	0.9983	18
	6	4.00	3.71	0.11	0.9988	18

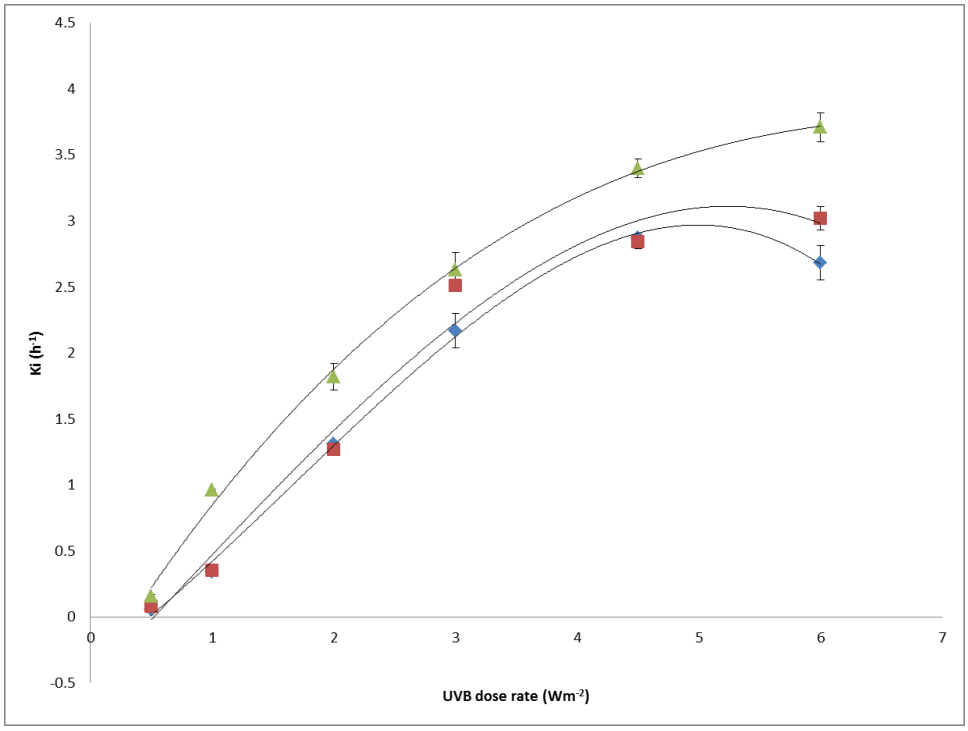


Figure 4-03: Measured MS2 inactivation rate K_i (h^{-1}) in buffered pH 7.5 RO water at 0.5, 1, 2, 3, 4.5 and 6 Wm^{-2} for 10ºC (♦), 20ºC (■) and 30 ℃ (▲)and experimental data 'fitted' using a cubic polynomial model

At 10°C, for MS2:

$$K_i\ (h^{-1}) = -0.0385D^3 + 0.2577D^2 + 0.2942D - 0.0737 \qquad \textbf{Equation 4–3}$$

$$R^2=0.9953,\ p<0.001$$

At 20°C, for MS2:

$$K_i\ (h^{-1}) = -0.0324D^3 + 0.2083D^2 + 0.4262D - 0.1035 \qquad \textbf{Equation 4–4}$$

$$R^2=0.9794,\ p<0.001$$

At 30°C, for MS2:

$$K_i\ (h^{-1}) = -0.0098D^3 - 0.0016D^2 + 0.9957D - 0.1117 \qquad \textbf{Equation 4–5}$$

$$R^2=0.9559,\ p<0.001$$

where D is the UVB dose rate (Wm^{-2}).

The dose rate of 6 Wm^{-2} was higher than reported environmental UVB dose rates.

The data suggest that the effect of UVB dose rate on K_i saturates at dose rates about 6 Wm^{-2}.

Statistical interrogation of the cubic polynomial model (Table 4-4) showed that there was a statistically significant ($p<0.0001$) difference in the value of the intercept term between incubations at 30°C and 10°C and between those at 20°C and 10°C. This infers a lower value for the MS2 dark inactivation rate constant for the lower temperature condition relative to the high temperature condition, confirming the findings for dark inactivation reported in Chapter 3.

Analysis of K_i (slope 1 of the cubic spline fit) confirmed the statistical analysis above, that there was a significant difference ($p=0.000066$) between UVB inactivation at 10°C and 30°C; however, the difference in K_i between 10°C and 20°C ($p=0.057$) was not significant. This is strong evidence that K_i increases more slowly as the UVB dose rate increases at 10°C and 20°C compared with 30°C.

Table 0-3: Interrogation of the third-order polynomial model used to fit K_i data obtained at UVB dose rates between 0.5 and 6.0 Wm^{-2} at 10°C, 20°C and 30°C
Note: The curve fit is shown in Figure 4.3.

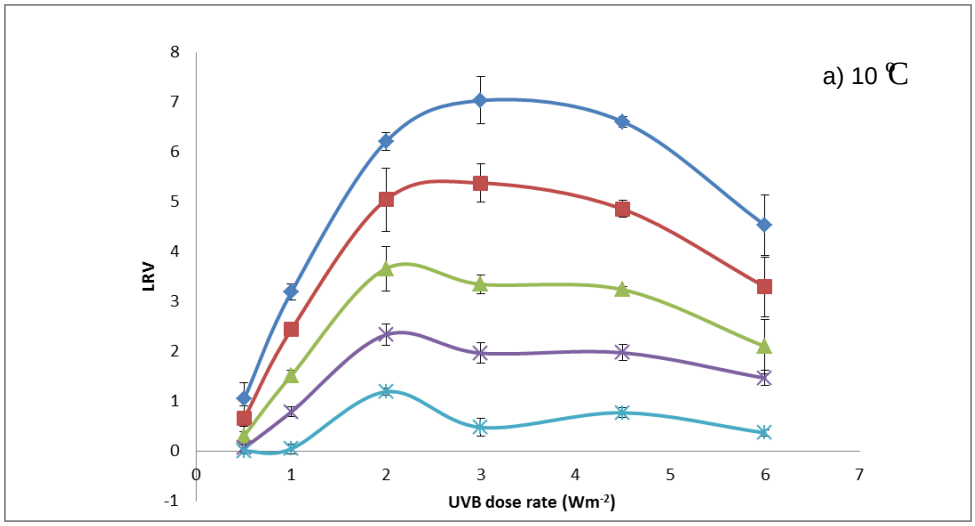
Parameter	Estimate	Std. Error	*t*-value	p
Intercept Temp 30°C vs 10°C	8.156	0.360	22.685	0.000
Intercept Temp 20°C vs 30°C	-3.259	0.377	-8.646	0.000
Intercept Temp 20°C vs 10°C	-0.705	0.381	-1.849	0.0644
Cubic Slope 1 Temp 20°C vs 10°C	0.953	0.500	1.907	0.0565
Cubic Slope 1 Temp 30°C vs 10°C	1.985	0.497	3.991	0.0000659

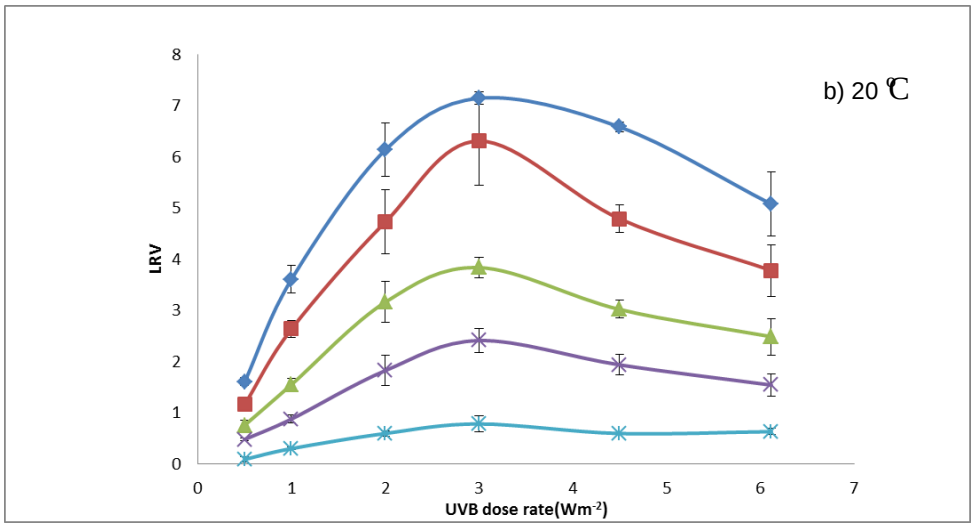
4.3.2 Interaction between UVB dose rate (Wm^{-2}) and UVB dose (Jm^{-2}) on MS2 UVB inactivation

The effect of UVB dose delivery on MS2 inactivation was explored in buffered (pH 7.5) RO water at 10 ℃, 20 ℃ and 30ºC. Samples were taken in accordance with the study design's time points (Table 4-1) to ensure that the UVB dose (Jm^{-2}) was

internally consistent at each sampling time in the sequence. Two-way ANOVA and follow-up least significant difference (LSD) analysis were applied to compare the significance of UVB dose and dose rates on MS2 log_{10} reduction value (LRV).

The MS2 LRV increased with increasing UVB dose (Jm^{-2}) at all incubation temperatures (Figure 4-4(a-c)). At a dose equivalent to the daily UVB dose ($61261Jm^{-2}$) recorded for a cloudy winter's day in South Australia, the MS2 LRV was <1.0 at all incubation temperatures. In contrast, at a UVB dose of 89490 Jm^{-2}, which was above the maximum UVB dose (86,490 Jm^{-2}) recorded on a sunny summer's day in South Australia, the MS2 LRV was up to 7-fold higher.





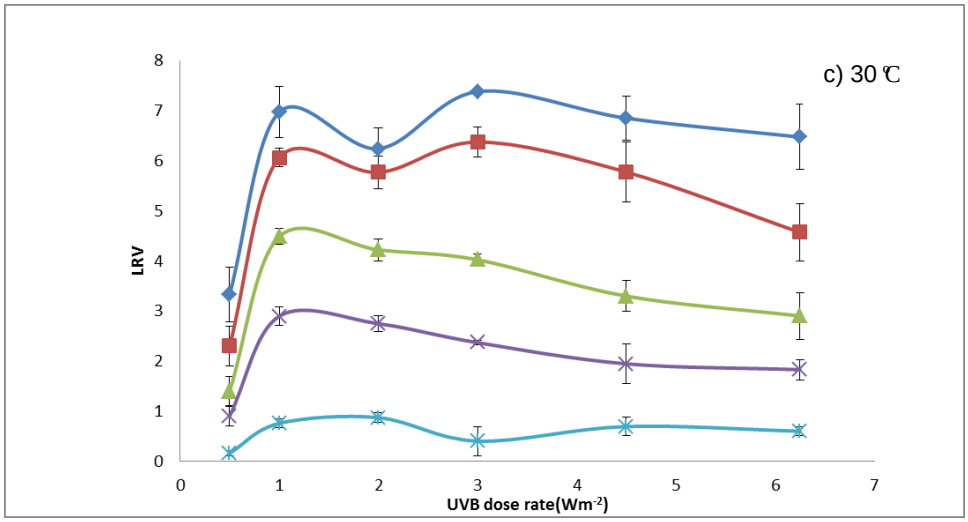


Figure 4-4: MS2 log_{10} reduction value (LRV) in buffered pH 7.5 RO water, using six UVB dose rates–time combinations: 0.5, 1, 2, 3, 4.5 and 6 Wm^{-2}, to achieve five UVB doses: 6126(*), 22049(×), 37973(▲), 62231(■) and 86490(◆) Jm^{-2} at (a) 10℃, (b) 20℃ and (c) 30℃

Notwithstanding, it is clear from Figure 4-4(a-c) that the manner in which the UVB dose (Wm^{-2}) was delivered, that is, the dose rate (Wm^{-2})–exposure time relationship employed to achieve the dose (Jm^{-2}) influenced the LRV at all doses and at all temperatures except for the lowest dose 6126 Jm^{-2} at 20°C and 30°C.The MS2 LRVs for all UVB doses in incubations at 10ºC and 20ºC increased sharply with increasing dose rates from 0.5–2.0 Wm^{-2}, which could be defined as a "dose rate-limited" stage: in the "dose rate-limited stage", the increase in dose rate increased the LRV. With the LRV plateaued at a dose rate between 2.0 Wm^{-2} and 6.0 Wm^{-2}, this suggests a "dose rate-saturated" stage, where an increase in UVB dose rate has little influence on LRV. Dissimilarly, at 30ºC, the dose rate-limited stage appeared shorter than 10°C and 20°C, from 0.5 Wm^{-2} to 1.0 Wm^{-2} for all UVB doses (Jm^{-2}). After 1 Wm^{-2}, all curves at 30ºC appeared "dose rate-saturated". This increase of LRV by increasing dose rate and adjusting incubation time to yield the same dose was more evident at doses >61261Jm^{-2} at all incubation temperatures. Interestingly, the LRV at all doses (Jm^{-2}) decreased as the dose rate–time combinations approached the environmental recorded maximum dose rate of 4.5 Wm^{-2}.

Varied UVB dose rates had a statistically significant influence on MS2 inactivation LRV under the same UVB doses for all three temperatures ($p<0.001$): also, significant interaction was found between the effect of UVB doses and UVB dose

rates on MS2 LRV.

As mentioned above, the UVB dose rates significantly influenced the LRV at the same UVB dose ($p<0.001$) at 10°C: the LRV increased from 0.5 Wm^{-2} to 2 Wm^{-2} or 3 Wm^{-2}, and then dropped until the end. The highest LRV achieved at each curve was at 2 Wm^{-2} or 3 Wm^{-2} while the lowest LRV was at 0.5 Wm^{-2}.

As shown on Figure 4-4 (b), at 20°C, although delivering UVB doses by different UVB dose rates significantly influences the LRV ($p<0.001$) overall, at the lowest UVB dose of 6126 Jm^{-2}, the effect of five UVB dose rates was found insignificant ($p=0.093>0.05$). This finding confirmed the conclusion that the UVB delivery manner effect was more apparent at higher UVB doses. The LRV rose from 0.5 Wm^{-2} to 3 Wm^{-2} and then decreased until 6 Wm^{-2}.

Figure 4-4 (c) shows the UVB delivery manner effect at 30°C in RO water. As at 20°C, the UVB delivery manner influenced LRV at all UVB doses except for the lowest one, 6126 Jm^{-2} ($p=0.13>0.05$). The peak LRV point occurred over a broad range of UV dose rates from 1 Wm^{-2} to 3 Wm^{-2}. This was not the same as the 10°C (Figure 4-4(a)) and 20°C (Figure 4-4(b)) curves where the peak LRV value clearly occurred at the UVB dose rate of 3 Wm^{-2}. Furthermore, at 0.5 Wm^{-2}, the LRV at each UVB dose was still the lowest one. This further supports the case that ambient temperature had a marked effect on MS2 die-off at temperatures above 20°C.

Overall, at almost all UVB doses, the UVB dose delivery manner, delivering the same UVB dose by six different UVB dose rates, significantly influenced the MS2 LRV at all three temperatures. The two stages, “dose rate-limited” and “dose rate-saturated”, were observed at all three temperatures. Commonly, at the high UVB dose, the effect of UVB dose delivery manner was more apparent than at low UVB doses. For all curves, the lowest LRV achieved were at 0.5 Wm^{-2}, and the highest occurred in the UVB dose rate range (1–3 Wm^{-2}).

4.3.3 UVB inactivation of *E. coli* and the effect of temperature

The current study explored the UVB inactivation of *E. coli* at two temperatures encompassing the range frequently measured in HRAPs operated in South Australia, 20°C and 30°C, in buffered (pH 7.5) RO water under a range of UVB doses (Jm^{-2}) achieved using a range of UVB dose rate (Wm^{-2})–time combinations. Two-way ANOVA and follow-up least significant difference (LSD) analysis were applied to compare the significance of temperature.

In pH 7.5 buffered RO water, no *E. coli* inactivation was recorded in the dark incubated controls at either temperature over the 48-hour incubation period. This result was the same as for MS2 (above).

Generally, as UVB dose rate increased, the *E. coli* inactivation rate increased. However, a few exceptions were recorded. At 20°C, an increase in dose rate from 1 Wm^{-2} to 2 Wm^{-2}, resulted in no significant difference in K_i ($p=0.441>0.05$). Similarly, at 30°C, an increase from 2 Wm^{-2} to 3 Wm^{-2} resulted in no significant increase in K_i ($p=0.857>0.05$). As with the data for MS2, no statistically significant ($p<0.05$) difference in *E. coli* inactivation rates was observed between 20°C and 30°C at the range of UVB dose rates investigated (Figure 4-5). The *E. coli* inactivation rates (K_i) at five UVB dose rates were modelled using $\log_{10}$-linear regression: the equations of the regression lines at 20°C and 30°C are shown in Equations 4-6 and 4-7, respectively.

Table 4-4: *E. coli* inactivation rates K_i (h^{-1}) in buffered pH 7.5 RO water at UVB dose rates of 0.5, 1, 2, 3 and 4.5 Wm^{-2} at 20ºC and 30ºC

Notes: K_i (h^{-1}); mean ± SD) was obtained from a $\log_{10}$-linear + tail model using the GInaFiT model (Geeraerd et al., 2005) for which the individual R^2 values are shown together with n, the number of data points included in the model.

Temperature (℃)	UVB dose rate (Wm^{-2})	Incubation time (h)	K_i (h^{-1})	SD	R^2	n
20	Dark Control	48	0.00	0.00	0.0012	12
	0.5	48.05	0.6	0.09	0.9817	27
	1	24.02	6.78	0.37	0.9948	27
	2	12.01	8.14	1.67	0.9469	21
	3	8.01	21.85	2.41	0.9856	18
	4.5	5.34	36.69	0.31	1.000	12
30	Dark Control	48	0.00	0	0.01	12
	0.5	48.05	1.28	0.39	0.9058	21
	1	24.02	7.13	0.51	0.9951	21
	2	12.01	17.64	1.71	0.9899	21
	3	8.01	18.57	1.23	0.9935	18
	4.5	5.34	31.68	3.95	0.9935	12

At 20°C, for *E. coli:*

$$K_i\,(h^{-1})\ 20°C = 7.3628 \times D \qquad \textbf{Equation 4–6}$$

(R^2=0.92158, p<0.001)

At 30°C, for *E. coli:*

$$K_i\,(h^{-1})\ 30°C = 6.9948 \times D \qquad \textbf{Equation 4–7}$$

(R^2=0.9559, p<0.007)

where D is the UVB dose rate (Wm^{-2}).

As no significant difference was found between the two lines, a linear regression analysis was performed on the combined K_i values obtained at 20°C and 30°C (as shown in Equation 4-8);

E. coli data at 20°C and 30°C combined,

$$K_i\,(h^{-1}) = 7.1788 \times \text{UVB dose rate } (Wm^{-2}) \qquad \textbf{Equation 4–8}$$

(R^2=0.9781)

This $\log_{10}$-linear model was applicable for temperatures between 20°C and 30°C.

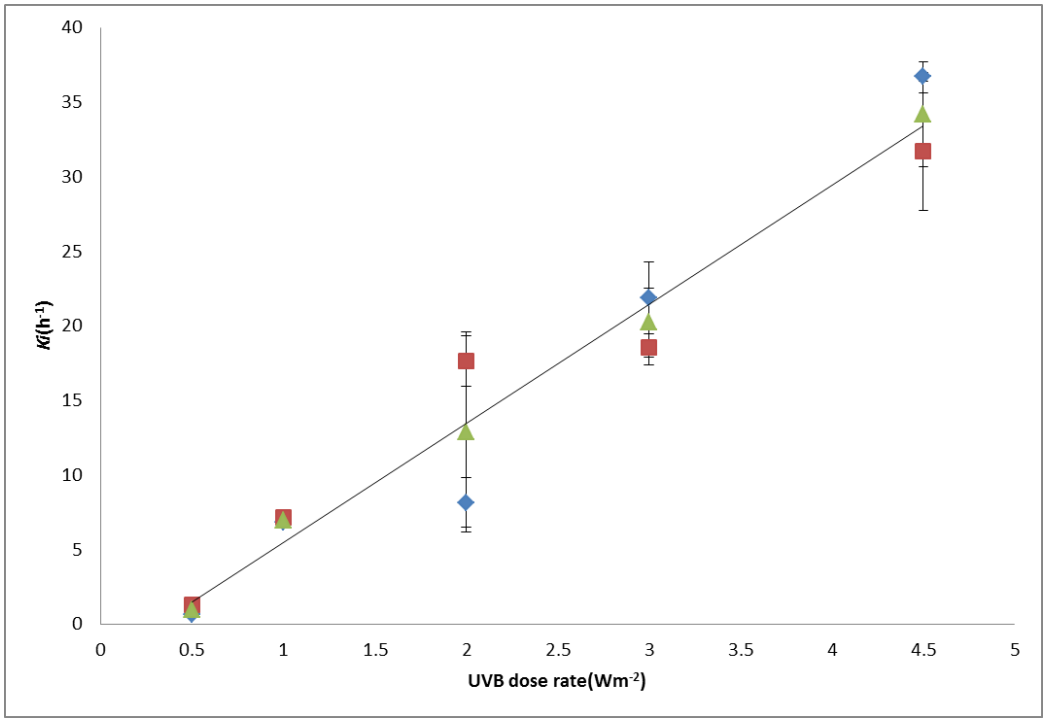
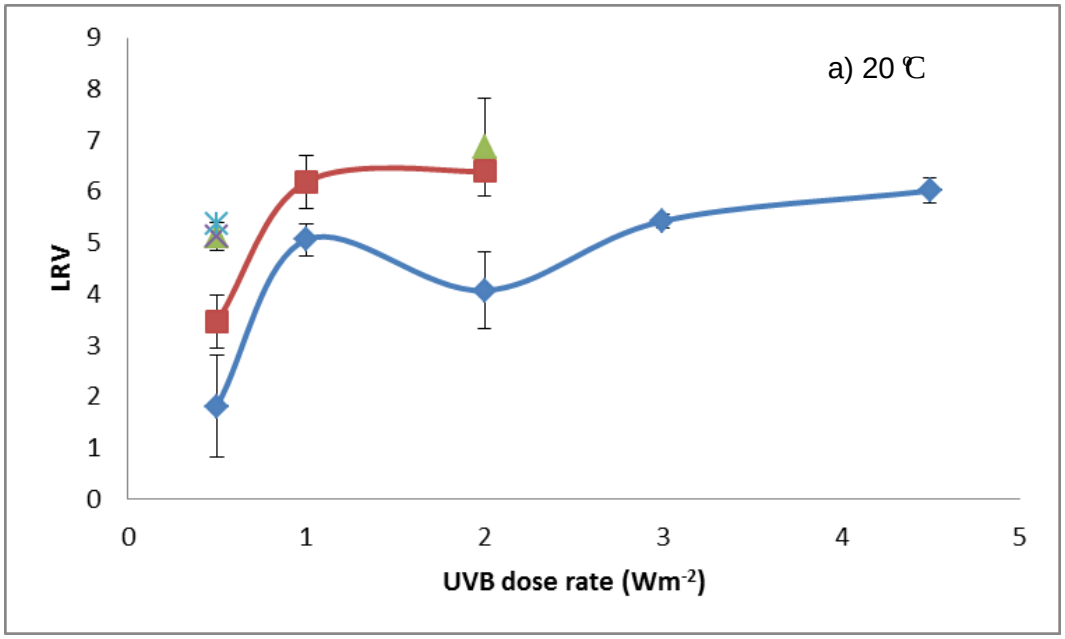


Figure 4–5: Comparison of *E. coli* inactivation rate (K_i) in buffered 7.5 pH RO water at 20ºC (♦) and 30ºC (■) the mean of the K_i at 20ºC and 30ºC (▲) and linear regression of the mean K_i (h^{-1}) at UVB dose rates of 0.5, 1, 2, 3 and 4.5 Wm^{-2}

4.3.4 Interaction between UVB dose rate (Wm^{-2}) and UVB dose (Jm^{-2}) on *E. coli* UVB inactivation

E. coli LRVs were determined at five UVB doses (Jm^{-2}), each achieved using five UVB dose rate (Wm^{-2})–exposure time) combinations. Samples were taken in accordance with the study design's time points (Table 4-5) to confirm that the UVB dose was the same at each time in the sequence. Two-way ANOVA and follow-up least significant difference (LSD) analysis were applied to compare the significance of UVB dose and dose rates on *E. coli* log reduction value (LRV).

E. coli was found to be more sensitive to UVB inactivation than MS2 in buffered pH 7.5 RO water. Consequently, only one LRV data point was available for either of the high UVB doses, 62231 Jm^{-2} or 86490 Jm^{-2} (Figure 4-5). The absent points were due to all *E. coli* being inactivated within the first exposure period.



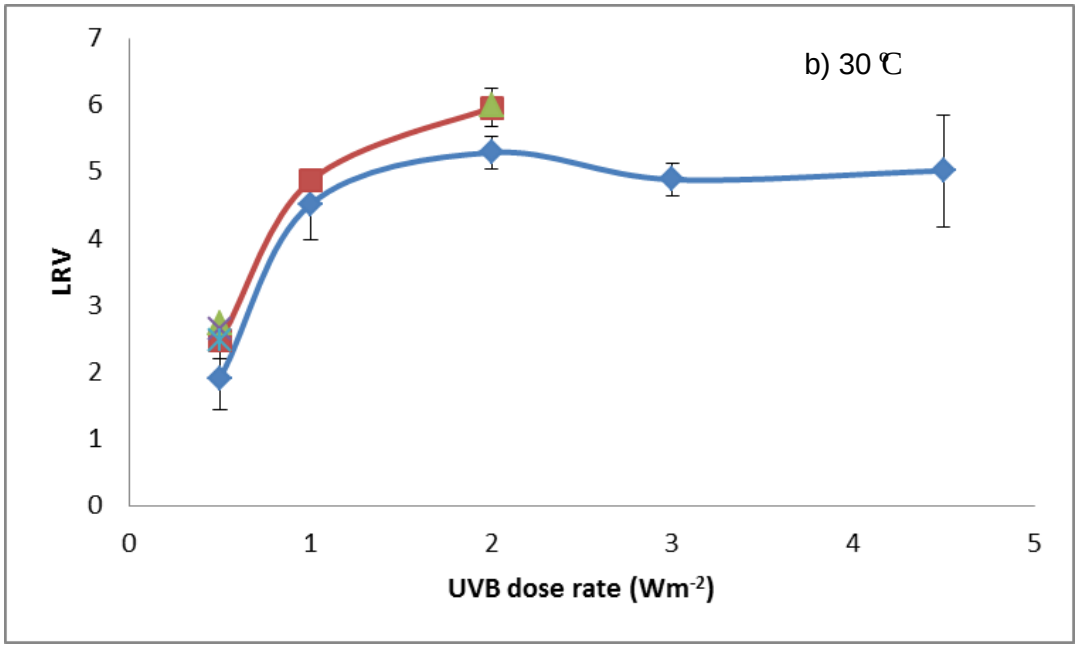


Figure 4-6: *E. coli* $\log_{10}$ reduction value (LRV) in buffered pH 7.5 RO water, using five UVB dose rates–time combinations: 0.5, 1, 2, 3 and 4.5 Wm^{-2}, to achieve five UVB doses: 6126 (◆), 22049 (■), 37973 (▲), 62231 (×) and 86490 (*) Jm^{-2} at (a) 20℃ and (b) 30℃

Similarly, at 22049 Jm^{-2}, the response to only three UVB dose rates (Wm^{-2}) was recorded. The sensitivity of *E. coli* UVB inactivation was such that only at the lowest UVB dose, 6126 Jm^{-2}, were LRVs able to be determined at all five dose rates.

These limitations make data interpretation more difficult and less conclusive. *E. coli* LRV significantly ($p<0.001$) increased with increasing UVB dose (Jm^{-2}). However, as

recorded for MS2, the dose rate (Wm^{-2})–exposure time combination used to achieve the dose (Jm^{-2}) influenced the *E. coli* LRV. At both 20ºC and 30ºC and UVB doses of 22049 Jm^{-2} and 61261 Jm^{-2}, the *E. coli* LRV increased rapidly when the dose rate used to achieve the same UVB dose was increased from 0.5 Wm^{-2} to ≥1 Wm^{-2}: this is termed “dose rate-limited”. At other doses, the *E. coli* LRV plateaued at dose rates >2 Wm^{-2}, a “dose rate-saturated” stage. These results were similar to those recorded for MS2 over a greater range of UVB doses (as discussed above).

4.4 Discussion

In this chapter, the effects on MS2 and *E. coli* inactivation and LRV were determined of both UVB dose (Jm^{-2}) and dose rates (Wm^{-2})—exposure time combinations to achieve specific UVB doses and incubation temperature. The conditions chosen were environmentally relevant to HRAP operating conditions in South Australia, including temperatures of 10ºC, 20ºC and 30ºC; UVB dose rates of 0.5, 1, 2, 3, 4.5 and 6 Wm^{-2}; and UVB doses of 6126, 22049, 39973, 62232 and 86490 Jm^{-2}.

4.4.1 Temperature effects on MS2 UVB inactivation

Temperature was selected for investigation as it is an important factor in natural wastewater treatment systems. Many pond design equations employ temperature as a key environmental parameter influencing performance. Temperature has been correlated with UV irradiance in many studies, for example, cyanobacteria growth (Roos et al., 1998); damage to the crustacean *Daphnia pulvinaria* (MacFadyen et al., 2004); and the inactivation of pathogens (Heaselgrave et al., 2006; Theitler et al., 2012). However, few studies focus specifically on the effect of temperature on UVB disinfection.

In the current study, no MS2 inactivation (K_i) was observed in the dark control incubations over the short incubation period. In contrast, temperature had a significant effect on MS2 UVB inactivation rates with K_i significantly influenced by temperature. The K_i values recorded at 30ºC were significantly greater than those recorded at either 10ºC or 20ºC in buffered (pH 7.5) RO water. There was, however, no statistically significant difference in the K_i recorded for MS2 incubations at 10ºC and 20ºC. Theitler et al. (2012) found a slight synergistic disinfection effect of heat and natural sunlight on MS2 in deionised water; however, the incubation temperature was as high as 59ºC. Other researchers also concluded that temperature only effectively influenced the pathogen’s inactivation rate under solar

UV beyond 50ºC (Sommer et al., 1997; Wegelin et al., 1994).

The most possible reason behind the difference in results from the previous literature is that among this UV research, the current study was also the only one which used UVB irradiance solely, together with different durations of exposure to UVB. Direct photoinactivation of MS2 by UVB results from the absorbance of UVB by RNA to form dimers of the pyrimidine molecule. The increase in temperature accelerates the formation of a double bond between pyrimidine molecules, and MS2 becomes more easily inactivated (USEPA, 1996). Heaselgrave et al. (2006) suggested that another explanation for temperature–UV synergy on protozoa inactivation was that elevated temperature leads to increasing permeability of the cyst walls in the presence of UV irradiance disinfection. Other potential reasons in the literature are that the incubation did not conduct constant mixing (Sommer et al., 1997; Wegelin et al., 1994), and that the temperature ranges adopted were higher than in the current study; for instance, the range was 30ºC to 60ºC for Theitler (2012) and 20ºC to 50ºC for Wegelin (1994).

4.4.2 Temperature effects on *E. coli* UVB inactivation

The *E. coli* UVB inactivation rate was determined at 20ºC and 30ºC. Unlike MS2 incubations, no significant temperature effect on *E. coli* inactivation rates was observed under a range of UVB dose rates between 20°C and 30°C ($p=0.686>0.05$). Wegelin (1994) used a rotating photoreactor to explore the influence of temperature on the process of solar water disinfection (SODIS): the *E. coli* UV survival behaviour did not change from 20ºC to 40ºC, but at 50ºC, the inactivation rate was almost three times high than that from 20-40°C. *Streptococcus faecalis* was also included in their study and its response was similar to *E. coli*; however, *Enterococcus* responded differently, with the inactivation rate only increasing above 55°C. Theitler (2012) also recorded up to 3-log *E. coli* inactivation difference for the synergistic effect of 50°C and 2000 KJm^{-2} under full wavelength natural sunlight or simulated solar irradiance when compared to the separate treatment. The endonuclease sensitive site (ESS) assay was applied in their study to quantitate the *E. coli* DNA damage: in their solar water disinfection (SODIS) experiment, constant mixing was also adopted.

One of the potentially important reasons that caused the difference in temperature effect between *E. coli* and MS2 is that more mechanisms are involved in *E. coli* inactivation. For *E. coli*, the outcome of inactivation under UVB irradiance is also

moderated by *E. coli* UV repair mechanisms. The UV-induced lesion on *E. coli* can be repaired by two mechanisms of DNA (Chan & Killick, 1995; Harm, 1968; Hill, 1965). Firstly, after the formation of lethal pyrimidine dimers, a photoreactivating light and a photoreactivating enzyme contribute to separating the dimers. Secondly, a dark repair system removes the dimers. The enzyme systems involved in repairing DNA damage have been shown to be temperature-dependent; thus, at higher temperatures, the repairing system works more efficiently, including the temperature range from 20°C to 30°C. At the same time, the salinity of the environment was proved to also decrease *E. coli* recovery (Chan & Killick, 1995; MacFadyen et al., 2004).

4.4.3 UVB dose rate effect on MS2 UVB inactivation rate

Six environmentally relevant UVB dose rates (Wm^{-2}) were selected to assess the influence of dose rate on MS2 inactivation rates in RO water (pH 7.5) at 10ºC, 20ºC and 30ºC. Considering that direct photoinactivation is the mechanism of UVB inactivation, it was predicted that the MS2 inactivation rate (K_i) should increase with increasing UVB dose rate, as, at a constant dose (Jm^{-2}), the UVB exposure per unit time increases, as would the rate of the formation of MS2 RNA pyrimidine dimer. This deduction worked in the current study when the UVB increased from 0.5 Wm^{-2} to 4.5 Wm^{-2} at all three temperatures, and the rate of change of K_i was consistent. However, an interesting finding was observed from 4.5 Wm^{-2} to 6 Wm^{-2}: a threshold was observed for the increased of K_i. The MS2 inactivation rate K_i did not perform the same at all three temperatures. When the UVB dose rate increased from 4.5 Wm^{-2} to 6 Wm^{-2}, at 10ºC, K_i decreased a little; at 20ºC, K_i did not change; while at 30ºC, K_i increased a little. However, all curves indicated that here was a threshold for K_i in the current study no matter how high the level of the UVB dose rate. The cubic polynomial model was observed to be the best model to express the relationship between UVB dose rate and MS2 inactivation rate K_i.

Few studies have explored the effect of UV dose rate on the waterborne pathogen inactivation rate. Sommer et al. (1998) tested the time–dose reciprocity of UVC disinfection in saline water using 10 low-pressure mercury UV lamps (253.7 nm) and a diaphragm and optical wire mesh to attain three UVC dose rates of 2, 0.2 and 0.02 Wm^{-2}. Combining dose rates and exposure times, for example, 2 Wm^{-2} for 50 seconds, 0.2 Wm^{-2} for 500 seconds, and 0.02 Wm^{-2} for 5000 seconds to yield a constant dose of 100 Jm^{-2}, three UVC doses 100, 300 and 500 Jm^{-2} were studied. It should be noted that these UVC doses are at least an order of magnitude less than

the UVB doses used in the current study. Seven different pathogen indicators, including MS2 and *E. coli*, were selected. An inactivation rate was not provided for each indicator. However, the exposure duration (seconds) and the $\log_{10}$ reduction value for each indicator were provided, which enabled subsequent calculation of the inactivation rate assuming first-order kinetics (Table 4-5). In terms of UVC, when the dose rate increased from 0.02 Wm^{-2} to 0.2 Wm^{-2} and 2 Wm^{-2}, an apparent increase in K_i was observed independent of the dose (Jm^{-2}). The MS2 inactivation rate increased >10-fold when the UVC dose rate increased from 0.02 Wm^{-2} to 0.2 Wm^{-2}, then the increase rate dropped 8.7 times when the dose rate rose from 0.2 Wm^{-2} to 2 Wm^{-2}. This observation was the same as in the current UVB study.

However, for the other two groups, 150, 1500 and 15000 seconds and 250, 2500 and 25000 seconds, the MS2 inactivation rate did not perform the same way; *K* kept increasing at the same rate or at an even higher rate with the expansion of UVC dose rate. The reason, perhaps, is that the UVC dose rate chosen in this study was not high enough to be close to the threshold. Another interesting finding from Table 4-5 is that, with the same UVC dose rate, the MS2 inactivation rate varied with the different experiment length. It indicated that the inactivation rate K_i gained from Chick's Law is not very accurate in many research studies(Chick, 1908). In a certain experiment, pathogen inactivation rate often varies over the time, so even under the same UV dose rate, different experiment duration changes the finally value of the inactivation rate. GInaFiT in the current research provides a range of curves to solve this problem.

Liu and Zhang (2006) assessed the effect of UVC dose rate and turbidity on pathogen indicator inactivation. In their experiments, low pressure mercury vapour lamps (253.7 nm) provided three UVC dose rates 0.08 Wm^{-2}, 1 Wm^{-2} and 1.8 Wm^{-2}, and kaolin was added to sterilised water to reach the three levels of turbidity, at 0.5 NTU, 4 NTU and 12 NTU. After analysing the MS2 $\log_{10}$ reduction value and time as above to gain the inactivation rate, a similar result emerged. This showed that when UVC dose rate increased, the MS2 inactivation rate also increased. However, no obvious slowdown was observed with regard to the MS2 inactivation rate *K* when the dose rate kept rising; in contrast, the MS2 inactivation rate increased faster with the higher UVC dose rate.

Table 4–5 MS2 UVC inactivation rates K_i (s^{-1}) in saline water calculated from dose rate exposure time and log_{10}reduction

Note: This is as recorded in research reported by Sommer et al. (1998).

Exposure time (s)	UVC dose rate (Wm^{-2})	UVC dose (Jm^{-2})	MS2 inactivation rate (s^{-1})
50	2	100	0.0096
500	0.2	100	0.0011
5000	0.02	100	0.000094
150	2	300	0.0108
1500	0.2	300	0.0011
15000	0.02	300	0.000121
250	2	500	0.0105
2500	0.2	500	0.001
25000	0.02	500	0.0001

The author is unaware of any other study which has systematically assessed and modelled the effect of an environmentally relevant UVB dose rate on the MS2 UVB inactivation rate. A cubic polynomial model was found to best express the correlation of UVB dose rate and MS2 inactivation rate. A threshold, or saturation dose rate, was noted for MS2 inactivation above which further increases in dose rate did not result in increases in MS2 inactivation rate.

This unique study, when combined with water column attenuation, will enable the development of a more accurate pathogen inactivation model in natural wastewater treatment systems, for example, HRAPs and WSPs.

4.4.4 UVB dose rate effect on *E. coli* UVB inactivation rate

Five environmentally relevant UVB dose rates were selected to assess the influence of dose rate on *E. coli* inactivation rates in pH 7.5 buffered RO water at 20ºC and 30ºC. As the UVB dose rate increased from 0.5 Wm^{-2} to 4.5 Wm^{-2}, the *E. coli*

inactivation rate also increased. A log-linear regression model described the correlation between UVB dose rate and *E. coli* inactivation rate.

Sommer et al. (1998) performed comparable research using three different strains of *E. coli*, exposed to UVC. The inactivation rate was calculated from their data for one of these strains, *E. coli* ATCC11229 (Table 4-6), which showed that the *E. coli* inactivation rate increased with increasing UVC dose rate and that a log-linear model best described this relationship. Moreover, the inaccuracy of Chick's Law on UV inactivation rate was more obvious for *E. coli* than for MS2. When the exposure increased from 20 to 35 seconds, at the same as the UVC dose rate increased to 2 Wm^{-2}, the *E. coli* inactivation rate grew more than two times: what happened there might be that the *E. coli* inactivation rate boosted from the 15th second to the 35th second. This finding proved the necessity of applying GInaFiT once a suitable microbiology inactivation rate had been obtained. Liu (2006) obtained *E. coli* experimental data for water with three levels of turbidity, with the study's results also supporting the same conclusion.

Table 4–6: *E. coli* ATCC11229 UVC inactivation rates K_i (h^{-1}) in saline water calculated from exposure time (seconds) to respective dose rate and log_{10} reduction value
Note: This is as measured by Sommer et al. (1998).

Exposure time (seconds)	UVC dose rate (Wm^{-2})	UVC dose (Jm^{-2})	*E. coli* inactivation rate (s^{-1})
20	2	40	0.0425
200	0.2	40	0.0049
2000	0.02	40	0.00063
35	2	70	0.113
350	0.2	70	0.0104
3500	0.02	70	0.000989
45	2	90	0.114
450	0.2	90	0.0094
4500	0.02	90	0.00094

The current study is the first study to systematically assess and model the environmentally relevant UVB dose rate effect on the *E. coli* UVB inactivation rate. The *E. coli* UVB inactivation rate was found to significantly increase with the increasing UVB dose rates from 0.5 Wm^{-2} to 4.5 Wm^{-2}, while a log-linear model was found to express the correlation of UVB dose rate and *E. coli* inactivation rate.

4.4.5 UVB dose, dose rate–time combinations and the influence on MS2 UVB inactivation and LRV

Five UVB doses (Jm^{-2}) were all delivered by six UVB dose rate (Wm^{-2})–exposure time combinations to explore the relationship between dose rate–exposure time and UVB dose on MS2 disinfection effect at three temperatures. UVB dose rate–exposure time was observed to significantly influence the MS2 disinfection effect in pH 7.5 buffered RO water.

In most studies of UV disinfection, the UV dose was recorded as prior information to describe UV irradiance (Chang, 1985; USEPA, 1996). Therefore, it is important to check if dose rate also plays an important role for UV disinfection performance. UVB was found to be able to kill micro-organisms directly by causing pyrimidine dimer formation in DNA or RNA (USEPA, 1999). Micro-organism nucleic acids are the most important absorbers of UV light in this mechanism (Jagger, 1967). Results presented here demonstrate that the MS2 LRV recorded at the same dose (Jm^{-2}) was dependent on how the dose was delivered, that is, the dose rate–exposure time relationship at all three temperatures. As a consequence, it is proposed that both the dose and dose rate–exposure time values should be reported for all UV disinfection-related research. Moreover, at all three temperatures, the “dose rate-limited” stage and “dose rate-saturated” stage were observed at almost all curves. “Dose rate-limited” described the part of the LRV value that is significantly influenced by increases in the dose rate. During this stage, the reception capacity of the UVB photons of MS2 in the mixing vessels improved with the increasing UVB dose rate; the capacity then reached a peak between 1 Wm^{-2} and 3 Wm^{-2}. After reaching the peak value, the curves acted as the “dose rate-saturated” stage: although the UVB dose rate kept increasing, the MS2 LRV hardly varied.

Related research has been performed for UVC disinfection (Sommer et al., 1998). In their study, they found that the LRV of MS2 did not change obviously for three UVC dose rates of 0.02 Wm^{-2}, 0.2 Wm^{-2} and 2 Wm^{-2}under UVC doses of 100 Jm^{-2}, 300 $Jm^{-2}$and 500 Jm^{-2} in sterile 0.85% saline. This finding is the same as reported

when delivering low UVB doses, for example, 6126 Jm^{-2} by five different UVB dose rates at either 20ºC and 30ºC in the current study. In contrast, Liu and Zhang (2006) observed that a higher UVC dose rate for a short time caused more MS2 LRV than the same dose for a longer time. In their study, the UVC dose rates selected were 1.8 Wm^{-2}, 1 $Wm^{-2}$and 0.08 Wm^{-2}, to achieve a constant dose of 400 Jm^{-2}. This phenomenon was also observed in the current study when the UVB dose rate increased from 0.5 $Wm^{-2}$to 1 Wm^{-2} or 2 Wm^{-2}at a high UVB dose, especially at 86490 Jm^{-2}.

The current study is unique in its systematic exploration of the influence on MS2 inactivation rates and LRVs of the method of delivering a fixed, environmentally relevant, UVB dose by combinations of dose rate and exposure time. Generally, within a constant dose, the UVB dose rate–time combination significantly influenced MS2 inactivation: the phenomenon was more obvious at a high UVB dose.

4.4.6 UVB dose, dose rate–time combinations and the influence on *E. coli* UVB inactivation and LRV

The manner in which a constant UVB dose was delivered, that is, the UVB dose rate–exposure time combination significantly influenced the *E. coli* disinfection.

E. coli was more sensitive to UVB exposure than MS2. Consequently, fewer data are available for *E. coli* inactivation at a high UVB dose as all the *E. coli* were inactivated early in the exposure. The increase in LRV with increasing UVB dose (Jm^{-2}) was statistically significant ($p<0.001$) at both temperatures. The results also show that increasing UVB dose rates from 0.5 Wm^{-2} to 2 Wm^{-2}, while maintaining the same UVB dose (Jm^{-2}), led to higher *E. coli* LRV. This is the same as the results recorded for MS2 at high UVB doses. In contrast, *E. coli* LRV was constant and independent of dose rate above 2 Wm^{-2}, and MS2 performed the same at the lowest UVB dose of 6126 Jm^{-2} at 20ºC and 30ºC. The “dose rate-limited” stage and “dose rate-saturated” stage were also observed in *E. coli* experiments.

4.4.7 MS2 and *E. coli* sensitivity comparison

In this chapter, MS2 and *E. coli* were both selected for exploring the effect of temperature and UVB dose delivery manner on disinfection. Under the same UVB doses and dose rates, it is obvious that *E. coli* was more sensitive to UVB irradiance than MS2. Firstly, under the same UVB dose rates from 0.5 Wm^{-2} to 4.5 Wm^{-2}, the inactivation rate K_i of *E. coli* was higher than the rate for MS2. The gap between the

inactivation rate of the two indicators kept increasing with the increased UVB dose rate: at 4.5 Wm^{-2}, the K_i of *E. coli* was already more than 10 times higher than the K_i of MS2. In addition, when delivering the same UVB doses by different UVB dose rates, *E. coli* died out at high UVB doses but MS2 did not.

The ability of a pathogen's indicator to survive during the disinfection process is an important parameter to consider when choosing a suitable surrogate indicator. In Chapter 3, it was reported that MS2 was more sensitive when incubated in the dark in agitated raw wastewater than *E. coli.* In contrast, here it has been shown that under UVB irradiance, *E. coli* died much faster than MS2. One of the important reasons that may contribute to this result is the larger physical size and greater amount of genetic material that *E. coli* has in comparison with MS2. These characteristics may result in *E. coli* receiving more photons, so that the pyrimidine dimer is more readily formed on *E. coli*'s DNA. A similar conclusion has been supported by much UV-related research (Bolton, 2011; Fisher et al., 2012; Theitler et al., 2012).

4.5 Conclusions

In buffered pH 7.5, distilled water:

- Ambient temperature has a significant effect with increasing dose rates (0.5, 1, 2, 3, 4.5 and 6 Wm^{-2}) on the UVB disinfection of MS2 from 10ºC to 30ºC. The effect was not observed from 10ºC to 20ºC.

- No significant effect was found on UVB disinfection of *E. coli* by increasing temperature from 20ºC to 30ºC ($p>0.05$).

- The log-linear + tail model is the best model to achieve a suitable inactivation rate of the indicators under UVB irradiance.

- The correlation between MS2 inactivation rates and UVB environmentally relevant dose rates was able to be modelled using a cubic polynomial: a threshold was observed for MS inactivation rates. Meanwhile, the *E. coli* inactivation rate could be modelled by a log-linear model.

- Under the same UVB dose, the UVB dose rate had a strong influence on UVB disinfection of both *E. coli* and MS2 regardless of temperature,

especially at high dose. Therefore, it is proposed that for all future reporting of UV disinfection research temperature, UV dose rate and UV dose should be recorded.

Gaining more understanding and modelling UVB inactivation on a pathogen indicator, such as MS2, is essential to public health not only for the natural wastewater treatment systems like HRAPs, but also for the natural drinking water UV disinfection processes.

These experiments were conducted in clear buffered water to allow a focus on the UVB direct disinfection mechanism, rather than confounding the effects due to attenuation. Further research needs to be done with a more complicated water medium, involving more disinfection mechanisms.

CHAPTER 5: DOSE DEPENDENCE ON UVB DISINFECTION IN FILTERED WASTEWATER AND IN FILTERED WASTEWATER IN THE PRESENCE OF THE ROS QUENCHER L-HISTIDINE

5.1 Introduction

The next step in this study, after having determined the dose and temperature dependence required for UVB disinfection of MS2 and *E. coli* in buffered (pH7.5), optically clear, reverse osmosis (RO) water, was to add complexity and determine the relationships in wastewater. As described in this chapter, this series of UVB experiments utilised filtered wastewater and filtered wastewater in the presence of L-histidine, a quencher of reactive oxygen species (ROS). Furthermore, following the conclusions drawn in Chapter 4, several modifications were made to the experimental design. As MS2 was obviously a more resistant pathogen indicator than *E. coli*, to UVB disinfection, the chosen pathogen indicator for study from this point onwards was solely MS2. Moreover, as the study showed that having a UVB dose rate outside that of the natural range (4.5–6 Wm^{-2}) did not make any significant difference, the dose rate range on which this study focused was 0–4.5 Wm^{-2}. The results of the potential involvement of exogenous photosensitisers and the effect of turbidity on UVB photoinactivation are reported in this chapter.

The main difference in characteristics between filtered wastewater and buffered RO water with regard to UV disinfection is the potential for exogenous photosensitisers to influence inactivation. The term ‘exogenous photosensitisers’ refers to compounds, which appear in natural wastewater as suspended solid, dissolved humic matter, photosynthetic pigments and dissolved organic matter (DOM), that are able to catalyse UV inactivation by photon-sensitised, energy transfer to $^1O_2$ and other reactive oxygen species (ROS) (Curtis, 1992; Davies-Colley et al., 1999; Kohn & Nelson, 2006). In wastewater, the humic substances that predominantly occur are organic matter resulting from the microbial and chemical decomposition of organic debris; although different resources result in different humic substances, they have similar colloidal status and adsorption abilities (Davies et al., 1998; Lipczynska-Kochany & Kochany, 2008). In the following chapters, the term ‘dissolved organic matter (DOM)’ refers to humic substances, while exogenous photosensitisers are referred to and qualified by DOM from filtered wastewater treated by wastewater stabilisation ponds (WSPs), alum flocculation and microfiltration.

There are three sunlight-mediated inactivation mechanisms, direct DNA/RNA damage: photoinacitvation, indirect endogenous photoinactivation and exogenous photoinactivation. The exogenous photosensitisers which contribute to the formation of ROS outside of the cells (Curtis, 1992) were considered in this research. Little research has been done on indirect photoinactivation as it has been hard to separate the endogenous and exogenous effects (Kohn & Nelson, 2006). However, the following general conclusions have been made, exogenous photosensitisers are more likely to be sensitised by long wavelength UV, for example, UVA or visible light (Curtis, 1992; Davies-Colley et al., 1999) and are highly dependent on DO, pH and salinity in both fresh and saline water (Bolton, 2011; Curtis, 1992; Davies-Colley et al., 1999; Reed, 1997; Sinton et al., 2002). However, more recently, UVB-induced damage has also been connected with exogenous ROS (Santos et al., 2012). In their research, the scavenger, sodium azide, was found to significantly attenuate the reduction of all four culturable bacterial groups studied, and singlet oxygen was found to be important in UVB-induced cell inactivation.

5.1.1 Reactive oxygen species (ROS)

Reactive oxygen species (ROS) comprise five forms: singlet oxygen ($^1O_2$); superoxide (O_2^-); hydroxyl radical (OH); peroxyl radical (RO_2^-); and hydrogen peroxide (H_2O_2), with these commonly produced by a sensitiser molecule absorbing UV radiation, or through energy transfer or electron-transfer reactions with oxygen (Kohn & Nelson, 2006). They attack the external viral capsid protein causing the damage. Unlike *E. coli,* MS2's protein capsid contained little organic matter content which is likely to participate in endogenous photoinactivation, which therefore can be excluded as a potential mechanism for MS2 inactivation.

Kohn and Nelson (2006) investigated the effect of different ROS on MS2 exogenous photoinactivation with filtered and unfiltered WSP water. They used different quenchers to suppress the selective ROS: formate (50 mM) for OH; L-histidine (20 mM) for $^1O_2$; superoxide dismutase (SOD) (2 U/mL) for O_2^-; and catalase (200 U/mL) for H_2O_2, with the experiment conducted in batch reactors, irradiated by a solar simulator with a collimated beam, equipped with a 1000 W xenon (Xe) lamp. The total irradiance provided by the lamp was the full wavelength from 280nm to 700 nm, and the dose rate was 368 Wm^{-2}. The results confirmed that $^1O_2$ was the most important ROS with regard to MS2 exogenous photoinactivation. The same conclusion was also reached by Curtis (1992). Therefore, to test the possible effect of exogenous photoinactivation, this study selected L-histidine to be added to filtered

wastewater.

This chapter presents a series of laboratory based experiments that were conducted using 0.2 µm filtered WSP wastewater, with and without the $^1O_2$ quencher, L-histidine. The objective of this work was to explore the effect of DOM exogenous photosensitisers and wastewater turbidity on UVB inactivation. In comparison with Chapter 4, the work in this chapter goes one more step towards the study of field wastewater.

5.2 Methods

5.2.1 Filtered wastewater collection

The wastewater used in this experiment was taken from the wastewater treatment plant (WWTP) located at Mt Barker, South Australia. The Mt Barker WWTP treats domestic wastewater from septic tanks by utilising a series of lagoons comprising a mixed aerated lagoon, a polishing lagoon followed by dissolved air flotation (DAF) unit and a continuous (0.2 µm) microfiltration (CMF) unit. For this study, the filtered wastewater was collected after the CMF unit and refiltered using a 0.2 µm filter in the laboratory to confirm that only dissolved exogenous photosensitisers remained in the wastewater. The filtered wastewater was then taken and stored at 4ºC in the dark and used within a two-week period.

5.2.2 UVB irradiation

Schott bottles (Chapter 2) were placed, as shown in Appendix 1. Five environmentally relevant UVB dose rates of 0.5, 1, 2, 3 and 4.5 Wm^{-2} were chosen in this study. For the dark control, the vessels were totally wrapped in aluminium foil and were simultaneously incubated in the temperature controlled, shaking water bath with the illuminated treatments at 20ºC.

5.2.3 Experiment design

The duration of the incubation to achieve the UVB dose at various dose rates was calculated (Figure 5-1).

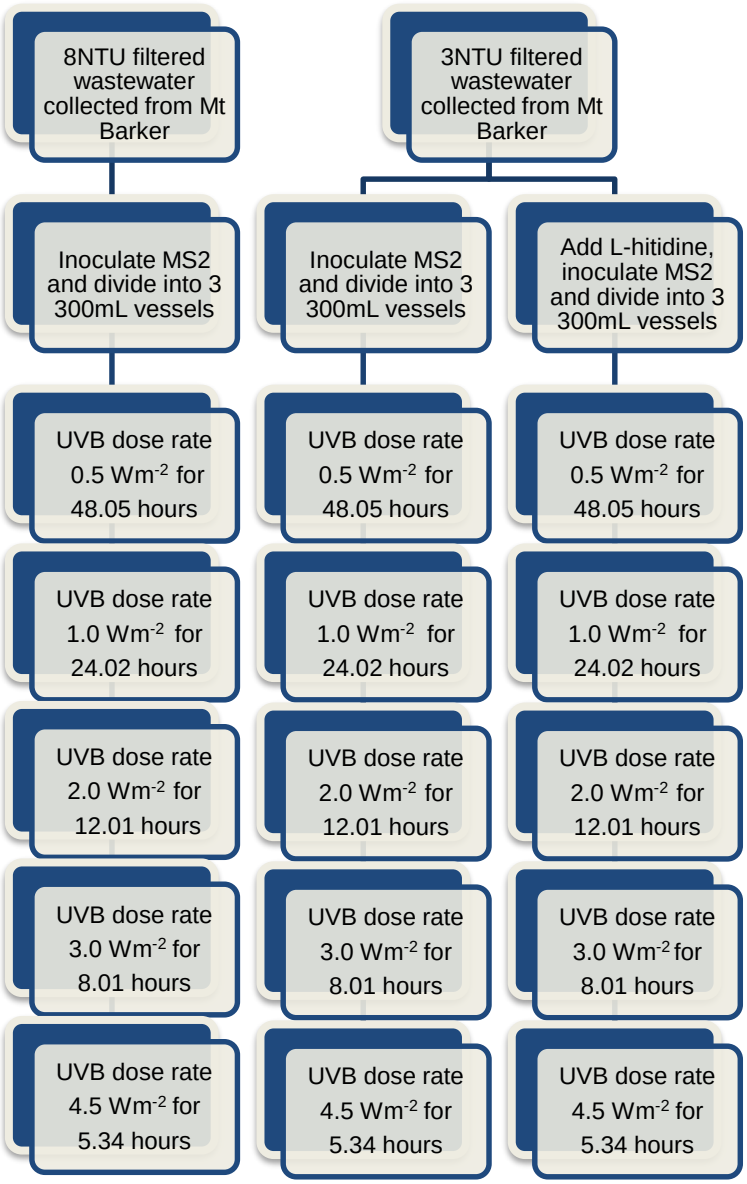


Figure 5–1: Flow chart for UVB irradiance experiments on filtered wastewater and filtered wastewater + L-histidine

5.2.4 Filtered wastewater characterisation

5.2.4.1 Dissolved organic carbon (DOC)

The concentration of dissolved organic carbon (DOC) was determined using a DOC-5000A (Shimadzu) total organic carbon (TOC) analyser. TOC standards (0-10 mg C L^{-1}) were prepared using $KHC_8H_4O_4$. Inorganic carbon (IC) standards (0-1 mg C $L^{-1)}$ were prepared using Na_2CO_3 and $NaHCO_3$. The method was APHA Test 2540 D

(Greenburg et al., 1992).

5.2.4.2 Turbidity

A HACH2100P turbidimeter (HACH Company, Loveland, Colorado, USA) was used to measure the turbidity (NTU).

5.2.5 MS2 stock preparation and quantification

Details about the MS2 stock preparation (ACTT#15597-B1) and quantification were described in Chapter 2. Briefly, the MS2 stock was made by incubating 5 mL of tryptone water over a plateful of MS2 plaques: the double layer method was applied to quantify the MS2 (Debartolomeis & Cabelli, 1991; Noble et al., 2004). Dilution, when necessary, was carried out in 9 ml of 0.5% tryptone water.

5.2.6 L-histidine

An $^{1}O_2$ quencher, 20 mmol of L-histidine (Sigma), was added to the low turbidity filtered wastewater (3NTU-W) samples prior to inoculating the MS2.

5.2.7 Statistical analysis

Results from previous studies (Chapter 4) had shown that the log-linear + tail model of Geeraerd and Van Impe's Inactivation Model Fitting Tool (GInaFiT) (Geeraerd et al., 2005) best modelled MS2 inactivation rates and consequently the same model was used for low turbidity filtered wastewater (3NTU-W), 3NTU-W + L-histidine and higher turbidity filtered wastewater (8NTU-W).

Two-way analysis of variance (ANOVA) was used to analyse the effect of dissolved organic matter (DOM), turbidity and the relationship between UVB dose rate (Wm^{-2}) – exposure time and UV dose (Jm^{-2}) on MS2 inactivation.

5.3 Results

5.3.1 Mt Barker wastewater characterisation

Filtered (0.2 µm) wastewater samples were obtained on two occasions from Mt Barker. Turbidity of 3 NTU and 8 NTU were recorded: the wastewater was defined as 3 NTU wastewater (3NTU-W) and 8 NTU wastewater (8NTU–W). The pH of both types of wastewater was 7.8. The concentrations of the dissolved organic carbon

(DOC) in 3 NTU-W and 8NTU-W were 13.48 mgC L^{-1} and 14.19 mgC L^{-1}, respectively. These DOC results were similar to those measured by Bolton (2011) from the same filtered wastewater resource (8.9 mg DOC L^{-1}). The UVB irradiance experiment was applied to both the filtered wastewater samples to determine the potential influence of slight turbidity difference the MS2 inactivation rate. The L-histidine was added to only the 3NTU-W to determine the influence of exogenous photosensitisers.

5.3.1.1 Effect of UVB dose rates

The study's experiments explored the effect of five UVB dose rates on the inactivation rates of MS2 in the 3NTU-W, in the same wastewater + L-histidine and in the 8NTU-W. The UVB inactivation rates from these incubations were compared with those in reverse osmosis (RO) water at 20ºC, described in Chapter 4.

No significant MS2 dark inactivation was observed over 48h in any of the three wastewater incubations. The results of modelling the MS2 UVB inactivation rate data using the $\log_{10}$-linear + tail model in GInaFiT (Geeraerd et al., 2005) are shown in Table 5-1. As the UVB dose rate increased from 0.5–4.5 Wm^{-2}, the MS2 inactivation rate K_i increased significantly in all three treatments (i.e. 3NTU-W and 3NTU-W+ L-histidine and 8NTU-W).

Corresponding results were found in the buffered (pH7.5) RO water from 0.5–4.5 Wm^{-2}. The response of the MS2 inactivation rate to increasing UVB dose rate in wastewater was different to that in the optically clear RO water (Figure 5-2). In the RO water, the K_i rate under a range of UVB dose rates was modified by the cubic polynomial model in which the rise of K_i could be divided into three phases, an indicative lag in inactivation response to UVB irradiation (0-0.5Wm^{-2}) followed by UVB light limited inactivation (0.5 – 3.0 Wm^{-2}) and a light-saturated MS2 inactivation (3.0 -6.0 Wm^{-2}). In contrast, the K_i maintained a steady rate of increase in the other three water resources from 0.5–4.5 Wm^{-2}, that is, 3NTU-W, 3NTU-W+ L-histidine 8NTU-W. This suggests that the inactivation rate measurements were within the UVB light limited phase of the inactivation rate curve, although curves fitted to the 3NTU-W and the 3NTU-W + L-histidine data (Equations 5-1 and 5-2, respectively) show some indication of the onset of light-saturated inactivation at >3.0 Wm^{-2}. The $\log_{10}$-linear relationship between UVB dose rate and inactivation rate in 8NTU-W is shown in Equation 5-3.

3NTU-W,

K_i (h^{-1}) = 0.5378 UVB dose rate (Wm^{-2}) + 0.0974 **Equation 5–1**

R^2 = 0.9664, P<0.001

3NTU –W + L-histidine,

K_i (h^{-1}) = 0.5269 UVB dose rate (Wm^{-2}) +0.0308 **Equation 5–2**

R^2 = 0.987, P<0.001

and for 8NTU-W,

K_i (h^{-1}) = 0.3684x UVB dose rate (Wm^{-2}) + 0.1647 **Equation 5–3**

R^2 = 0.9344, P<0.001

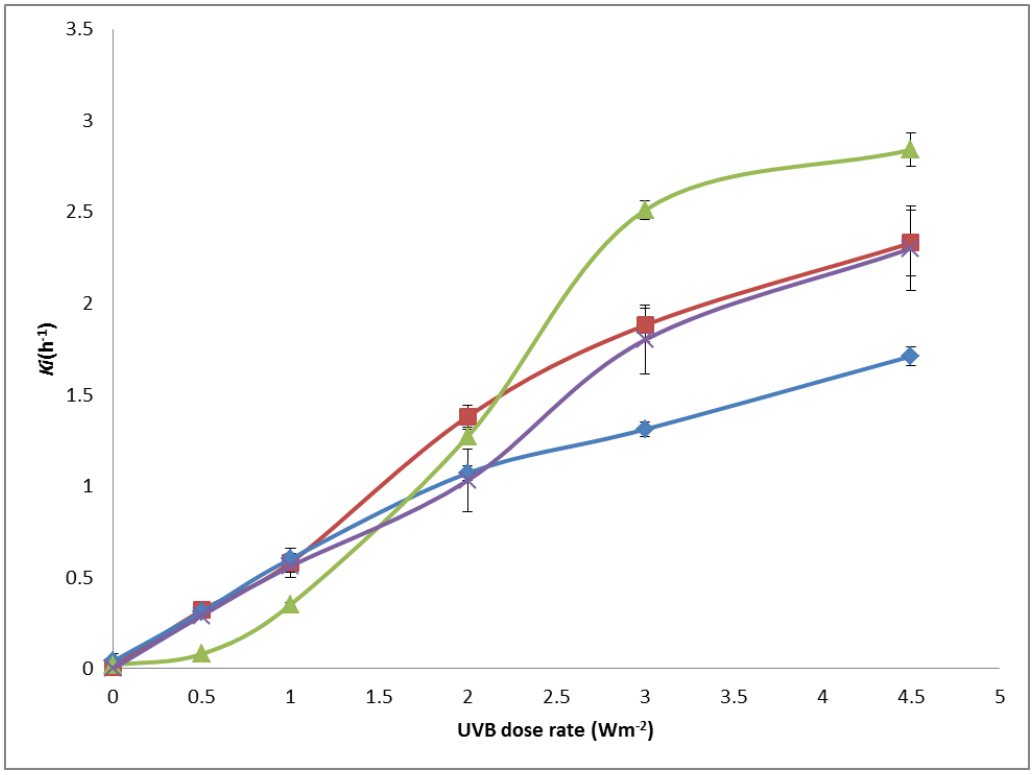


Figure 5–2: MS2 inactivation rates K_i (h^{-1}) in buffered 7.5 pH RO water (▲) at 20ºC, low turbidity filtered wastewater (■, 3NTU-W), 3NTU-W + L-histidine (x) and high turbidity filtered wastewater (♦, 8NTU-W) at UVB dose rates of 0.5, 1, 2, 3 and 4.5 Wm^{-2}

Table 5–1: MS2 inactivation rates K_i (h^{-1}, mean ± standard deviation, SD) at 20 ℃, in low turbidity filtered wastewater (3NTU-W), 3NTU-W + L-histidine and high turbidity filtered wastewater (8NTU-W) at UVB dose rates of 0.5, 1, 2, 3 and 4.5 Wm^{-2}

Note: n=number of data points.

Wastewater source	UVB dose rate (Wm^{-2})	Incubation time (h)	K_i (h^{-1})	SD	R^2	n
3NTU-W	Dark Control*	48	0.01	0.02	0.0213	18
	0.5	48.05	0.32	0.02	0.9951	18
	1	24.02	0.58	0.08	0.9786	18
	2	12.01	1.38	0.06	0.9969	18
	3	8.01	1.88	0.09	0.9969	18
	4.5	5.34	2.33	0.18	0.9874	18
3NTU-W + L-histidine	Dark Control*	48	0.00	0.01	0.0255	18
	0.5	48.05	0.29	0.01	0.9965	18
	1	24.02	0.56	0.03	0.9964	18
	2	12.01	1.03	0.17	0.9649	18
	3	8.01	1.8	0.19	0.9848	18
	4.5	5.34	2.3	0.23	0.9875	18
8NTU-W	Dark Control*	48	0.04	0.04	0.1845	18
	0.5	48.05	0.31	0.01	0.9992	18
	1	24.02	0.6	0.03	0.9984	18
	2	12.01	1.07	0.04	0.9984	18
	3	8.01	1.31	0.04	0.9985	18
	4.5	5.34	1.71	0.05	0.9991	18

Notes: K_i under UVB irradiance was calculated by the $\log_{10}$-linear + tail model of GInaFiT; K_i of the dark incubated control group was calculated by the $\log_{10}$-linear model of GInaFiT.

5.3.2 Effect of DOM

The study's experiments then explored the effect of DOM presence on five UVB dose rates in relation to the inactivation rates of MS2 in two filtered wastewater (NTU=3, DOC=13.48 mg/L, and NTU=8, DOC=14.19 mg/L,). The results from the UVB experiment in reverse osmosis (RO) water at 20ºC, as described in Chapter 4, were selected for the comparison. Two-way ANOVA and follow-up least significant difference (LSD) analysis was conducted for MS2 inactivation in the RO water, 3NTU-W and 8NTU-W. Overall the presence of DOC, has a significant influence on MS2 inactivation rates under UVB irradiance ($p<0.001$)

Comparing these three water groups, DOM was found to have a statistically significant interaction between UVB dose rates (F (8, 30) = 26.276, $p < 0.001$); therefore, in this experiment, the effect of UVB dose rates on K_i also depended on the concentrations of DOM. From the univariate tests, an analysis of the simple main effect (LSD) of DOM concentration on MS2 inactivation indicated statistical significance. For the simple main effect, DOM was found to be statistically significant at all five (5) UVB dose rates: 0.5 Wm^{-2} (F (2, 30) = 3.605, $p = 0.04 < 0.05$); 1 Wm^{-2} (F (2, 30) = 4.034, $p = 0.028 < 0.05$); 2 Wm^{-2} (F (2, 30) = 5.038, $p = 0.013 < 0.05$); 3 Wm^{-2} (F (2, 30) = 70.698, $p < 0.001$); and 4.5 Wm^{-2} (F (2, 30) = 69.014, $p < 0.001$).

Separately, the difference of MS2 inactivation rates caused by DOM between RO water and 3NTU-W ($p=0.058$) was found not as obvious as difference between RO water and 8NTU-W ($p<0.001$), so the effect of DOM on MS2 inactivation rate was more evident at higher DOM concentration. Pairwise comparisons were conducted to examine the effect of 3NTU-W, 8NTU-W and each UVB dose rate on K_i, with the 95% confidence intervals (CI) and p-values (LSD) reported. In terms of the K_i comparison for the RO water and 3NTU-W, at low UVB dose rates of 0.5 Wm^{-2} and 1 Wm^{-2}, K_i in the RO water was significantly lower than K_i in the 3NTU-W ($p=0.029$ and $p =0.023$, respectively). However, at UVB dose rate of 2 Wm^{-2}, the MS2 inactivation difference between RO water and 3NTU-W became insignificant ($p=0.294>0.05$). At the high UVB dose rates of 3 Wm^{-2} and 4.5 Wm^{-2}, UVB inactivation rates were significantly higher in the RO water and 3NTU-W (in both, $p<0.001$). The variable effect on K_i in the RO water and 3NTU-W was probably due to the inverse effect of ROS and turbidity caused by DOM exogenous photosensitisers in 3NTU-W. Similar observation was found between RO water and 8NTU-W.

Comparing K_i for MS2 in the 3NTU-W and 8NTU-W, significant difference was found (P<0.001) between K_i of these two groups. At the lower UVB dose rates of 0.5 Wm^{-2} and 1 Wm^{-2}, almost no difference was found, with p = 0.949 and p = 0.986. In contrast, significant difference occurred at 2 Wm^{-2} (p = 0.004 <0.05), 3 Wm^{-2} (p < 0.001) and 4.5 Wm^{-2} (p < 0.001). Overall, the negative effect of high DOM concentration on MS2 inactivation rates K_i under the environmentally relevant UVB dose rates increased as the UVB dose rates rose from 2 Wm^{-2} to 4.5 Wm^{-2}.

5.3.3 Effect of L-histidine

This part of the study explored the effect of L-histidine on five UVB dose rates in relation to the inactivation rates of MS2 by comparing experiments in the 3NTU-W (NTU=3, DOC=13.48 mg/L), the 3NTU-W + L-histidine and MS2 inactivation rates in RO water (DOC=0 mg/L, NTU=0) at 20ºC.

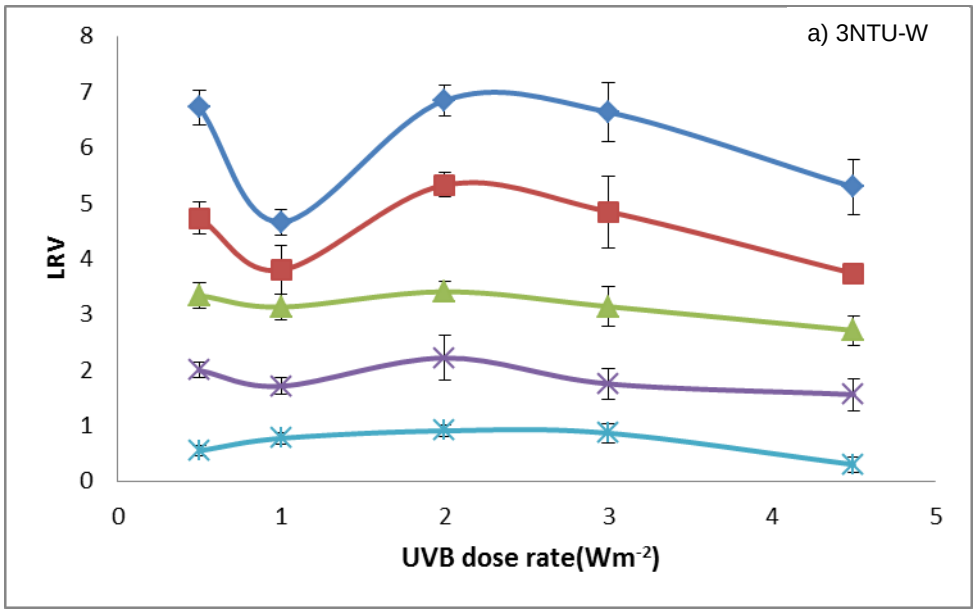
The rationale for the addition of L-histidine was that it would suppress any inactivation associated with the production of ROS through the interaction between UVB and exogenous photosensitisers. This would result in the inactivation rates in the presence of L-histidine being lower than those in the 3NTU-W alone. A two-way ANOVA and follow-up least significant difference (LSD) analysis was conducted on the MS2 inactivation in 3NTU-W and 3NTU-W + L-histidine and the RO water to identify the effect L-histidine on UVB disinfection. There was a statistically significant interaction between UVB dose rates and the MS2 inactivation within RO water the respective types of wastewater, namely, 3NTU-W and 3NTU-W + L-histidine (DOM exogenous photosensitisers) (i.e. F (8, 30)=11.761 and p<0.001, which means that the effect of DOM on MS2 inactivation rates K_i depends on the level of the UVB dose rates, thus clearly indicating the existence of exogenous photo inactivation.

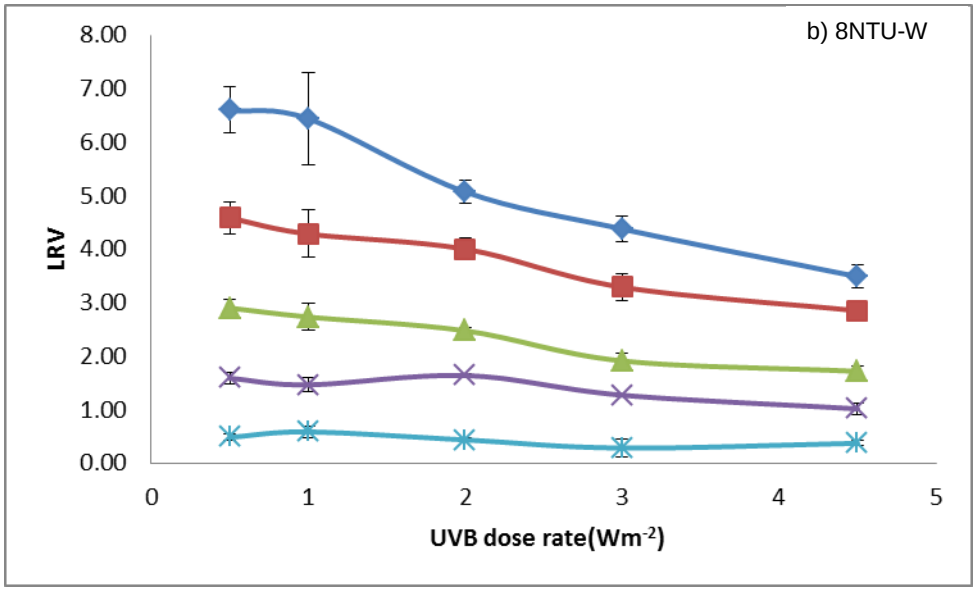
Compared to 3NTU-W alone, the addition of L-histidine to 3NTU-W changed the MS2 inactivation rates significantly overall (p = 0.004), with the influence of L-histidine independent from that of the UVB dose rates. Under the low UVB dose rates of 0.5 Wm^{-2} and 1 Wm^{-2}, no statistically significant difference was observed, with p-values of 0.788 and 0.687 and differences between the means being 0.028 and 0.042, respectively. In comparison to the differences between the RO water and 3NTU-W, almost no difference occurred to K_i in the 3NTU-W and 3NTU-W + L-histidine. As the UVB dose rates increased, the means of K_i became significantly different at 2 Wm^{-2} (p = 0.02 < 0.05), the differences reaching peak values at 0.352 (95% CI, from 0.142 to 0.563). However, the K_i of these two groups remained

insignificant at UVB dose rates of 3 Wm^{-2} (p = 0.331) and 4.5 Wm^{-2} (p = 0.497): the differences also reduced to 0.102 (95% CI, from 0.109 to 0.312) and 0.071 (95% CI, from 0.139 to 0.281). Therefore, of the five UVB dose rates, the addition of L-histidine caused the most evident significant decrease in K_i at a UVB dose rate of 3 Wm^{-2}.

5.3.4 Manner of UVB dose delivery

The effect of varying dose rate (Wm^{-2}) and exposure time (h) to deliver the same UVB dose (Jm^{-2}) on MS2 inactivation was explored in 3NTU-W, 3NTU-W + L-histidine and in 8NTU-W (Figure 5-3). Two-way ANOVA and follow-up least significant difference (LSD) analysis were applied to compare the significance of UVB dose (Jm^{-2}) and dose rates (Wm^{-2}) on MS2 log_{10} reduction value (LRV).





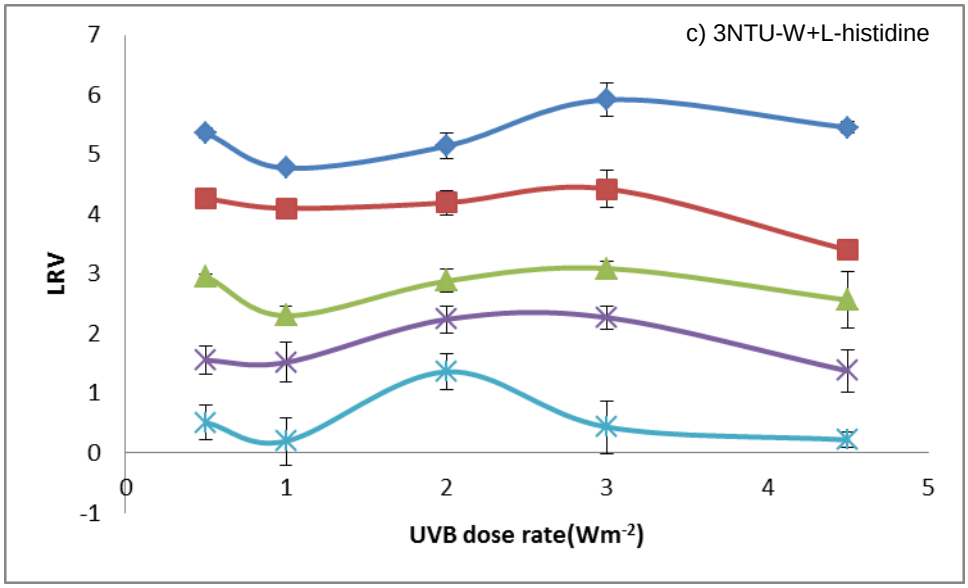


Figure 5–3: MS2 $\log_{10}$ reduction values (LRV) in (a) 3NTU-W (b) 8NTU-W and (c) 3NTU-W + L-histidine using 5 UVB dose rates–time combinations: 0.5, 1, 2, 3, 4.5 and 6 Wm^{-2} to achieve 5 UVB doses: 6126 (*), 22049 (×), 37973 (▲), 62231 (■) and 86490 (◆) Jm^{-2} at 20˚C

As with the experiment for MS2 $\log_{10}$ reduction value (LRV) under UVB irradiance in the RO water at 20ºC, in the 3NTU-W, UVB dose rates (Wm^{-2}) were found to significantly interact with the UVB dose (Jm^{-2}) and influence MS2 log reduction ($p <$ 0.001). However, for the three lowest UVB doses no significant difference was found between inactivation rate (K_i) and dose rate within UVB doses of 6126, 22049 and 37973 Jm^{-2} overall (p =0.097, p = 0.82 and p = 0.61, respectively). That is the LRV at a particular dose was generally similar irrespective of the dose rate-exposure time combination used to deliver that dose (Figure 5-3). This phenomenon was only

observed at 6126 Jm^{-2} in the RO water at 20ºC. Significant differences of the LRV were observed at the two highest UVB doses: 86490 Jm^{-2} ($p < 0.001$) and 62231 Jm^{-2} ($p < 0.001$). These two UVB dose curves showed a similar tendency with the LRV decreasing sharply from 0.5 Wm^{-2} to 1 Wm^{-2}, then increasing rapidly reaching a peak at 2 Wm^{-2}. The LRV then remained almost unchanged from 2 Wm^{-2} to 3 Wm^{-2}, and then decreased to the minimum 4.5 Wm^{-2}.

In summary, in the presence of DOM exogenous photosensitisers, at relatively low UVB doses, the dose rate-exposure time combination used to deliver the dose did not influence the MS2 inactivation. The MS2 LRV increased with increasing UVB dose and ranged from 1.0 to 7 LRV between doses of 62231 Jm^{-2} and 86490 Jm^{-2} respectively, which were equivalent to winter and summer UVB doses recorded in South Australia. However, at high UVB doses, the UVB dose rates had significant influence on the MS2 log reduction value (LRV). Compared to the MS2-UVB experiments in the RO water at 20ºC, the effect of UVB dose rates on the MS2 LRV was less obvious in the 3NTU-W under the same UVB doses.

Figure 5-3(b) shows the MS2 log reduction value (LRV) at five UVB doses and five UVB doses rates in 8 NTU filtered wastewater (8NTU-W). In 8NTU-W, the UVB dose rates were also found to have a significant interaction with UVB doses in regard to MS2 inactivation overall ($p < 0.001$). While the effect of the dose rate on the MS2 LRV at a dose of 6126 Jm^{-2} was found to be insignificant ($p = 0.685 > 0.05$), for the other four (4) UVB doses, the dose rates were observed to have a statistically significant influence on the mean differences of MS2. For the four highest UVB dose curves, it was obvious that the LRV decreased with the rise in UVB dose rate.

Overall, the MS2 LRV in the 8NTU-W showed that increasing the environmentally relevant UVB dose rates (Wm^{-2}) and adjusting exposure time to achieve the same UVB dose resulted in less MS2 inactivation. This was observed at >3Wm^{-2} in 3NTU-W whereas the decrease in LRV at all does was observed at >1 Wm^{-2} in 8NTU-W.

As shown in Figure 5-3(c), the MS2 log reduction value (LRV) was explored under five UVB doses and five UVB dose rates in the 3NTU-W + L-histidine. Based on two-way ANOVA, the UVB dose rates caused significant mean differences within the same UVB doses. Taken separately, significant mean differences were detected for all five curves ($p < 0.05$). These five curves in the 3NTU-W + L-histidine displayed a similar trend to that shown by the two highest UVB doses in the 3NTU-W as the

UVB dose rates increased. This could be divided into three phases: the first phase was a decrease from 0.5 Wm^{-2} to 1 Wm^{-2}; followed by increase in LRV to the peak of each curve at 2 Wm^{-2} or 3 Wm^{-2}; with a final decrease through to the end of the UVB dose curve.

5.4 Discussion

As described in this chapter, the study continued to explore the effect of UVB doses and UVB dose rates on different mechanisms of the UVB photoinactivation of MS2, in wastewater. In Chapter 4, all experiments were conducted in the RO water, in which the direct inactivation mechanism was the only one effecting MS2 die-off. Therefore, in the current chapter, filtered wastewater was selected to determine the potential for exogenous photo-oxidation to influence MS2 inactivation when irradiated with UVB. Dissolved exogenous photosensitisers are able to absorb UVB irradiance to form lethal ROS, thus promoting MS2 inactivation, but the presence of dissolved exogenous photosensitisers also enhances the water's turbidity. In the current study, UVB-MS2 experiments were conducted in low turbidity filtered wastewater (3NTU-W) (DOC=13.48 mg/L, NTU=3), 3NTU-W with L-histidine and higher turbidity filtered wastewater (8NTU-W) (DOC=14.19 mg/L, NTU=8). The results are discussed below.

The NTU and DOM, of filtered wastewater had a significant effect on MS2 UVB inactivation ($p < 0.001$). The K_i of 3NTU-W was higher at UVB dose rates>1.0 Wm^{-2} than that of the 8NTU-W, which also had a slightly higher DOM concentration. This suggests that small changes in turbidity can have a significant influence on inactivation.

Two attenuation related equation developed by Bolton (2011) supported the deduction above:

$$Z_{1\%} = \frac{\mathrm{In}(100)}{\mathrm{Ka}} \qquad \textbf{Equation 5–4}$$

$$\mathrm{KaUVB} = (2.9 log_{10}\mathrm{NTU})^2 + 7.3 \qquad \textbf{Equation 5–5}$$

where,

$Z_{1\%}$ = the 1% penetration depth,

Ka = the attenuation coefficient.

The equations predicted and calculated attenuation of UVB based on a range of surface water environments measurement. According to Equations 5-4 and 5-5, the increase of NTU would enhance UVB attenuation significantly.

The specific effect of turbidity also appeared to depend on the UVB dose rate. This maybe because at the low UVB dose rates small differences in low K_i values are more difficult to detect than at high dose rates with higher relative K_i values. The relative K_i values, RO>3NTU-W>8NTU-W, reflect the NTU of the RO water and wastewater and by inference the effect of increasing UVB attenuation.

The two filtered wastewater samples had turbidity of 3 and 8, which were within the common turbidity ranges used in other research (Liu & Zhang, 2006; Oppenheimer, 2002). Few studies on the effect of turbidity have focused on UVB, with UVC being more frequently investigated. Passantino et al. (2004) concluded that, by adding low turbidity (montmorillonite clay, 12 NTU) with algae (42000 cells/mL) to unfiltered drinking water, the MS2 inactivation rates did not change under UVC irradiance. Although the level of turbidity chosen was higher than in the current study, the inactivation ability of UVC is known to be greater than that of UVB: therefore, it is consistent for Passantino et al. (2004) to conclude that one possible reason for their result was the relatively low levels of turbidity and algae. In contrast, Liu and Zhang (2006) tested the influence of three levels of turbidity, 0.5, 4 and 12 NTU, on UVC inactivation of MS2 and other bacteria. Three UVC dose rates of 1.8, 1.0 and 0.08 Wm^{-2} were chosen. In contrast to the finding for UVB presented here, they reported that at a high UVC dose rate, the influence of turbidity was less obvious than at a low UVC dose rate, especially for MS2. They argued that the reason why the high UVC dose rate overcame the negative influence of turbidity was because MS2 did not associate with the particles.

The presence of L-histidine in 3NTU-W was found to significantly decrease the overall MS2 inactivation rate K_i ($p = 0.004 < 0.05$) when compared with 3NTU-W in the absence of L-histidine. Furthermore, the difference between the values of the mean of K_i in these two groups was also significant ($p < 0.001$). The greatest reduction in K_i in the presence of L-histidine was observed at 2 Wm^{-2}. It could be considered that, for the current vessel geometry, turbidity and DOC concentration, that the formation rate of $^1O_2$ reached its peak, among the natural environmentally relevant UVB dose rates, at 2 Wm^{-2}. The ROS $^1O_2$ is short-lived and is easily quenched by water the relative rates of production and quenching would be expected to influence the equilibrium and availability of $^1O^2$ and effective indirect

photoinactivation (Cory et al., 2008; Zepp et al., 1977).

The addition of the $^1O_2$ quencher L-histidine to 3NTU-W provided evidence that an interaction between UVB and exogenous photosensitisers could result in the production of ROS which contributes to the photoinactivation of MS2. Although the results presented here provide supporting evidence for ROS production by UVB with exogenous photosensitisers the magnitude of the influence of indirect UVB photoinactivation of MS2 remains unclear. The results presented here suggest that direct UVB photoinactivation of MS2 was the predominant mechanism. In contrast, Kohn and Nelson (2006) using four different ROS quenchers concluded that, for UVB light, exogenous photoinactivation was more significant than direct photoinactivation, and further, that among all four ROS that were formed, $^1O_2$ (quenched by L-histidine) was the most important. It may be inferred from the results recorded here, where inactivation was greater in 3NTU-W than in RO water, at dose rates of 0.5 Wm^{-2} and 1 Wm^{-2}, that ROS other than $^1O_2$, were potentially formed.

Other pertinent research studies have supported the current study's findings at 0.5 m^{-2} and 1 Wm^{-2} in low turbidity filtered wastewater. Davies-Colley et al. (1999) explored inactivation rates for the F-specific RNA bacteriophage (F-RNA bacteriophage) under natural sunlight in unfiltered wastewater and in filtered wastewater. Using a step-by-step process, the wastewater from a wastewater stabilisation pond (WSP) was filtered by a 10 µm pre-filter, then by a 1.2 µm grade GF/C filter, followed by a 0.7 µm grade GF/F filter. They observed that the F-RNA bacteriophage was inactivated faster when WSP dissolved matter, even WSP solids, was present: both the dissolved matter and solids were considered to be potential exogenous photosensitisers in their experiment.

Bolton (2011) also collected filtered wastewater samples from Mt Barker WWTP, and tested the MS2 and ϕX174 (phage) inactivation rates under UVB, UVA and visible light (Vis) irradiance. However, her study employed only a single UVB dose rate of 1.1 Wm^{-2}. Similar to the findings reported here, she also reported that in the presence of DOM in Mt Barker wastewater, the MS2 UVB inactivation rate was significantly lower than that of RO water. The MS2 inactivation rate K_i measured by Bolton was 0.254 to 0.289 h^{-1}, which was almost half the K_i (0.58h^{-1}) reported here. This may have been due to the differences in DOC between the two filtered wastewater samples used in the respective studies. The DOC in the filtered wastewater used by Bolton (8.9 $mgCL^{-1}$) was almost half that of the DOC in the wastewater used here. A higher concentration of DOC likely produces more reactive

oxygen species (ROS). The difference in K_i may also be due to the different duration of the experiments with Bolton's experiment running for eight (8) hours while the current study ran for 24 hours. Furthermore, different models were used by the studies to 'best fit' the data from MS2-UVB experiments.

The results presented here, and consideration of those of others, highlights the complexity of the influence of turbidity and DOM on the magnitude of MS2 UVB inactivation. The inactivation rate is influenced by the magnitude of UVB attenuation by DOM and the production of ROS via photosensitisation.

Many research studies have reported that, when the same UVC doses were delivered at different dose rates, the micro-organisms' log reduction values (LRVs) were different (Liu & Zhang, 2006; Murakami et al., 2006; Sommer et al., 1998). However, few studies have been conducted on UVB dose rates. Therefore, in the current study, the experiments were set up to determine the influence on MS2 LRV of the manner of the UVB dose delivery, delivering five UVB doses at five different UVB dose rates in 3NTU-W, 8NTU-W and 3NTU-W + L-histidine. In both RO water and 8NTU-W at 20ºC, it was found that for all UVB doses, except the lowest (6126 Jm^{-2}), the UVB dose rate (Wm^{-2}) used to achieve the dose (Jm^{-2}) influenced MS2 LRV. At the same UVB doses, the MS2 LRV in the 3NTU-W + L-histidine was similarly influenced by dose rates. In the 3NTU-W, however, UVB dose rate changes had less impact on LRV at the same UVB doses.

5.5 Conclusion

In this chapter, the effects on MS inactivation of DOM, turbidity and the manner of the UVB dose delivery were investigated. Experiments were conducted on three groups, namely, 3NTU-W, 3NTU-W + L-histidine and HTFW (8NTU-W) under five different UVB doses and five different UVB dose rates. To the author's knowledge, this is the first study that has systematically investigated the effect of such a range of environmentally relevant UVB doses and dose rates on inactivation of MS2 in wastewater.

The following conclusions were made:

- MS2 inactivation was inversely related to NTU and associated increases in UVB attenuation.
- The negative effect of NTU on the inactivation rate constant was more obvious at high UVB dose rates (Wm^{-2}).

- Exogenous photoinactivation was confirmed to be a process contributing to the UVB disinfection process.
- The dose rate (Wm^{-2}) used to deliver the UVB dose (Jm^{-2}) significantly influenced MS2 LRV in 3NTU-W, 3NTU-W + L-histidine and 8NTU-W.

In the next chapter, the effect of raw wastewater and raw wastewater + L-histidine on MS2 inactivation at a range of UVB doses and dose rates is presented.

CHAPTER 6: DOSE DEPENDENCE ON UVB DISINFECTION IN RAW WASTEWATER AND RAW WASTEWATER + L-HISTIDINE

6.1 Introduction

Ultraviolet B (UVB) inactivation in raw wastewater is affected by far more complicated factors than is the case in reverse osmosis (RO) water and unfiltered wastewater. It is largely dependent on the characteristics of the wastewater,in field conditions, the wastewater characteristics are seen to be variable with time. Several physical, chemical and biological factors, for example, temperature, pH, dissolved oxygen and algal production in raw wastewater influence the UV inactivation process (Shilton, 2005a), and these factors perform overlapping, correlated and sometimes competing roles in high-rate algal ponds (HRAPs) (Buchanan, 2014). For instance, attenuation is a very significant concept to consider in relation to UV inactivation in a water environment. Attenuation is defined as the effect of light absorption and scattering in water: in raw wastewater, it depends on physicochemical characteristics like suspended solids (SS), humic substances, algal production and turbidity. This chapter describes the MS2 inactivation experiments in wastewater and wastewater + L-histidine under different UVB doses and dose rates which were conducted to explore the effect of raw wastewater(unfiltered) constituents on UVB inactivation. Reverse osmosis (RO) water and filtered wastewater data were selected in these experiments for comparison.

Several important constituents in wastewater were measured and recorded before and after the experiments, including pH, dissolved oxygen (DO), dissolved organic carbon (DOC), SS, chlorophyll *a* and turbidity. These measurements likely influence the UV inactivation process positively or negatively. Kirk (1994) concluded that in a natural water environment, all the UV absorption could be attributed to four components: water, dissolved yellow pigments, the photosynthetic biota and inanimate particulate matter, with light attenuation the result of both absorption and the scattering process. In this study, DOC, chlorophyll *a* and SS refer to three of these components, respectively: dissolved yellow pigments, the photosynthetic biota and particulate matter (comprising both biotic and abiotic solids). Generally, an increase in DOC, SS and algae undoubtedly increases the UV attenuation, especially for UVB due to its short wavelength (Bolton, 2011; Bolton et al., 2011). De Lange (2000) measured the relationship between attenuation and water properties

of 19 different inland waters in the Netherlands, concluding that, for UVB irradiance attenuation, on average, 30% can be attributed to ash weight (SS), 30% was absorbed by the water itself, 20% was attributed to dissolved absorption (DOC) with 20% also attributed to chlorophyll *a*.

However, conflicting results have been found regarding the contributors to UV attenuation in natural water environments. Morris et al. (1995) included in their study of attenuation measurement at 65 sites from 59 lakes in Alaska and Colorado in the United States (USA). Their results suggested that more than 85% of variation in UV transparency could be solely explained by bulk concentration of dissolved organic carbon (DOC). Scully and Lean (1994) reached almost the same conclusion. In another study, Balogh, Németh and Vörös (2009) investigated the role of chromophoric dissolved organic matter (CDOM), algal-free suspended solids (SS) and algae in sunlight attenuation. For UVB irradiance attenuation, they found that CDOM played the major role in water bodies (54–100%), next was algal-free attenuated suspended solids (SS) (55–75%), while the effect of algae was negligible in most samples from lakes. However, the effect of algal concentration in light attenuation was observed (1994) for wastewater stabilisation pond (WSP) water. Buchanan (2014) tried to summarise the relationship between algal production and suspended solids (SS) in WSPs and HRAPs: interestingly, algal concentration was not always a good predictor for SS concentration in WSPs but, in HRAPs, it was a good predictor. Furthermore, the increase of UV attenuation caused by these constituents does not necessarily reduce the UV radiance inactivation of micro-organisms in wastewater. Kohn and Nelson (2006) found that MS2 die-off was faster in unfiltered WSP water than in filtered WSP water under a solar stimulator. Other factors in WSP wastewater such as sedimentation and ingestion by higher organisms may also potentially influence the MS2 inactivation rates (Shilton, 2005a).

The confusing effects of separate wastewater constituents are not the focus in the current study: instead, the study explores their combined effect on UVB inactivation which is meaningful for modelling HRAP pathogen inactivation. Rarely has the research focus on UVB inactivation mechanism specifically in raw wastewater. Therefore, the aims of the experiments described in this chapter were to:

- Assess the effect of dose rate and dose of UVB on MS2 inactivation in raw wastewater.
- Compare the effect of UVB inactivation on MS2 in raw wastewater to that in RO water and filtered wastewater to assess the effect of wastewater

constituents on MS2 inactivation.

6.2 Methods

6.2.1 Raw wastewater collection

The raw wastewater was collected in January 2015 from the wastewater treatment plant (WWTP) at Mount Barker, South Australia. The Mount Barker WWTP receives domestic sewage from on-site septic tanks, so the large solids had already been removed. This WWTP's average daily flow (ADF) is approximately 2.5 ML. As mentioned in the previous chapter, the Mount Barker WWTP consists of a large anaerobic effluent pond (200 m × 130 m × 3.5 m deep), a polishing lagoon, a dissolved air flotation (DAF) unit and a continuous microfiltration (CMF) unit. The exact sampling location chosen for collecting the raw wastewater was at one corner of the effluent pond which was close to the inlet pipe. Approximately 20 litres of raw wastewater were taken and stored at 4ºC in the dark in the laboratory, and were used for the experiments within two weeks of collection. All the experiments were run at 20ºC.

6.2.2 UVB irradiation

As shown in Appendix 1, vessels were placed under the UVB lamps. Five environmentally relevant UVB dose rates of 0.5, 1, 2, 3 and 4.5 Wm^{-2} were chosen in this study. For the dark incubated, control incubations, the vessels were totally wrapped in aluminium foil.

6.2.3 Experiment design

The duration of the incubation to achieve the UVB dose at various dose rates in raw wastewater was calculated (Figure 6-1).

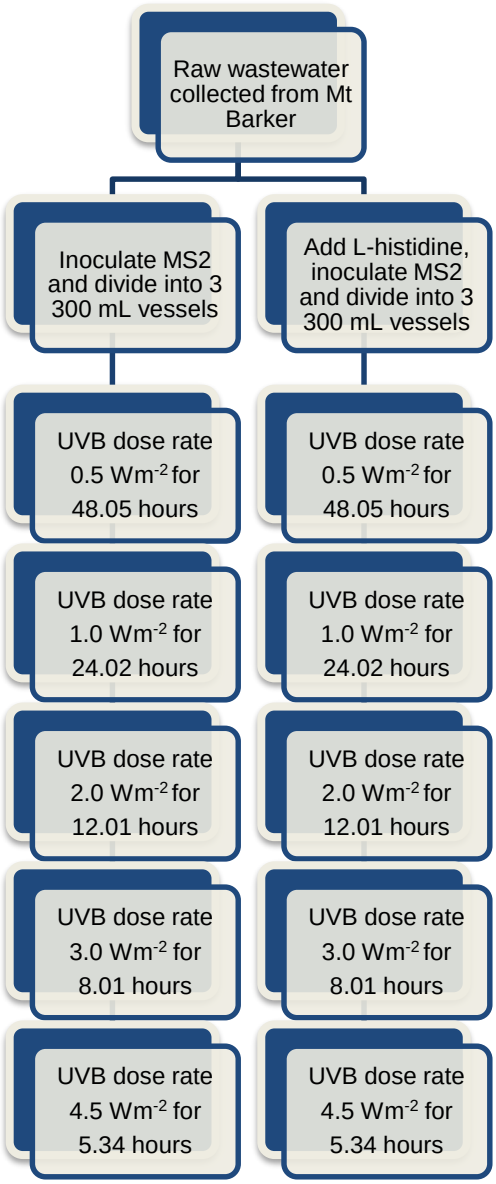


Figure 6–1: Flow chart for conducting the MS2 UVB inactivation experiments in raw wastewater and raw wastewater + L-histidine

6.2.4 Raw wastewater characterisation

Details about raw wastewater characterisation measurement were described in Chapter 2, including the concentration of dissolved organic carbon (DOC), turbidity,

suspended solids (SS) and chlorophyll *a*.

6.2.5 MS2 stock preparation and quantification

Details about the MS2 stock preparation (ACTT#15597-B1) and quantification were described in Chapter 2. Briefly, the MS2 stock was made by incubating 5 mL of tryptone water over a plateful of MS2 plaques on a lawn of host *E. coli*: the double layer method (Debartolomeis & Cabelli, 1991; Noble et al., 2004) was applied to quantify the MS2.

6.2.6 L-histidine

A singlet oxygen ($^1O_2$) quencher, comprising 20 mmol of L-histidine (Sigma), was added to the raw wastewater samples prior to inoculating the MS2.

6.2.7 Statistical analysis

Geeraerd and Van Impe's(2005) Inactivation Model Fitting Tool (GInaFiT) was applied to all data to obtain the MS2 inactivation rates under UVB and in the dark to find the best fitting model. The log_{10}-linear model was found to be the best fit for the dark incubated control group in the wastewater experiment, while the log_{10}-linear + tail model was the best fit for the UVB incubated experiments. To compare the influence of raw wastewater constituents on UVB inactivation of MS2 with those of RO water, filtered wastewater and filtered wastewater + L-histidine, two-way ANOVA in the Statistical Package for the Social Sciences (SPSS) was applied.

6.3 Results

6.3.1 Mt Barker raw wastewater characterisation

The characteristics of the Mt Barker WWTP's raw wastewater were measured twice, before and after the experiments. As anticipated, the measurements did not vary significantly; the values shown below are the average of the two measurements. The average pH value was 7.7, similar to that of the buffered RO water (Chapter 4; pH7.5) and the filtered wastewater (Chapter 5). The average DO was 7.21 mg L^{-1}, a little lower than that for RO water (8.56 mg L^{-1}) and filtered wastewater (9.1–9.6 mg L^{-1}). The average DOC measured in the wastewater was 62.23 mg L^{-1} which was more than four times higher than that for the filtered wastewater samples in Chapter 5. The turbidity measured was 94.9 NTU, far higher than for the filtered wastewater (3–8 NTU): this was due to high DOC, SS (60 mg L^{-1}) and algae (chlorophyll *a* 737.4 µg L^{-1}).

6.3.2 MS2 dark inactivation rate (control group) in Mt Barker WTTP raw wastewater

It was important to check whether the MS2 dark inactivation rate was significant in the Mt Barker WWTP wastewater, and to compare this with the results for HRAP wastewater, RO water and filtered wastewater.

MS2 dark inactivation was detected in the Mt Barker raw wastewater and raw wastewater + L-histidine, the average inactivation rates, determined over three days, were 0.04 and 0.03 K_i (h^{-1}) respectively. In comparison with the MS2 dark inactivation rate in the HRAP inlet wastewater (K_i 0.163 h^{-1} measured over six days), the inactivation rate for MS2 was obviously slower in the Mt Barker WWTP raw wastewater.

6.3.3 Effect of UVB dose rates

Five environmentally relevant UVB dose rates were applied to the MS2 inactivation experiments in the raw wastewater and raw wastewater + L-histidine to assess the effect of dose rates on UVB inactivation. The results were compared with those obtained from the RO water experiment results at 20ºC (Chapter 4), filtered wastewater (3NTU-W), 3NTU-W + L-histidine and filtered wastewater (8NTU-W; Chapter 5).

As shown in Figure 6-2, the MS2 inactivation rates (K_i) generally increased as the UVB dose rate (Wm^{-2}) increased in raw wastewater and raw wastewater + L-histidine, this finding was the same as for the RO water and filtered wastewater (3NTU-W and 8NTU-W) experiments. The relationship between inactivation rate (K_i) and dose rate recorded for the RO water group was similar when compared with the other treatments. However, in the RO water incubations the K_i showed an initial lag phase, followed by a UVB dose rate-limited phase (0.5–3.0 Wm^{-2}) and a UVB dose rate-saturated phase (3.0–4.5 Wm^{-2}). A similar shaped curve was evident for all incubations but appeared to be moderated by increasing complexity of the water matrix.

The log_{10}-linear model was applied to 3NTU-W, 3NTU-W + L-histidine and 8NTU-W in the experiments described in Chapter 5. In the current chapter, it was also found that the log_{10}-linear model was the best fit for the raw wastewater (Equation 6.1)

$$K_i\,(h^{-1}) = 0.2421 * \text{UVB dose rate } (Wm^{-2}) + 0.084 \qquad \textbf{Equation 6–1}$$

$R^2 = 0.963$, $P<0.001$

and for raw wastewater + L-histidine (Equation 6-2),

$$K_i\ (h^{-1}) = 0.2492*\text{UVB dose rate}\ (Wm^{-2}) + 0.0402 \qquad \text{Equation 6–2}$$

$R^2 = 0.981$, $P<0.001$

The log_{10}-linear slope was less in the two types of raw wastewater. The K_i (h^{-1}) values for the raw wastewater, wastewater + L-histidine were 1.07 ± 0.08 and 1.12 ± 0.09, respectively, which were also much lower rates than those recorded for the other treatments.

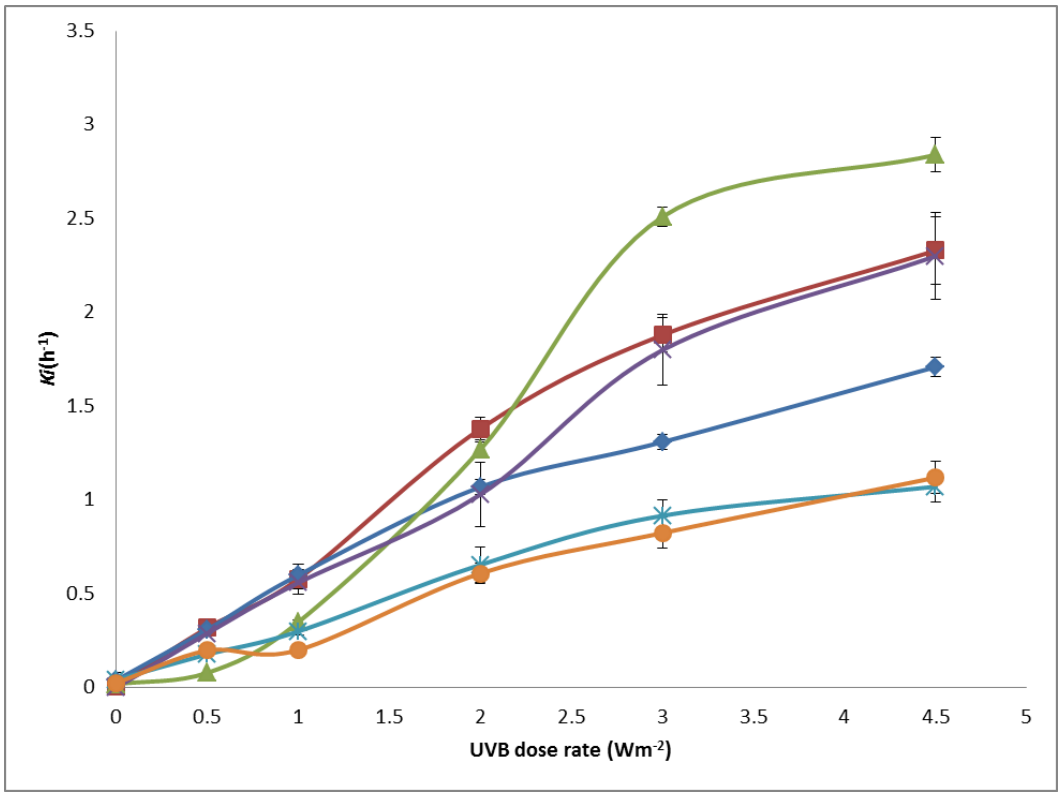


Figure 6-2: MS2 inactivation rates K_i (h^{-1}) in raw wastewater (✱), raw wastewater + L-histidine (●), compared with buffered 7.5 pH RO water (▲) at 20ºC, 3NTU-filtered wastewater (■), 3NTU-wastewater + L-histidine (x) and 8NTU-filtered wastewater (♦) under 0, 0.5, 1, 2, 3 and 4.5 Wm^{-2} UVB irradiance

Table 6–1: MS2 UVB inactivation rates K_i (h^{-1}) in in raw wastewater (RAW WW), raw wastewater + L-histidine (RAW WW + L-histidine), compared with buffered 7.5 pH RO water at 20ºC, 3NTU filtered wastewater (3NTU-W), 3NTU-W + L-histidine and 8NTU filtered wastewater (8NTU-W) under 0.5, 1, 2, 3 and 4.5 Wm^{-2} UVB irradiance

Water source	UVB dose rate (Wm^{-2})	Incubation time (h)	K_i	SD	R^2	N
RO water (20ºC)	0	48	0.02	0	0.9725	18
	0.5	48.05	0.08	0	0.9976	18
	1	24.02	0.35	0.01	0.9994	18
	2	12.01	1.27	0.04	0.9982	18
	3	8.01	2.51	0.05	0.9993	18
	4.5	5.34	2.84	0.09	0.9988	18
3NTU-W	0	48	0.01	0.02	0.0213	18
	0.5	48.05	0.32	0.02	0.9951	18
	1	24.02	0.58	0.08	0.9786	18
	2	12.01	1.38	0.06	0.9969	18
	3	8.01	1.88	0.09	0.9969	18
	4.5	5.34	2.33	0.18	0.9874	18
3NTU-W + L-histidine	0	48	0	0.01	0.0255	18
	0.5	48.05	0.29	0.01	0.9965	18
	1	24.02	0.56	0.03	0.9964	18
	2	12.01	1.03	0.17	0.9649	18
	3	8.01	1.8	0.19	0.9848	18
	4.5	5.34	2.3	0.23	0.9875	18
8NTU-W	0	48	0.04	0.04	0.1845	18
	0.5	48.05	0.31	0.01	0.9992	18
	1	24.02	0.60	0.03	0.9984	18
	2	12.01	1.07	0.04	0.9984	18
	3	8.01	1.31	0.04	0.9985	18
	4.5	5.34	1.71	0.05	0.9991	18
RAW WW	0	61.42	0.04	0.00	0.995054	12
	0.5	48.05	0.18	0.01	0.998986	18
	1	24.02	0.30	0.02	0.994954	18
	2	12.01	0.66	0.10	0.976636	18
	3	8.01	0.92	0.08	0.991605	18
	4.5	5.34	1.07	0.08	0.995124	18
	0	61.42	0.03	0.00	0.974114	12
	0.5	48.05	0.20	0.01	0.99894	18
	1	24.02	0.20	0.03	0.988085	18

RAW WW + L-histidine	2	12.01	0.61	0.06	0.993596	18
	3	8.01	0.83	0.08	0.990249	18
	4.5	5.34	1.12	0.09	0.995806	18

6.3.4 Effect of wastewater constituents

The experiments described in this chapter investigated MS2 inactivation rates at a range of UVB dose rates in raw wastewater from the Mt Barker WWTP (DOC=62.23 mgL^{-1}, NTU=94.9, SS=60 mgL^{-1}, chlorophyll *a*=737.4 µg L^{-1}) to assess the entire effect of these wastewater constituents on UVB inactivation mechanisms. The RO water, 3NTU-W and 8NTU-W were selected here for comparison. Two-way ANOVA and, as a follow-up, least significant difference (LSD) analysis were applied to analyse the statistical significance of the differences.

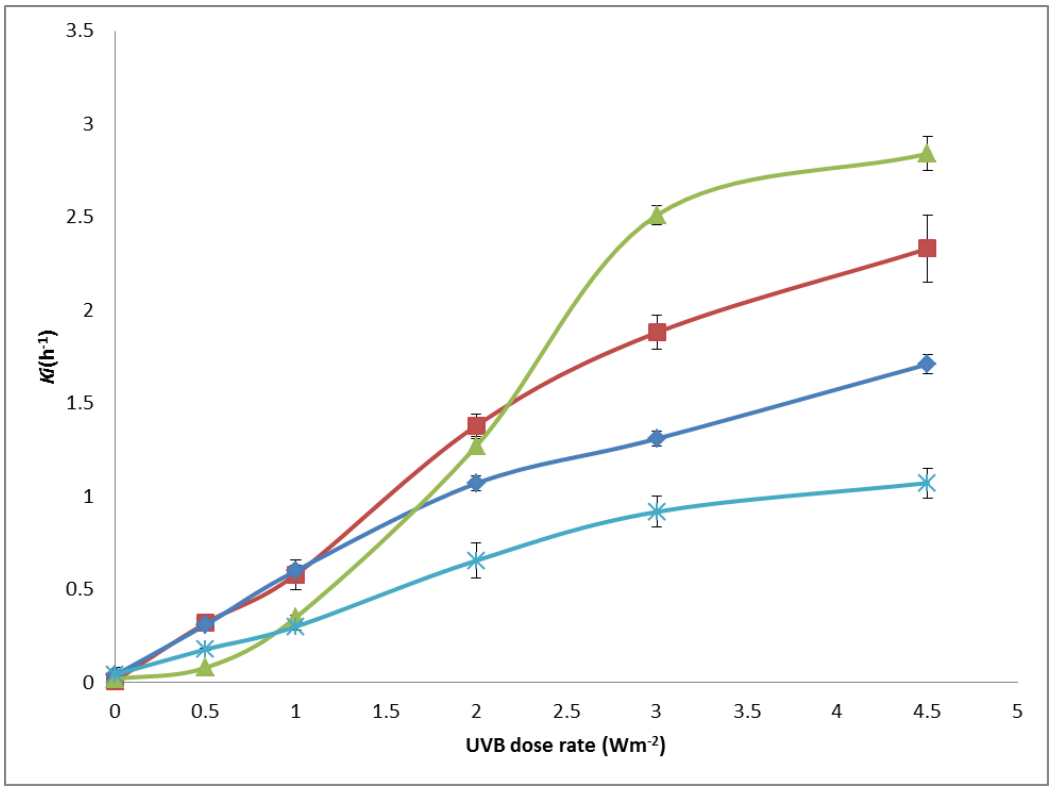


Figure 6–3: Comparison of MS2 inactivation rates (K_i) in raw wastewater (*), buffered 7.5 pH RO water at 20ºC (▲), and 3 NTU filtered wastewater (■, 3NTU-W) and 8 NTU filtered wastewater (♦, 8NTU-W) under five UVB dose rates: 0.5, 1, 2, 3 and 4.5 Wm^{-2}

The results showed that the raw wastewater constituents ($p < 0.001$) had a significant influence on the MS2 inactivation rates K_i compared to RO water and

filtered wastewater. In addition, a statistically significant interaction was observed between UVB dose rates and wastewater constituents (i.e. F [12, 40] = 36.202 and $p < 0.001$. This finding was the same as the findings for 3NTU-W and 8NTU-W, which indicated raw wastewater matrix and UVB dose rates influence K_i. Pairwise comparisons were conducted to examine the influence of the water matrix (raw wastewater, 3NTU-W, 8NTU-W and RO water) at each UVB dose rate. Firstly, the raw wastewater MS2 inactivation rates K_i were compared with those for the RO water at low UVB dose rates. Interestingly, at low dose rates no significant difference was found between the K_i for raw wastewater and the RO water. At 0.5 Wm^{-2}, the mean difference in K_i was 0.096 h^{-1} ($p = 0.299 > 0.05$); at 1 Wm^{-2}, the mean difference was even smaller at 0.053 h^{-1} ($p = 0.566 > 0.05$). In contrast, at 2 Wm^{-2}, the MS2 inactivation rate K_i was significantly ($p<0.001$) higher in the RO water than it was in raw wastewater. The gap between these two K_i curves increased gradually as the UVB dose rates increased, the mean differences were 0.614 h^{-1},1.559 h^{-1} and 1.802 h^{-1} at 2.0, 3.0 and 4.5 Wm^{-2} respectively. The similarity in K_i in RO water and raw wastewater at low UVB doses may suggest that the doses are about a minimum threshold for inactivation both in optically clear water (RO water) and turbid wastewater. Alternatively, at low UVB doses the indirect photo-inactivation via ROS production compensates for the reduction in direct inactivation by UVB associated with these types of turbid wastewater. This was supported by the depression of K_i at 1.0 Wm^{-2} in raw wastewater in the presence of the ROS quencher L-histidine to values less than those reported in RO water at the same dose rate (Figure 6-4).

The MS2 inactivation rates K_i at each UVB dose rate was significantly lower in raw wastewater than in either 3NTU-W or 8NTU-W (Figure 6-3). As was also observed when comparing raw wastewater with RO water, the difference between the K_i curves increased as the dose rate increased. Therefore, the negative effect on K_i of raw wastewater was more apparent at a high UVB dose rate than at a low dose rate. In addition, the difference between the K_i in raw wastewater and those in 8NTU-W generally increased with increasing dose rate, 0.135 h^{-1}, 0.300 h^{-1}, 0.410 $^{-1}$, 0.366 h^{-1} and 0.625 h^{-1} at 0.5, 1, 2, 3 and 4.5 Wm^{-2}, respectively. The difference between K_i in raw wastewater and 8NTU-W was not as large as the difference between raw wastewater and 3NTU-W. These differences largely reflect the composition of the water matrices with K_i values in the order RO>3NTU-W>8NTU-W>95 NTU raw wastewater; an order equivalent to the DOC, SS and chlorophyll *a* content of the respective waters.

6.3.5 Effect of L-histidine

To investigate the importance of the reactive oxygen species (ROS), $^1O_2$, in the UVB photoinactivation process in raw wastewater, the $^1O_2$ quencher, L-histidine, was added to the Mt Barker WWTP raw wastewater to supress the ROS's positive effect on UVB inactivation. The MS2 inactivation rates determined data in raw wastewater, 3NTU-W and 3NTU-W + L-histidine are compared in Figure 6-4.

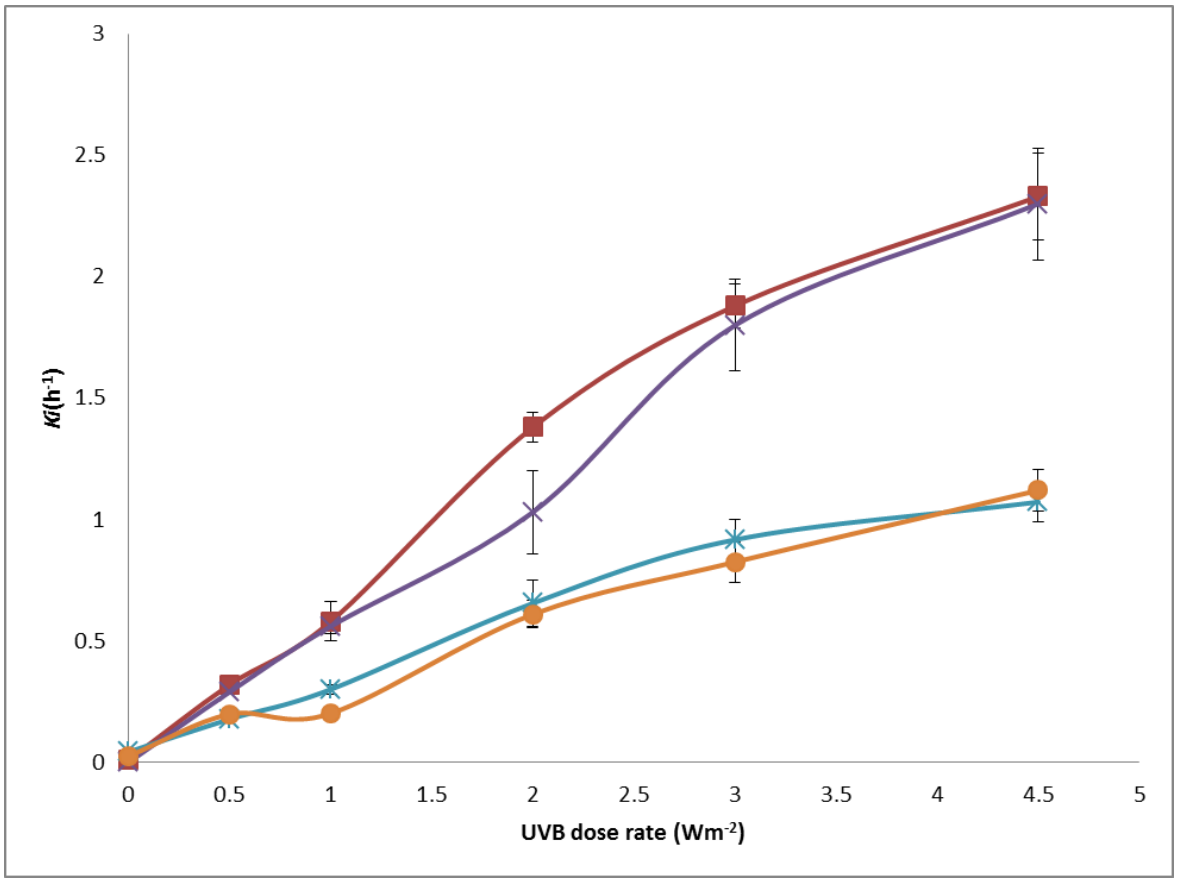


Figure 6–4: Comparison of MS2 inactivation rates (K_i) in 3 NTU filtered wastewater (■) 3 NTU filtered wastewater + L histidine (x), raw wastewater (*) and raw + L-histidine (●) at UVB irradiance between 0.5 and 4.5 Wm^{-2}

Two-way ANOVA was applied to analyse the significance of the effect of L-histidine on UVB photoinactivation in raw wastewater. Unlike the significant influence of L-histidine on the MS2 inactivation rates (K_i) 3NTU-W ($p < 0.05$), especially at 2 Wm^{-2}, overall, L-histidine was observed as not significant in influencing UVB photoinactivation in raw wastewater ($p = 0.163 > 0.05$). The interaction between L-histidine and dose rate was also insignificant ($p = 0.41 > 0.05$). When considering each dose rate separately, at every UVB dose rate (i.e. 0.5, 1, 2, 3 and 4.5 Wm^{-2}), the effect of L-histidine was found not to be significant when compared with raw wastewater. The largest K_i mean differences occurred at 1 Wm^{-2} and 3 Wm^{-2}, but

neither of these were very large at 0.91 (p = 0.128). Whereas the results for 3NTU-W identified an influence of ROS on MS2 UVB inactivation this influence was not detected for raw wastewater. This may be due to the influence of greater attenuation in raw wastewater decreasing the UVB irradiance absorbed by suspended solids which may not be exogenous photosensitisers.

6.3.6 Effect of manner of UVB dose delivery

The effect of UVB dose (Jm^{-2}) was investigated on MS2 inactivation in raw wastewater and raw wastewater + L-histidine. The dose rate (Wm^{-2}) exposure time combinations were used as described were carried out in accordance with the study design's time points, as described in Chapter 4, to confirm that the UVB total dose The results and effect of the manner of UVB dose delivery in the RO water at three temperatures, and in 3NTU-W, 3NTU-W + L-histidine and 8NTU-W were discussed in the previous chapter. Two-way ANOVA and least significant difference (LSD) analysis were applied here to compare the significance of the UVB dose and dose rates on the MS2 $\log_{10}$ reduction value (LRV) in raw wastewater and raw wastewater + L-histidine.

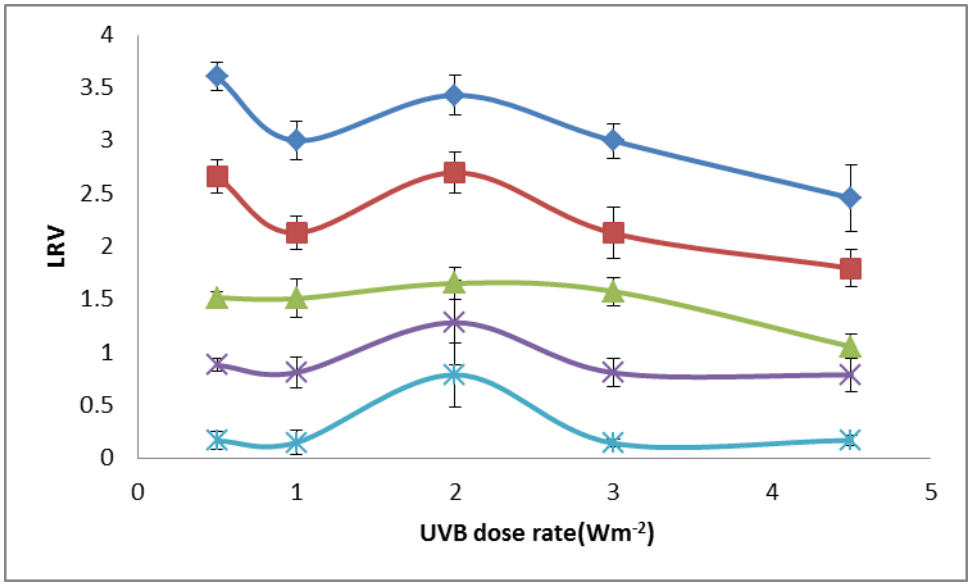


Figure 6–5: MS2 $\log_{10}$ reduction value (LRV) in raw wastewater, using 5 UVB dose rates–time combinations: 0.5, 1, 2, 3 and 4.5 Wm^{-2}, to achieve 5 UVB doses: 6126 (*), 22049 (×), 37973 (▲), 62231 (■), 86490 (◆) Jm^{-2}

Figure 6-5 shows the MS2 $\log_{10}$ reduction value at five UVB doses and five UVB dose rates in raw wastewater (RAW WW). Firstly, the UVB dose and the UVB dose

rate were observed to statistically significantly influence the MS2 $\log_{10}$ reduction value in raw wastewater ($p < 0.001$). In addition, significant interaction was found between the influence of UVB dose and the UVB dose rate ($p < 0.001$) on MS2 $\log_{10}$ reduction value. Separately, for all UVB doses, the UVB dose rate still had significant influence on LRV ($p < 0.001$ for 6126 Jm^{-2}, $p = 0.006$ for 22049 Jm^{-2}, $p = 0.002$ for 37973 Jm^{-2}, $p < 0.001$ for 62231 Jm^{-2}, $p < 0.001$ for 86490 Jm^{-2}): the same was found in 3NTU-W and 3NTU-W + L-histidine. The $\log_{10}$ reduction value for MS2 increased with increasing UVB dose (Jm^{-2}). At all doses the LRV decreased when the dose rate increased from 0.5 to 1.0 Wm^{-2}. At all doses the maximum LRV was recorded at 2.0 Wm^{-2} and the LRV in raw wastewater subsequently decreased for all doses at dose rates >2.0 Wm^{-2}. The highest LRV reached in raw wastewater was around 3.6, which was much less than the highest LRV in the RO water, 3NTU-W, 3NTU-W + L-histidine and 8NTU-W.

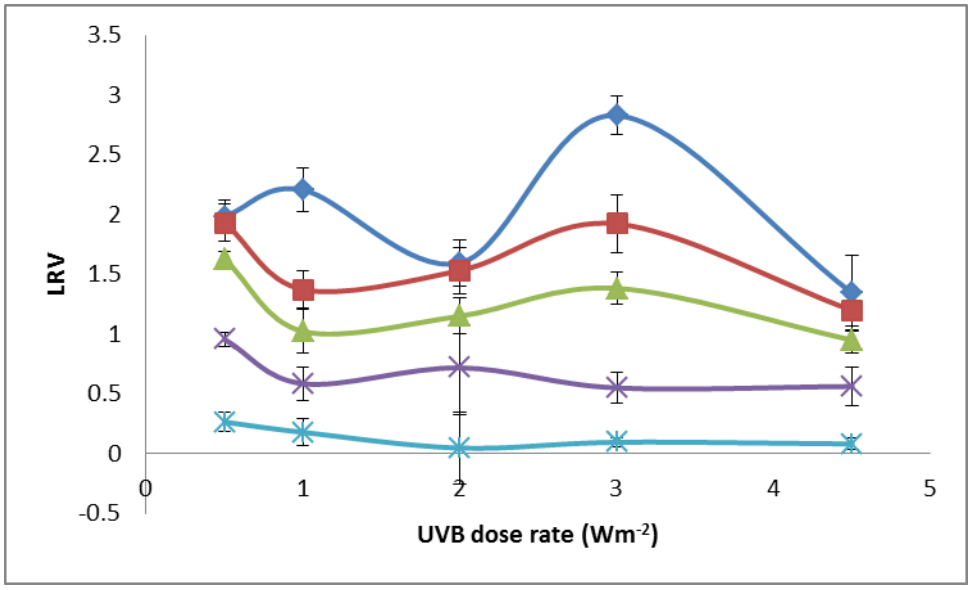


Figure 6–6: MS2 $\log_{10}$ reduction value (LRV) in raw wastewater + L-histidine, using 5 UVB dose rates–time combinations: 0.5, 1, 2, 3 and 4.5 Wm^{-2}, to achieve 5 UVB doses: 6126 (*), 22049 (×), 37973 (▲), 62231 (■), 86490 (◆) Jm^{-2}

Figure 6-6 shows the MS2 $\log_{10}$ reduction value at five UVB doses and five UVB dose rates in raw wastewater + L-histidine. As was found in raw wastewater, in raw wastewater + L-histidine, the UVB dose and UVB dose rate significantly influenced MS2 inactivation, and the interaction between the UVB dose and UVB dose rate was also significant. However, it was not the same significance for each curve: at the lowest UVB dose of 6126 Jm^{-2}, the five UVB dose rates were observed not to significantly influence the LRV ($p = 0.312$). However, for other UVB doses, the UVB

dose rates were found to significantly influence the $\log_{10}$ reduction value (LRV). The variation trends of the five UVB doses in raw wastewater + L-histidine were not constant. At the lowest UVB dose of 6126 Jm^{-2}, not much change was observed: for the middle three UVB doses, they performed similarly to the raw wastewater group. The highest UVB dose curve (86490 Jm^{-2}) reached the peak LRV (2.8) at 3 Wm^{-2}. Generally, the MS2 LRV in raw wastewater + L-histidine were the lowest among all of the experimental groups: the RO water at three temperatures, 3NTU-W, 3NTU-W + L-histidine, 8NTU-W, raw wastewater and raw wastewater + L-histidine. While the engagement of ROS in MS2 UVB inactivation was confirmed statistically in 3NTU-W it may only be implied from the results for raw wastewater from the incubations including L-histidine recording the lowest LRVs.

6.4 Discussion

The study continued to investigate the effect of UVB irradiance on MS2, in raw wastewater, a more complex water matrix. In Chapter 4, buffered RO water was used as the water medium for the UVB experiment, which meant that the direct photoinactivation was the single mechanisms to influence MS2 inactivation. Chapter 5 included consideration of the involvement of turbidity, exogenous sensitisers (DOC), evaluated using the ROS quencher, L-histidine. In this chapter, raw wastewater, the final target investigated, was used for further research. The following sections discuss the MS2 dark inactivation in raw wastewater, as well as the combined and separate effects of DOC, SS, chlorophyll *a* and turbidity.

The MS2 dark inactivation rate K_i in raw wastewater was 0.03 h^{-1}, which was much lower than the K_i in the HRAP inlet wastewater at 20 ℃ (i.e. 0.163 h^{-1}). Several factors contribute to micro-organisms' dark inactivation in natural wastewater environments, such as: sedimentation which has been demonstrated as important for faecal coliform inactivation in lakes (Auer & Niehaus, 1993; Gannon et al., 1983); lack of nutrition (Gann et al., 1968); potential algal toxic cyanobacteria in WSPs with this observed as potentially toxic to bacteria (Oufdou et al., 2001); antagonistic ingestion by higher organisms, which has been confirmed by some research studies (Decamp & Warren, 1998; Manage, 2002); and particulates' adsorption function (Ohgaki, 1986). Therefore, the most probably reason have impacted on the different MS2 inactivation rates in the two wastewater resources is the wastewater components. One obvious difference is the algal concentration, Young(2014) measured 1.97 mgL^{-1} chlorophyll *a* from the same HRAP, which is three times higher than the chlorophyll a concentration in WSP raw wastewater reported here.

At all UVB dose rates (Wm^{-2}) in the raw wastewater, the log_{10}-linear + tail model in GInaFiT was the model with the best fit amongst 10 different models. To date, the log_{10}-linear + tail model has proved to be the best fit for all MS2-UVB inactivation experiments, including those in the RO water, 3NTU-W, 3NTU-W + L-histidine, 8NTU-W, raw wastewater and raw wastewater + L-histidine. Many research studies on sunlight-related inactivation of micro-organisms have found the same tail at the final phase of their UV disinfection experiments, in rainwater solar disinfection(Amin & Han, 2011) and food industry disinfection(Baysal et al., 2013; Izquier & Gómez-López, 2011); however, the mechanism leading to the 'tail' remains unclear. The presence of a 'tail' implies a sub-population of MS2 more resistant to UVB irradiance or with greater ability to repair UVB damage regardless of the water bodies in which they were suspended.

UVB attenuation by raw wastewater constituents was the most likely cause of lower MS2 inactivation rates in this matrix. UVB irradiance has been found to poorly penetrate natural lakes, wetlands and wastewater stabilisation ponds (WSPs) (Arts et al., 2000; Bolton et al., 2011; Curtis & Curtis, 1994). Although there is considerable debate as to which of the constituents, e.g.DOC, water, SS or algae, makes the most contribution to wastewater attenuation (Curtis & Curtis, 1994; De Lange, 2000; Morris et al., 1995; Scully & Lean, 1994), However, conflicting results was found by Kohn and Nelson (2006) who reported that MS2 inactivation was faster in unfiltered wastewater than in filtered wastewater under full wavelength sunlight. The possible explanation was that UVA and visible light were involved in Kohn and Nelson's experiment, with these able to penetrate more deeply than UVB in raw wastewater. In addition, the high concentration of exogenous sensitisers of raw wastewater may have absorbed UVA and visible light to enhance MS2 inactivation by producing reactive oxygen species (ROS).

The raw wastewater constituents did not only influence UVB photoinactivation in a negative way. The sole exception for K_i comparison occurred at 0.5 Wm^{-2}, for which the MS2 inactivation rate K_i in raw wastewater was found to be higher than the K_i in the RO water. This finding confirms the conclusion in Chapter 5 that, at the lowest UVB dose rate, the negative effect of attenuation was not apparent but the exogenous photoinactivation was apparent. Therefore, only at 0.5 Wm^{-2} was the positive effect of wastewater exogenous photoinactivation able to overcome the strong attenuation of UVB, thus resulting in a high level of MS2 inactivation. In addition to demonstrating that DOC performs as an exogenous sensitiser, SS may

also act as an exogenous sensitiser (Davies-Colley et al., 1999).

The ROS $^1O_2$ quencher, L-histidine, was added to raw wastewater to check the importance of UVB exogenous photoinactivation. Although no significant effect on MS2 inactivation rate in raw wastewater, either separately or at all five (5) UVB dose rates was found, and no significant differences at all were found between the raw wastewater and raw wastewater + L-histidine groups. It was found the average LRV in raw wastewater was the lowest compare to RO water and filtered wastewater. This partly confirmed that L-histidine significantly influenced the UVB inactivation rate K_i in 3NTU-W as described in Chapter 5.

The manner of UVB delivery was significantly influenced the MS2 LRV in the RO water, 3NTU-W, 3NTU-W + L-histidine and 8NTU-W. The manner of UVB delivery was also found to significantly influence the MS2 LRV in raw wastewater and raw wastewater + L-histidine. In terms of raw wastewater, the significant effect was found at every UVB dose, and the curve of each UVB dose performed very similarly to the curve for the 3NTU-W + L-histidine group, looking like a “down-and-up wave”, with the highest LRV achieved at 0.5 or 2 Wm^{-2}. To date, for all the water bodies in which DOC is involved, 0.5 Wm^{-2} was almost always the dose rate to achieve the highest MS2 $\log_{10}$ reduction value (LRV). This finding suggests that UVB exogenous photoinactivation is detectable at relatively low UVB dose rates. In addition, the LRV in raw wastewater was commonly lower than for other water bodies due to the high attenuation. The lowest LRV at each UVB dose and dose rate was recorded in the wastewater + L-histidine treatment; implying a role for ROS produced via UVB interaction with DOC.

Most of the literature on the manner of UV dose delivery has agreed that micro-organisms die faster with a high UV dose rate at the same UV dose (Liu & Zhang, 2006; Murakami et al., 2006; Sethi, 2009; Sommer et al., 1998). A few researchers have debated that long-term UV irradiance was more efficient (Sommer, 1996). After systematic experiments were undertaken to explore the manner of the UVB dose delivery, a more complex conclusion was able to be reached that the manner of UVB dose delivery has a significant effect on MS2 inactivation for most of the cases; however, whether it is a high or a low dose rate that enhances the inactivation largely depends on the water characteristics and the dose rates chosen.

6.5 Conclusion

This chapter has explored the effects of raw wastewater constituents on MS2 inactivation and has investigated the manner of UVB dose delivery. Experiments were conducted on two groups, namely, raw wastewater and raw wastewater + L-histidine under five (5) different UVB doses and five (5) different UVB dose rates. Comparison with the RO water, 3NTU-W, 3NTU-W + L-histidine and 8NTU-W experiments was undertaken. The following conclusions were made:

- GInaFiT's $\log_{10}$-linear + tail model was shown to be the best fit for all UVB-MS2 die-off curves, including the RO water, filtered wastewater, filtered wastewater + L-histidine, raw wastewater and raw wastewater + L-histidine.
- The $\log_{10}$-linear model was also the best fit for raw wastewater and raw wastewater + L-histidine to express the relationship between the MS2 inactivation rate K_i and UVB dose rates, so two log-linear equation were developed.
- The manner of UVB dose delivery (Wm^{-2}) significantly influenced the MS2 LRV under the same UVB doses (Jm^{-2}) in raw wastewater and raw wastewater + L-histidine, relatively narrow range fluctuations were observed when dose rates changes within the same doses.
- The raw wastewater constituents have high turbidity and strong attenuation effects, with the effects significantly decreasing the UVB photoinactivation in comparison to findings from the experiments with other RO and filtered wastewater.
- L-histidine was observed to have no statistically significant on UVB photoinactivation in raw wastewater; although the lowest LRV and K_i values were recorded for this treatment.

CHAPTER 7: DISCUSSION AND CONCLUSIONS

7.1 Discussion

The study reported in this thesis mainly focuses on gaining an understanding and modelling the effect of a series of environmentally relevant factors on the dark and UVB inactivation, primarily of the F-RNA coliphage MS2. The new knowledge and models provide insight to assist the future design of natural wastewater treatment systems including HRAPs and WSPs (Bolton, 2011; Buchanan, 2014; Buchanan et al., 2011a; Cromar et al., 1996; Fallowfield et al., 1996; Fallowfield et al., 1999; Mbonimpa et al., 2012; Shilton, 2005a).

The environmental factors investigated in this study were relevant to practical field situations associated with the operation of HRAPs and WSPs in South Australia: these factors were temperature, UVB dose and UVB dose rates. The effect of wastewater constituents likely to influence UVB attenuation or the production of ROS and, consequently, MS2 UVB inactivation, for example, DOM, suspended solids and chlorophyll *a* were also a focus. The temperatures applied in the current study ranged from 10ºC to 30ºC, based on Buchanan's (2014) one-year temperature measurement of an HRAP located at Kingston-on-Murray in South Australia. Six ordinal UVB dose rates 0.5, 1, 2, 3, 4.5 and 6 Wm^{-2} and seasonal representative UVB doses of 6126, 22049, 39973, 62232 and 86490 Jm^{-2} were chosen from data collected over 28 randomly assigned days in 2009 in South Australia (Bolton, 2011). The wastewater matrices used in the study were all collected from natural wastewater treatment systems, for example, an HRAP located at Kingston-on-Murray (S 34 14', E 140 20') and wastewater treatment plant (WWTP) located at Mt Barker, South Australia. MS2 and *E. coli* was chosen as pathogen indicators in this study: they are widely suggested and used as bacteria and virus indicators (Bolton, 2011; Buchanan, 2014; Fallowfield et al., 1996; Fiers et al., 1976; Fisher et al., 2012). The major findings of the study are summarised and presented below in the order corresponding to the aims of the respective chapters.

> *Aim 1: Assess and model the influence of a range of typical environmental temperatures on the dark die-off rate of MS2 and Escherichia coli (E. coli) in mixed, unfiltered wastewater.*

Chapter 3 reported the dark inactivation rates, over six days, of natural strains MS2 and *E. coli* in mixed raw wastewater incubated at 10ºC, 20ºC and 30ºC. The

inactivation rates were derived from the experimental data using the $\log_{10}$-linear + shoulder + tail model, GInaFiT (Geeraerd et al., 2005). Compared to research related to sunlight disinfection, relatively little research has been conducted on dark disinfection processes relevant to WSPs and HRAPs. However, dark disinfection is an important inactivation process particularly in unmixed WSPs in which suspended solids and greater depth causes significant light attenuation resulting in much of the pond depth residing in the dark (Bolton et al., 2011). Temperature has been recognised as one of the most significant secondary environmental factors in relation to disinfection in natural wastewater treatment systems (Chan & Killick, 1995; Fisher et al., 2012) and consequently is frequently used in design equations (Mara & Cairncross, 1989; Mara & Pearson, 1998). The results of the studies that are available on dark inactivation are contradictory regarding the effect of temperature (Auer & Niehaus, 1993; Craig et al., 2001, 2004; Feng, 2003; Flint, 1987; Moeller & Calkins, 1980). Possible causal factors contributing to these contradictions are the great dissimilarities in experimental design, for example, duration, water characteristics, indicator strain and the reporting of the micro-organisms' inactivation rates, including *K*, T90 and T99.

The inactivation rates of MS2 and *E. coli* reported in the current study increased significantly with increases in temperature from 10ºC to 30ºC ($p<0.0001$). Two equations (Equations 3-2 and 3-3) were developed to model the effect of temperature on MS2 and *E. coli* dark inactivation from 10ºC to 30ºC: they were partly validated using data from the related literature (Buchanan et al., 2011b; Craig et al., 2004; Feng, 2003; Flint, 1987). The models confirmed the fact that an increase in temperature positively influenced pathogen indicators' inactivation rates, for both bacteria and virus. The application of these models will assist HRAP design for pathogen removal where beneficial wastewater reuse is a required outcome of treatment. No influence of pH or DO on dark inactivation was apparent. The 10 curve-fitting models provided by GInaFiT (Geeraerd et al., 2005) were interrogated and the inactivation rates were subsequently derived using a $\log_{10}$-linear + shoulder + tail model which provided the best, statistically significant 'fit' to the experimental data. The author recommends the wide adoption of GInaFiT for the determination of the inactivation rate constant K_i. This would facilitate better comparison of results obtained by different researchers. In addition, the natural strain *E. coli* survived better than MS2 in dark, mixed wastewater: consequently *E. coli* is recommended as the indicator for future dark inactivation studies on wastewater.

No significant MS2 dark inactivation was observed over two days of measurement at 20ºC in RO water, 0.2 µm filtered wastewater or 0.2 µm filtered wastewater + L-histidine. In wastewater from an WSP-aerated lagoon and wastewater + L-histidine, the MS2 inactivation rates were 0.04 h^{-1} and 0.03 h^{-1} over two days, respectively. These rates were 24.5% of MS2 inactivation rate (0.163 h^{-1}) measured over six days in HRAP raw wastewater. The differences between MS2 dark inactivation in HRAP wastewater and WSP wastewater were most likely due to the differences in the duration of the incubations and wastewater components.

Further work is needed to integrate the dark disinfection models with those for sunlight disinfection to better model and describe the disinfection process within WSPs and HRAPs.

> *Aim 2: Assess and model the influence of a range of environmentally relevant temperature, UVB doses (Jm^{-2}) and dose rates (Wm^{-2}) on the inactivation of E. coli and MS2 under UVB irradiance in optically clear RO water.*

Chapter 4 reported the influence of temperatures ranging from 10ºEFFC to 30ºC, UVB dose from 6126 Jm^{-2} to 86490 Jm^{-2}, UVB dose rate from 0.5 Wm^{-2} to 6 Wm^{-2} on the UVB disinfection of *E. coli* and MS2 in RO water. Sunlight is considered as the most important factor influencing disinfection performance in pond systems (Bolton, 2011; Craggs, 2004; Maynard et al., 1999; Mayo, 1995). As UVB (280-315 nm) is recognised as the most significant part of the spectrum contributing to disinfection (Bolton et al., 2011; Kirk, 1994), it was chosen as the priority wavelength for research reported in this thesis. Although the final application target of the current study was the treatment of raw wastewater, gaining an understanding of UVB disinfection mechanism in optically clear, RO water was necessary to better understand the influence of components of the wastewater on UVB inactivation. Temperature and the manner in which UV dose is delivered, that is, the dose rate are two important factors which influence UV disinfection (Chan & Killick, 1995; Roos et al., 1998; Šolić & Krstulović, 1992; Sommer et al., 1998; Theitler et al., 2012). However, there is a paucity of information regarding the influence of these factors on UVB disinfection. This study sought to redress this lack of information.

Applying GInaFiT (Geeraerd et al., 2005), the log_{10}-linear + tail was found to be the best fitting model for gaining MS2 and *E. coli* inactivation rates under UVB irradiance in RO water. The “tail” part of the curves indicated no further MS2 and *E. coli* inactivation with an increasing UVB dose. The “tail” normally occurred after at

least 99.9% of the inactivation had occurred. A similar "tail" phase has been observed in full wavelength solar light disinfection (Amin & Han, 2011) and UVC disinfection (Baysal et al., 2013; Hijnen et al., 2006; Izquier & Gómez-López, 2011). This is the subject of debate as to its cause: hypotheses considered have included a resistant subpopulation, experimental bias and hydraulics (Hijnen et al., 2006). To the author's knowledge, the research reported here is the first that has confirmed the presence of "tail" in an inactivation rate measured under solely UVB irradiance.

Ambient temperature increases from 10ºC to 30ºC were observed to have a significant positive effect on MS2 UVB inactivation rates: the effect was more apparent as temperature was increased from 20ºC to 30ºC than from 10ºC to 20ºC. However, no effect of temperature was observed for *E. coli* inactivation rates when temperature increased from 20ºC to 30ºC in RO water. The difference in physical size, and in genetic and capsid/membrane material may be the cause of the different results. More importantly, three cubic polynomial equations (MS2 at three temperatures) and one log-linear equation (*E. coli*) were developed to describe the relationship between inactivation rates and UVB dose rates for further comparison.

When the same UVB doses (Jm^{-2}) were delivered by a range of dose rates (Wm^{-2}), remarkably different MS2 and *E. coli* inactivation outcomes (LRV) were observed. Two phases, "dose rate-limited inactivation" and "dose rate-saturated inactivation", were defined to describe the UVB dose delivery effect. The "dose rate-limited inactivation" phase was where MS2 or *E. coli* inactivation increased, within the same dose delivered by increasing dose rates. This was observed at lower dose rates from 0.5 Wm^{-2} to 2 Wm^{-2} at 10ºC and 20ºC, and from 0.5 Wm^{-2} to 1 Wm^{-2} at 30ºC. The "dose rate-saturated inactivation" phase described the part of the MS2 or *E. coli* inactivation curve where increasing dose rate did not result in an increase in inactivation rate. *E. coli* was found to be more sensitive to UVB irradiance in RO water than MS2.

The UVB experiments in RO water confirmed the hypothesis that temperature, UVB dose rates and UVB dose significantly influenced UVB inactivation of MS2 and *E. coli*. MS2 was chosen as the preferred indicator for further UVB experiments as it was more resistant to UVB inactivation that *E. coli*.

> *Aim 3: To assess and model the effect of DOM, exogenous photosensitisers and suspended solids concentration in wastewater and the interaction between UVB doses (Jm^{-2}) and dose rates (Wm^{-2}) on the MS2 inactivation.*

Chapters 5 and 6 reported the effect of UVB doses, UVB dose rates and the ROS quencher L-histidine on MS2 inactivation at 20ºC in a range of wastewater matrices, including 3NTU-W, 8NTU-W and raw wastewater.

The difference between RO water and double 0.2 µm filtered wastewater was the presence of dissolved organic matter (DOM), measured by dissolved organic carbon (DOC). The DOM plays an important role in exogenous photoinactivation as it potentially acts as an exogenous photosensitiser (Curtis, 1992; Kohn & Nelson, 2006). Exogenous photosensitisers are able to catalyse UV inactivation by photon-sensitised, energy transfer to $_1O^2$ and other reactive oxygen species (ROS) outside of the cell: the ROS is highly reactive and attacks membranes, thus causing inactivation (Curtis, 1992; Davies-Colley et al., 1999; Kohn & Nelson, 2006). Traditionally, exogenous photosensitisers are recognised as more likely to be sensitised by long wavelength light, for example, UVA or visible light (Curtis, 1992; Davies-Colley et al., 1999), but recently UVB was also implicated in exogenous photoinactivation (Santos et al., 2012). To assess whether exogenous photoinactivation was involved in UVB inactivation was an aim of this study. L-histidine was used as a selective quencher of the ROS, $_1O^2$, since this ROS has been shown to be the most significant.

While DOM may enhance UVB inactivation via exogenous photoinactivation, it may, together with suspended solids and chlorophyll *a* in raw wastewater, also increase UVB attenuation, adversely affecting UVB disinfection throughout the water column. There has been considerable debate on the relative significance of these components and their influence on UV attenuation and subsequent inactivation (Curtis & Curtis, 1994; De Lange, 2000; Morris et al., 1995; Scully & Lean, 1994). This study used 0.22 µm filtered wastewater and MS2 in the presence and absence of L-histidine to determine the relative contribution of direct UVB photoinactivation and exogenous photoinactivation. Subsequently, the complexity of the matrix was increased using raw wastewater, which incorporated not only DOM but also suspended solids (SS and chlorophyll *a*, enabling a determination of the relative effect of these additional components on MS2 UVB inactivation. Tables 7-1 and 7-2 compare the MS2 UVB inactivation rate (K_i) and LRV respectively in these matrices using inactivation in RO water as the reference.

Table 7-1 shows the equation of the $\log_{10}$-linear GInaFiT to the experimental data and the statistical significance of the fitted equation for RO water and all the wastewater matrices studied. The value of the MS2 inactivation rate constant

determined in the wastewater matrices was compared, as a percentage, to that obtained in RO water under the same experimental conditions. The MS2 inactivation rate in 0.22 µm filtered wastewater with relatively low DOM and a turbidity of 3 NTU (3NTU-W) decreased 25% compared to that measured in optically clear RO water. The addition of L-histidine to the 3NTU-W decreased K_i by a further 1.52% compared to the 3NTU-W alone. The quenching of ROS by L-histidine and a resultant decrease in K_i suggests a role for UVB-induced exogenous photoinactivation of MS2 in wastewater. When the turbidity of filtered wastewater increased to 8 NTU, the MS2 K_i was reduced by >20% compared to that measured for 3NTU-W and was 51.37% of that recorded in RO water; confirming that small increases in turbidity can result in significant decreases in inactivation. The additional presence of suspended solids (SS) and algae (expressed as chlorophyll *a*) in the raw wastewater further depressed the MS2 K_i by >20% compared to the effect in 8NTU-W; furthermore, the K_i was only 33% of that recorded in optically clear RO water. There was no evidence for involvement of UVB-induced reactive oxygen species (ROS) in the inactivation of MS2 in raw wastewater.

In Table 7-2, the LRVs recorded for MS2 in the RO water, within the dose rate's stationary phase of inactivation, at doses representative of the South Australian summer, winter and the mean annual UVB dose, are compared with data from the various wastewater matrices. The LRVs recorded confirmed the conclusion made for MS2 UVB inactivation rates above, that the turbidity was a significant factor influencing LRVs. The LRVs in 0.22 µm filtered wastewater with a turbidity of 3 NTU (3NTU-W) were 90% those of LRVs recorded in optically clear RO water. Once again, a small increase in turbidity to 8 NTU reduced the LRV to between 65% and 78% of the LRVs recorded in RO water. Raw wastewater, including DOM, suspended solids (SS) and algae reduced the MS2 LRVs to about 45% of those recorded in RO water.

The importance of UVB-induced ROS, evidenced by the use of the L-histidine quencher, was further emphasised by considering the LRVs. The LRVs in 0.22 µm filtered wastewater with a turbidity of 3 NTU (3NTU-W) were 90% those of LRVs recorded in optically clear RO water. The contribution of ROS to the LRV in 3NTU-W, estimated by the reduction in LRV in the presence of L-histidine, was between 10% and 20%, with the greater contribution being made at the lower UVB dose, which was equivalent to that experienced in a South Australian winter. In contrast to the inactivation rate data, the contribution to UVB-induced ROS to MS2 LRV in raw

wastewater was again between 10% and 20%, consistent with that recorded in ROS-quenched 3NTU-W.

Table 7–1: Log_{10}-linear models describing the relationship between MS2 inactivation rate and UVB dose rate from 0.5 to 4.5 Wm^{-2} in RO water and a range of wastewater matrices

Notes: The range of wastewater matrices comprised 3NTU-W, 3NTU-W + L-histidine, 8NTU-W, WTTP raw wastewater, WTTP raw wastewater + L-histidine. The inactivation rate constants recorded in the wastewater matrices are compared as percentages to the constants recorded in RO water.

Water medium	MS2 inactivation rate model[a]	R^2	p	K_i relative to RO water[b]
RO water	K_i = 0.7171D - 0.1363	0.9477	p<0.001	100%
3NTU-W	K_i = 0.5378D + 0.0974	0.972		75.00%
3NTU-W+L-histidine	K_i = 0.5269D + 0.0308	0.9876		73.48%
8NTU-W	K_i = 0.3684D + 0.1647	0.968		51.37%
WTTP raw wastewater	K_i = 0.2421D + 0.084	0.963		33.76%
WTTP raw wastewater + L-histidine	K_i = 0.2492D + 0.0402	0.981		34.75%

[a] K_i is the MS inactivation rate constant (h^{-1}), D is the UVB dose rates from 0 Wm^{-2} to 4.5 Wm^{-2}.

[b] It is assumed that the MS2 inactivation rate model slope in RO water is 100%.

Table 7–2: MS2 log_{10} reduction value (LRV) measured by three UVB doses (86490, 37973, 6126 Jm^{-2}) in a range of water matrices

Notes: The range of water matrices were RO water, 3NTU-W, 3NTU-W + L-histidine, 8NTU-W, raw wastewater, raw wastewater + L-histidine, with the doses delivered by dose rates from 0.5 to 4.5 Wm^{-2} except for RO water from 2 to 4.5 Wm^{-2}. The LRVs recorded in the wastewater matrices are compared as percentages to the LRVs recorded in RO water in the dose rate-saturated phase of inactivation.

Water medium	UVB doses (Jm^{-2})	Average LRV	SD	LRV relative to RO water[a]
RO water	86490	6.63	0.51	100.00%
	37973	3.34	0.44	100.00%
	6126	0.66	0.11	100.00%
3NTU-W	86490	6.03	0.99	90.98%
	37973	3.14	0.27	94.06%
	6126	0.68	0.25	102.39%
3NTU-W + L-histidine	86490	5.33	0.42	80.43%
	37973	2.76	0.32	82.54%
	6126	0.55	0.47	82.57%

Water medium	UVB doses(Jm^{-2})	Average LRV	SD	LRV relative to RO water[a]
8NTU-W	86490	5.19	1.33	78.35%
	37973	2.34	0.51	70.12%
	6126	0.43	0.11	64.77%
Raw wastewater	86490	3.10	0.45	46.79%
	37973	1.46	0.23	43.80%
	6126	0.29	0.28	43.27%
Raw wastewater + L-histidine	86490	1.99	0.58	30.06%
	37973	1.23	0.28	36.69%
	6126	0.13	0.09	20.23%

[a] Assumes that the MS2 LRV in RO water is 100%, the percentage value achieved by other water mediums.

This study has built a comprehensive database on UVB inactivation of MS2 from laboratory studies. The Flinders University group of researchers uniquely has access to an HRAP treating wastewater in South Australia with which the laboratory-derived results for MS2 can be compared. Young (2014) studied the 0.32 m deep, 200 m^2, HRAP at Kingston-on-Murray (KoM) (E 140 20' S 34 14'). Over a year-long period, Young (2014) determined the MS2 LRV in the HRAP and measured the in situ water temperature and surface irradiance to gain an understanding of disinfection performance.

Figure 7-1 shows the MS2 daily LRV in the HRAP at Kingston-on-Murray in 2014 (Young, 2014). The mean annual LRV measured in the HRAP was 1.59 ± 0.82, which is comparable to the MS2 LRV recorded in the laboratory in raw wastewater of 1.46 ± 0.23 at an annual average UVB dose of 37973 Jm^{-2}. The small difference between the LRVs may be due to the additional wavelengths, for example, UVA and visible light present in the natural sunlight spectrum. An interesting finding (shown in Figure 7-1) was that the lowest MS2 LRV observed in the HRAP was in summer (December), and the highest LRV was in winter (July). (Young, 2014) derived a statistically significant overall log_{10}-linear relationship between the MS2 LRVs and chlorophyll *a* concentration (mg L^{-1}; Figure 7-2). This relationship may be surrogate evidence of involvement of UVB-induced ROS in MS2 inactivation as identified in the laboratory studies reported here.

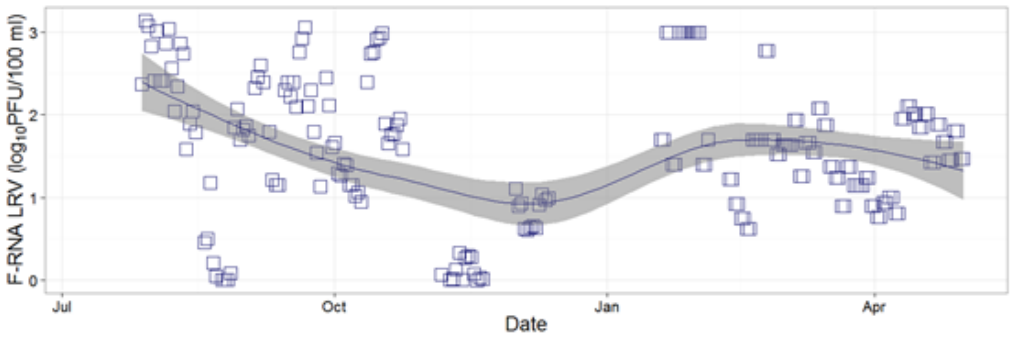


Figure 7-1: Measured daily MS2 (F-RNA) LRV in the HRAP at Kingston-on-Murray, SA between 10 July 2013 and 1 May 2014 (Young, 2014)

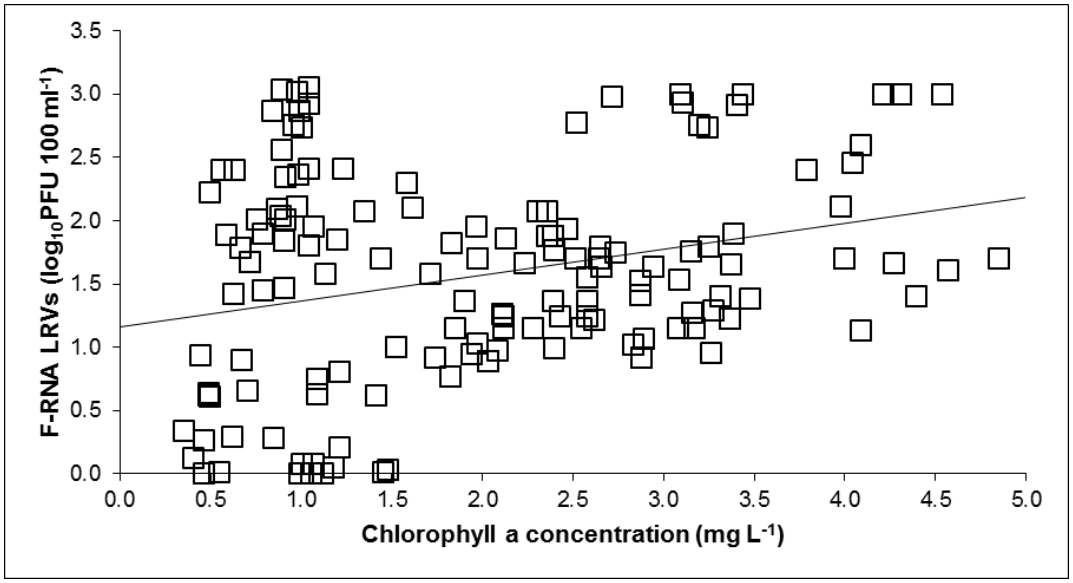


Figure 7-2: Linear relationship between the MS2 (F-RNA) LRVs and chlorophyll *a* concentration (mg L^{-1}) in the HRAP at Kingston-on-Murray, SA between 10 July 2013 and 1 May 2014 (Young, 2014)

The interpretation of the inactivation rate data and the LRV above has assumed that the UVB doses are surface irradiances. However, a range of UVB doses will occur throughout the depth of an HRAP due to attenuation. Micro-organisms will be exposed to these doses for various time periods through intentional mixing by the paddle wheel incorporated into the HRAP design. The challenge is to integrate these exposures and associated inactivation rate constants and LRVs into a model of disinfection for HRAPs.

7.2 Conclusions

- The dark inactivation rates of MS2 and *E. coli* increased significantly with increases in temperature from 10ºC to 30ºC in mixed, raw HRAP wastewater.
- As the wastewater was mixed, dark inactivation of MS2 and *E. coli* in mixing raw wastewater was not caused by sedimentation.
- The dark inactivation rates were best modelled using a $\log_{10}$-linear + shoulder + tail model. Based on this model, the value of the rates highly depended on experiment duration and wastewater composition.
- The MS2 and *E. coli* die-off curves under UVB irradiance were best modelled by GInaFiT $\log_{10}$-linear + tail.

- The temperature increase from 10ºC to 30ºC significantly enhances the MS2 inactivation rates at a range of UVB dose rates in RO water. In contrast, no influence on inactivation rates was observed for *E. coli* when temperature increased from 20ºC to 30ºC.

- Generally, the delivery of the same UVB doses by different UVB dose rates had a strong influence on UVB disinfection on both *E. coli* and MS2 regardless of temperature, both “dose rate-limited” (LRV increased with the increase of dose rates) and “dose rate-saturated” (LRV did not increase with the increase of dose rates) stages were observed at almost all locked UVB doses.

- UVB-induced ROS plays a not insignificant role in MS2 inactivation in wastewater. The study estimated between 10% and 20% of the MS2 LRV recorded in wastewater could be attributed to the generation of ROS.

- Small increases in turbidity resulted in significant decreases in UVB inactivation of MS2.

- Significantly, laboratory measurement of MS2 UVB $\log_{10}$ reduction values (1.46 ± 0.23) at irradiances equivalent to the annual mean were comparable with the annual mean values (1.59 ± 0.82) recorded in a research-demonstration HRAP treating wastewater in rural South Australia.

The proposed future directions can be summarized as follows:

- Assess the validation of equations achieved in this thesis by applying them into more practical HRAP data, combined with the properties of wastewater and attenuation.
- Collect the UVA and visible inactivation database similar to UVB’s database in this thesis. And explore their combined disinfection effect on pathogens. More pathogens like adenovirus rather than solely MS2 can be tested.
- Identify the molecular UV inactivation mechanisms to gain the understanding of the significance of UV delivery manner.

APPENDICES

Appendix 1

Tris (hydroxymethyl) aminomethane (Tris HCl) buffer

Stock solutions:

A: 0.2 M (24.2 g L^{-1}) tris (hydroxymethyl) aminomethane (Sigma)

B: 0.2 M HCl

Desired pH achieved with 250 mL of A + X mL of B, diluted to a total of 1 L.

X	pH
61.0	8.6
82.5	8.4
114.5	8.2
134	8.0
162.5	7.8
192	7.6
200	7.5
207	7.4

Appendix 2

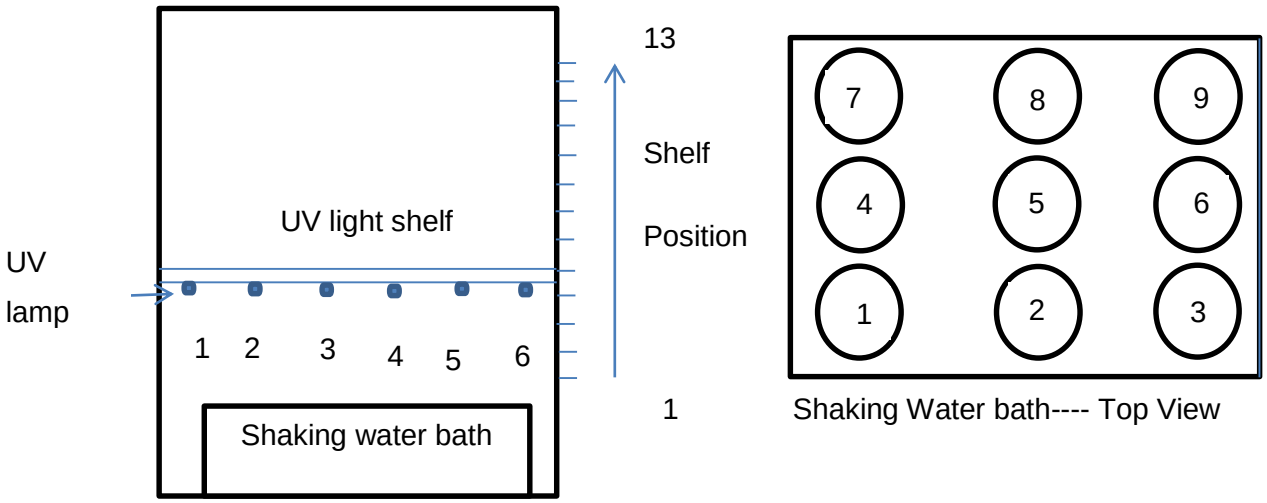


Figure A2–1: Characterisation of UV cabinet: UVB irradiance

Ideal Value (Wm^{-2})	Quantity of UVB lamps	Position of UVB lamps	Height of shelf	3 vessel position	Irradiance separately (Wm^{-2})			Available instantaneous Dose (Wm^{-2})	SD
0.5	1	6	13	2.5.7	0.49	0.53	0.48	0.50	0.03
0.5	1	6	12	2.4.7	0.51	0.48	0.46	0.48	0.03
0.5	1	6	6	2.4.7	0.56	0.44	0.37	0.45	0.09
1.0	1	1	6	3.6.9	0.88	1.02	0.92	0.94	0.09
1.0	1	5	6	2.4.7	1.04	0.86	0.81	0.90	0.12
1.5	1	4	6	2.4.7	1.65	1.49	1.48	1.54	0.10
1.0	1	2	10	2.6.9	0.97	0.99	0.97	0.97	0.06
1.0	1	3	10	1.2.3	0.94	1.01	0.98	0.97	0.06
1.0	1	4	10	2.5.7	1.04	1.08	0.99	1.04	0.05
1.0	1	5	10	3.5.8	1.01	0.94	1.02	0.99	0.06

Ideal Value (Wm^{-2})	Quantity of UVB lamps	Position of UVB lamps	Height of shelf	3 vessel position	Irradiance separately (Wm^{-2})			Available instantaneous Dose (Wm^{-2})	SD
1.5	2	3.4	13	2.4.7	1.44	1.53	1.44	1.47	0.10
2.0	2	3.4	10	1.2.4	1.90	2.09	2.20	2.07	0.20
5.5	2	3.4	6	5.6.9	5.37	5.66	5.69	5.57	0.42
6.5	2	3.4	4	3.4.7	6.72	6.19	6.50	6.47	0.48
3.0	4	2.3.4.5	13	3.5.6	3.06	3.06	2.94	3.02	0.16
10.0	4	2.3.4.5	6	5.6.9	9.71	9.98	10.06	9.92	0.70
15.0	4	2.3.4.5	4	5.8.9	15.1	14.87	14.80	14.92	1.05
4.0	6	All	13	3.4.5	4.04	4.07	4.10	4.07	0.27
4.5	6	All	12	3.4.5	4.73	4.51	4.77	4.67	0.25
5.0	6	All	12	6.8.9	5.09	5.09	4.84	5.01	0.24
25	6	All	2	4.6.8	24.0	24.30	23.97	24.09	1.50
30	6	All	1	4.8.9	29.1	29.93	29.83	29.62	2.16

Table A2-1: Combination of achieving ideal UVB dose rates by justifying different numbers of lamps, board level of UVB lamps and positions of vessels

Ideal UVB dose rate (Wm^{-2})	Number of UVB lamps	Positions of UVB lamps	Height level of shelf	3 vessel positions In water bath	Dose rate at each position (Wm^{-2})			Actual average UVB dose rate (Wm^{-2})	SD
0.5	1	6	13	2.5.7	0.49	0.53	0.48	0.50	0.03
1.0	1	4	10	2.5.7	1.04	1.08	0.99	1.04	0.05
2.0	2	3.4	10	1.2.4	1.90	2.09	2.20	2.07	0.20
3.0	4	2.3.4.5	13	3.5.6	3.06	3.06	2.94	3.02	0.16
4.5	6	All	12	3.4.5	4.73	4.51	4.77	4.67	0.25
6.0	2	3.4	4	3.4.7	5.75	6.19	6.25	6.06	0.22

Appendix 3

The log-linear + tail application formation is:

$$\log10(\mathrm{N}) = \log10\left[\left(10^{log10(N0)} - 10^{log10(Nres)}\right) * e^{-k_{max}t} * \left(\frac{e^{k_{max}S_1}}{1+(e^{k_{max}S_1}-1)*e^{-K_{max}t}}\right) + 10^{log10(N_{res})}\right] \quad \textbf{Equation A(1)}$$

where:

- N_{res}: the residual micro-organisms number at the given time (CFU/mL)
- N_0: the initial micro-organisms number (CFU/mL)
- t: the experiment duration
- K_{max}: the rate of inactivation (1/time unit)

This application model was modified from (Geeraerd et al., 2005):

$$\mathrm{N(t)} = (\mathrm{N(0)} - N_{res}) * e^{-\mathrm{k}_{max}t} * \left(\frac{e^{k_{max}S_1}}{1+(e^{k_{max}S_1}-1)e^{-k_{max}}}\right) + N_{res} \quad \textbf{Equation A(2)}$$

This was based on a couple of equations (Geeraerd et al., 2000):

$$\frac{\mathrm{dN}}{dt} = -k_{max} * N * (\frac{1}{1+C_c})(1 - \frac{N_{res}}{N}) \quad \textbf{Equation A(3)}$$

$$\frac{dC_c}{dt} = -k_{max} * C_c \quad \textbf{Equation A(4)}$$

where C_c is related to the cell physiological state; by changing the Cc with $e^{kmaxs1-1}$, formation 2 was gained; dN/dt model is the log-linear part of the inactivation part while the last part N_{res} stands for the more resistant subpopulation.

It should be noted that the equation is not a fundamental description but an empirical model. The tail part or survival part of the curve is referred for a population of micro-organisms that remain at a constant number, or do not process any following inactivation in time. The mechanism behind the appearance of the tail was still unclear: it could be a normal feature connected with the mechanism of resistance; the survivor was the result of mutation and adaption (Cerf, 1977).

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