EVALUATING THE POTENTIAL OF ULTRAVIOLET IRRADIATION FOR THE DISINFECTION OF MICROBIOLOGICALLY POLLUTED IRRIGATION WATER

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DECLARATION

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ABSTRACT

Fresh produce irrigation water from Western Cape Rivers carries faecal coliforms (FC) (*Escherichia coli*) at concentrations which often exceed the suggested limit of 1 000 FC per 100 mL and presents a health risk to consumers. On-farm ultraviolet (UV) irradiation presents several advantages for water disinfection but is an uncommon practice in South Africa. The aim of this study was to investigate the use of UV irradiation for river water disinfection prior to irrigation.

Escherichia coli (*E. coli)* strains were exposed to low-pressure (LP) UV (4 $mJ.cm^{-2}$) and UV/Hydrogen peroxide (H_2O_2) (4 $mJ.cm^{-2}$/20 $mg.L^{-1}$) treatments in Sterile Saline Solution (SSS). Strain variation in reductions was observed and ranged from 1.58 to 3.68 and 1.34 to 3.60 log for the UV and UV/H_2O_2 treatments, respectively. The UV/H_2O_2 treatment (4 $mJ.cm^{-2}$/20 $mg.L^{-1}$) was more effective, compared to UV singly, against some of the *E.* coli strains. Selected strains showed increased sensitivity at higher UV doses (8, 10 and 13 $mJ.cm^{-2}$) and H_2O_2 concentrations (100 and 200 $mg.L^{-1}$ with 4 $mJ.cm^{-2}$) but a 3 log target reduction was not always reached. For all UV and UV/H_2O_2 treatments maximum resistance was shown by an environmental strain. Reference strains should, therefore, not be used for the optimisation of UV based disinfection parameters.

At 10 $mJ.cm^{-2}$ an American Type Culture Collection (ATCC) reference strain and an environmental strain (ATCC 25922 and F11.2) were both significantly less inactivated in sterilised river water compared to SSS. Enhanced water quality allowed for improved inactivation of the ATCC strain. Also, the efficiency of LP UV (5, 7 and 10 $mJ.cm^{-2}$) and medium-pressure (MP) UV (13, 17 and 24 $mJ.cm^{-2}$) radiation was investigated using water from the Plankenburg River. Water was sampled and treated on three respective days (Trials 1 to 3). Physico-chemical and microbiological water quality was always poor. The FC concentration reached a maximum of 6.41 log $cfu.100\ mL^{-1}$ while UV transmission was always below 38%. For LP and MP UV irradiation increased doses resulted in increased disinfection but a 3 log reduction of FC was only attained when MP UV light was used in Trial 1. Disinfection efficiency was dependent on water quality and on the characteristics of the microbial population in the water. Since FC were never reduced to below 3 log $cfu.100\ mL^{-1}$, the water did not adhere to guidelines for produce irrigation.

Photo-repair following irradiation was investigated in river water using MP UV doses of 13 and 24 $mJ.cm^{-2}$ and 3.5 kLux reactivating light, initially. Ultraviolet transmission was close to 50% and total coliform (TC) reduction exceeded 3 log, even at 13 $mJ.cm^{-2}$. However, TC were reactivated from below 1 000 $cfu.100.mL^{-1}$ to 3.93 and 4.41 log $cfu.100\ mL^{-1}$ for the 13 and 24 $mJ.cm^{-2}$ treatments, respectively. A higher MP dose (40 $mJ.cm^{-2}$) and a different treatment regime (2 x 20 $mJ.cm^{-2}$) inhibited photo-repair (compared to 13 and 24 $mJ.cm^{-2}$) but TC were always recovered to a final concentration surpassing 3 log $cfu.100\ mL^{-1}$, even under lower light intensities (1.0 to 2.0 kLux).

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In the current study UV irradiation did not produce water of acceptable standards for produce irrigation, mainly as a result of extremely poor water quality. However, on farm-scale, UV efficiency could be enhanced by improving water quality before irradiation. Also, stronger lamps that deliver higher UV doses may result in adequate disinfection, irrespective of water quality. Higher UV doses and the use of combination treatments (such as UV/Chlorine and UV/Peracetic acid) should be further investigated also to determine its disinfection efficiency and possible capability to inhibit post-disinfection repair.

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UITTREKSEL

Varsproduk besproeiingswater vanuit Wes-Kaapse riviere bevat fekale kolivorme (FK) (*Escherichia coli*) in konsentrasies wat dikwels die voorgestelde limiet van 1 000 FK per 100 mL oorskry en hou `n gesondheidsrisiko vir verbruikers in. Plaasvlak ultraviolet (UV) bestraling bied verskeie voordele met verwysing na water dekontaminering, maar word selde aangewend in Suid-Afrika. Die doel van hierdie studie was om die gebruik van UV bestraling vir die dekontaminering van rivierwater, voor besproeiing, te ondersoek.

Escherichia coli (*E. coli*) isolate is blootgestel aan lae-druk (LD) UV (4 $mJ.cm^{-2}$) en UV/Waterstofperoksied (H_2O_2) (4 $mJ.cm^{-2}$/20 $mg.L^{-1}$) behandelings in Steriele Sout Oplossing (SSO). Isolaat variasie in reduksies is waargeneem en het gewissel tussen 1.58 tot 3.68 en 1.34 tot 3.60 log vir die UV en UV/H_2O_2 behandelings, onderskeidelik. In vergelyking met UV bestraling alleen was die UV/H_2O_2 behandeling (4 $mJ.cm^{-2}$/20 $mg.L^{-1}$) meer effektief teen sommige *E. coli* isolate. Geselekteerde isolate was meer sensitief tot hoër UV dosisse (8, 10 en 13 $mJ.cm^{-2}$) en H_2O_2 konsentrasies (100 en 200 $mg.L^{-1}$ met 4 $mJ.cm^{-2}$), maar `n 3 log teikenreduksie was nie altyd haalbaar nie. Vir alle UV en UV/$H_2O_2$ behandlinge was die meeste weerstand deur `n omgewingsisolaat gebied. Verwysingsisolate behoort daarom nie aangewend te word vir die optimisering van UV-gebaseerde behandelingsparameters nie.

By 10 $mJ.cm^{-2}$ was beide `n ATCC verwysingsisolaat en `n omgewingsisolaat (ATCC 25922 en F11.2) betekenisvol minder gedeaktiveer in rivierwater as in SSO. Verbeterde waterkwaliteit het verhoogde inaktivering van die ATCC isolaat toegelaat. Die doeltreffendheid van LD UV (5, 7 en 10 $mJ.cm^{-2}$) en medium-druk (MD) UV (13, 17 en 24 $mJ.cm^{-2}$) bestraling is ook ondersoek deur watermonsters vanuit die Plankenburg Rivier te gebruik. Watermonsters was getrek en behandel op drie verskillende dae (Proewe 1 tot 3). Fisies-chemiese en mikrobiologiese kwaliteit van die water was deurentyd swak. Die FK konsentrasie het `n maksimum van 6.41 log kve.100 $mL^{-1}$ bereik terwyl UV transmissie altyd laer as 38% was. Vir beide LD en MD UV bestraling het verhoogde dosisse gelei tot verbeterde dekontaminering, maar `n 3 log reduksie is slegs bereik toe MD UV lig gebruik is in Proef 1. Die effektiwiteit van die behandelings was afhanklik van waterkwaliteit en die eienskappe van die mikrobiese populasie in die water. Aangesien FK nooit tot onder 3 log kve.100 mL^{-1} verminder is nie het die water nie voldoen aan riglyne vir varsproduk-besproeiing nie.

Fotoherstel na bestraling was ondersoek in rivierwater deur aanvanklik gebruik te maak van MD UV dosisse van 13 en 24 $mJ.cm^{-2}$ en 3.5 kLux heraktiverende lig. Ultraviolettransmissie het byna 50% bereik en reduksie van totale kolivorme (TK) het 3 log oorskry, selfs by 13 $mJ.cm^{-2}$. Totale kolivorme was egter geheraktiveer van onder 1 000 kve.100.mL^{-1} tot 3.93 en 4.41 log kve.100 mL^{-1} vir die 13 en 24 $mJ.cm^{-2}$ behandelings, onderskeidelik. In vergelyking met 13 en 24 $mJ.cm^{-2}$ het `n hoër MD dosis (40 $mJ.cm^{-2}$) en `n veranderde bestralingstegniek

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(2 x 20 mJ.cm^{-2}) fotoherstel onderdruk, maar TK was in elke geval geheraktiveer tot `n finale konsentrasie hoër as 3 log kve.100 mL^{-1}, selfs onder laer intensiteit lig (1.0 tot 2.0 kLux).

In hierdie ondersoek het UV bestraling nie water van aanvaarbare standaarde vir varsproduk besproeiing gelewer nie, hoofsaaklik as gevolg van swak waterkwaliteit. Nietemin, op plaasvlak mag die effektiwiteit van UV bestraling verhoog word deur waterkwaliteit voor bestraling te verbeter. Die gebruik van sterker lampe, om hoër UV dosisse te produseer, mag verder bydra tot voldoende dekontaminasie, ongeag van waterkwaliteit. Hoër UV dosisse en die gebruik van kombinasie behandelinge (soos UV/Chloor en UV/Perasynsuur) moet ook verder ondersoek word om die dekontaminasie effektiwiteit, en vermoë daarvan om heraktivering na dekontaminering te onderdruk, vas te stel.

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Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

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ABBREVIATIONS

ANOVA	Analysis of Variance
AOP	Advanced Oxidation Process
API	Analytical Profile Index
ATCC	American Type Culture Collection
CES	Chromocult® Coliform Agar Enhanced Selectivity
cfu	Colony Forming Units
COD	Chemical Oxygen Demand
CPD	Cyclobutane Pyrimidine Dimer
DBPs	Disinfection By-products
d_p	Particle Diameter
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
E. coli	*Escherichia coli*
EC	Electrical Conductivity
FAD	Flavin Adenine Dinucleotide
FC	Faecal Coliforms
H_2O_2	Hydrogen Peroxide
HP1	Hydroperoxidase 1
HP2	Hydroperoxidase 2
HPC	Heterotrophic Plate Count
kLux	Kilolux
kve	Kolonie Vormende Eenhede
L-EMB	Levine's Eosin Methylene-Blue Lactose Sucrose Agar
LP	Low-pressure
LPM	Litres Per Minute
LSD	Least Significant Difference
MF	Membrane Filtration
MP	Medium-pressure
MPFs	Minimally Processed Foods
NCIMB	National Collections of Industrial, Marine and Food Bacteria
NER	Nucleotide Excision Repair
NTU	Nephelometric Turbidity Units
PBS	Phosphate Buffered Saline
PCA	Plate Count Agar
SANS	South African National Standards
SSS	Sterile Saline Solution

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TC	Total Coliforms
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
U	Catalase Activity Units
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
UVT%	Ultraviolet Transmission Percentage
VRBA	Violet Red Bile Agar
VSS	Volatile Suspended Solids
WHO	World Health Organisation

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Chapter 1
INTRODUCTION

Owing to its inclusion in the water-energy-food nexus, water as a resource is key to sustaining the activities and satisfying the needs of an ever-growing population (Ahuja, 2015). Large volumes of water are required annually for agricultural irrigation, which is estimated to contribute up to 70% of global water usage (Renner, 2012; Taft, 2015). Currently, however, population and economic growth, industrialisation and environmental concerns limit the availability of water for irrigational purposes and food production (Hanjra & Qureshi, 2010; Norton-Brandäo *et al.*, 2013). In the South African context, water is extremely scarce and continual pollution further compromises the usable yield of the available surface waters (DEAT, 2011). While several sectors contribute to water use in the country, agricultural irrigation dominates by using 62% of the accessible fresh water (DWAF, 2009; Basson, 2011). In this regard, the quality of the natural surface water resources in South Africa is of critical importance (Le Roux *et al.*, 2012).

Various researchers have reported that an increase in the number of produce-related foodborne disease outbreaks is currently observed (Lynch *et al.*, 2009; Velázquez, 2009; Warriner *et al.*, 2009). A propos, irrigation water has been identified as a major pre-harvest contributor of microbiological contamination of fresh produce (Pachepsky *et al.*, 2011). Gastrointestinal illness is increasingly related to the intake of such products, while a vast amount of money is spent annually in respect of this problem. In the United States of America, produce-associated illnesses were responsible for 46% of all foodborne outbreaks reported in the period 1998 to 2008 (Painter *et al.*, 2013). Between 2001 and 2005, in Australia, 4% of all illness outbreaks were linked to fresh produce consumption (Kirk *et al.*, 2008).

Although *Escherichia coli* (*E. coli)* and *Salmonella* are regarded as the predominant threats, an array of pathogenic protozoa, viruses and bacteria may occur on irrigated foodstuffs (Aruscavage *et al.*, 2006; Warriner *et al.*, 2009). Nonetheless, an *E. coli* O157:H7 outbreak, associated with uncooked radish sprouts, claimed the lives of 12 people in Japan in 1996 after 12 000 cases were reported (Michino *et al.*, 1999). Furthermore, *E. coli* O145 resulted in 26 known infections across multiple states in the USA in 2010, owing to the ingestion of contaminated lettuce (CDC, 2010). In one of the most tragic outbreaks yet, *Escherichia coli* O104:H4 was responsible for 4 000 confirmed infections and 47 deaths in Germany in 2011 (EFSA, 2011). The event was associated with the consumption of fenugreek seeds which later also instigated 16 illnesses in France (Olaimat & Holley, 2012). With regard to *Salmonella* found on irrigated products, 1 442 persons across the United States and Canada contracted Salmonellosis after consuming hot peppers in 2011 (Mody *et al.*, 2011). Adding to this, *Salmonella* Saintpaul associated with cucumbers instigated 84 illnesses across the US in 2013 (CDC, 2013). Considering such disease outbreaks, it suggested that the elderly, infants and individuals with poor immunity, particularly, are vulnerable targets (Britz *et al.*, 2012). This becomes problematic in South Africa, where the

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population presents a large percentage of immuno-compromised people, because of poor nutrition and HIV infection (Britz *et al*., 2012).

With reference to the microbiological quality of water used for fresh produce irrigation, the Department of Water Affairs (DWA) and the World Health Organisation (WHO) have suggested a guideline limit of 1 000 colony forming units (cfu) per 100 mL (3 log cfu.100 mL^{-1}) for faecal coliforms (FC) (WHO, 1989; DWAF, 1996). In this regard, South African rivers are extremely polluted and have become a great source of concern. Faecal contamination of local waters frequently occurs as inadequate waterworks cannot accommodate the requirements of urbanisation and a rapidly growing population (Van Vuuren, 2009; Ijabadeniyi & Buys, 2012). As a result, *E. coli* counts exceeding 500 000 cfu.100 mL^{-1} have been detected in irrigation water in the Western Cape (Paulse *et al*., 2009). Lötter (2010) reported on faecal coliform levels of 160 000 and 460 000 cfu.100 mL^{-1} in the Plankenburg and Mosselbank Rivers, respectively. In addition, in 2013, Britz *et al.* detected coliforms and faecal coliforms at levels reaching 4.897 MPN.100 mL^{-1} in the Eerste River. These results suggest that effective disinfection methods are required to reduce the microbial load in river water prior to its use for the irrigation of agricultural food products. In this regard, a target reduction of 3 – 4 log units has been suggested by Britz *et al.* (2013).

In order to quantify the level of faecal pollution (and subsequent disinfection efficiency) in water, *E. coli* is often used as indicator microorganism (Moussa & Massengale, 2008; Britz *et al*., 2012). This microorganism complies with most of the criteria of a good indicator and naturally occurs in the intestines of mammals (Odonkor & Ampofo, 2013). Furthermore, faecal coliforms (*E. coli*) are often encountered in water quality guidelines such as those proposed for fresh produce irrigation by WHO (1989) and DWAF (1996). The use of *E. coli* in laboratory-scale disinfection experiments is, therefore, highly appropriate.

Techniques for disinfecting contaminated water can generally be classified as being chemical, mechanical or photochemical in nature (Raudales *et al*., 2014). The functionality of these are usually influenced by water quality, which may be highly variable in surface water. As a result, not all methods will be equally suitable for disinfection purposes prior to irrigation (Jones *et al*., 2014). In recent years, the preferred treatment of contaminated surface water and wastewater has been rooted in the use of chemicals, in particular chlorine, due to its ease of application, fairly low cost and its ability to offer residual activity (Teksoy *et al*., 2011). However, the formation of potentially harmful disinfection by-products (DBPs) and the presence of chemical residues have encouraged the use of new-generation techniques either singly or in combination with current methods (Quek & Hu, 2008; Guo *et al*., 2011).

As an alternative method of water disinfection, ultraviolet (UV) irradiation is now well-accepted and gaining popularity when compared to conventional techniques (Poepping *et al*., 2014; Kollu & Örmeci, 2015). Ultraviolet systems are easily operated and are said to be effective against an array of pathogenic microorganisms (Vélez-Colmenares *et al*., 2011). In addition, the process does not lead to the generation of potentially hazardous DBPs (Liu *et al*., 2002; Guo *et al*.,

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2009; Turtoi, 2013). Typically, UV radiation is produced by using either low-pressure (LP) or medium-pressure (MP) mercury vapour lamps, which emit light at a single wavelength of 253.7 nm and within a range of 200 to 600 nm, respectively (Kowalski, 2009; Gayán *et al*., 2014).

Nevertheless, as with any disinfection method, UV irradiation is not entirely flawless. The predominant mechanism of UV disinfection is based on the absorption of UV energy by microbial genetic materials (Guo *et al*., 2013). More specifically, when nucleotides absorb UV light, the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine 6-4 pyrimidones (6-4 PPs) occur within the DNA strand (Gayán *et al*., 2014; Poepping *et al*., 2014). Consequently, mutagenesis will lead to cell death (Friedberg *et al*., 2006). A major drawback associated with UV disinfection is the reported capability of bacteria to repair UV-induced DNA damage following irradiation. Several routes of DNA repair may be followed, but the most extensively studied mechanism occurs in the presence of visible and near UV light and is known as photoreactivation or photo-repair (Hijnen *et al*., 2006; Guo *et al*., 2011). In this process, photolyase enzymes harness the energy of visible light to reverse the formation of CPDs, specifically (Gayán *et al*., 2014). In contrast, a phenomenon known as dark-repair involves the recovery of DNA damage in the absence of light and is performed by UvrABC exinuclease (Rastogi *et al*., 2010). The influence of dark-repair, however, is more difficult to study and is reported to be much less of a concern following UV irradiation.

Furthermore, while UV irradiation is regarded as a fairly effective method of water disinfection, several additional factors may complicate the investigation of its lethality. Water quality, in terms of those parameters affecting UV transmission, has been identified as being particularly influential (Brahmi *et al*., 2010). Essentially, water quality indicators such as the chemical oxygen demand (COD), UV transmission percentage (UVT%), turbidity, total suspended solids (TSS) content and conductivity may govern the efficiency of the process. Furthermore, variation in UV sensitivity of different microorganisms, and even different strains of the same species, has been reported (Hijnen *et al*., 2006; Gayán *et al*., 2014). Such differences have also been reported for the potential of post-disinfection DNA repair (Quek & Hu, 2008; Hu *et al*., 2012). As a result, some microorganisms may show greater UV resistance compared to others, due to differences in both intrinsic and extrinsic parameters.

It is clear that several factors will influence the disinfection efficiency of UV irradiation. In addition to investigating the technique on laboratory-scale (using isolated microorganisms) it is important to evaluate its effectiveness on a larger scale. This will introduce variability in the irradiated microbial population as well as in the quality of the water to be treated, thereby generating results that would be more representative of the effectiveness of the process. Furthermore, laboratory-scale UV equipment usually employs LP lamps whereas larger UV systems mostly use MP lamps. The difference in the emission spectra of the two types of lamps may also introduce variation in the observed disinfection capabilities of UV light. Medium-pressure UV lamps have been reported to be more effective, as they allow for lower levels of repair (Zimmer

& Slawson, 2002; Hu *et al.*, 2005). Therefore, the influence of the equipment used in UV disinfection studies should be duly noted. The impact of photoreactivation on disinfection efficiency should also be considered. Water to be disinfected on a larger scale would probably be exposed to sunlight before being used for irrigation. Depending on factors such as time of exposure and the intensity of the sunlight, photo-repair may influence disinfection efficiency.

The greater aim of the current research was to evaluate the potential of UV irradiation for the disinfection of microbiologically contaminated irrigation water. Several studies were performed focussing on: the effect of LP UV irradiation and UV based advanced oxidation processes (AOPs) on environmental and American Type Culture Collection (ATCC) reference *E. coli* strains; the influence of several parameters of river water quality on potentially effective UV treatments and AOPs; the potential of laboratory-scale (LP) and pilot-scale (MP) UV irradiation for the disinfection of river water containing a naturally occurring microbial population; and the influence of DNA repair mechanisms on the disinfection efficiency of MP (pilot-scale) UV irradiation of contaminated river water.

REFERENCES

Ahuja, S. (2015). Chapter 1: Nexus of Food, Energy, and Water. In: *Food, Energy, and Water: The Chemistry Connection* (edited by S. Ahuja). Pp. 1-20. USA: Elsevier.

Aruscavage, D., Lee, K., Miller, S. & LeJeune, J.T. (2006). Interactions affecting the proliferation and control of human pathogens on edible plants. *Journal of Food Science*, **71**, 89-99.

Basson, M.S. (2011). Water development in South Africa. [Internet document]. URL http://www.un.org/waterforlifedecade/green_economy_2011/pdf/session_1_economic_instruments_south_africa.pdf. Accessed 05/07/2015.

Brahmi, M., Belhadi, N.H., Hamdi, H. & Hassen, A. (2010). Modeling of secondary treated wastewater disinfection by UV irradiation: effects of suspended solids content. *Journal of Environmental Sciences,* **22**(8), 1218-1224.

Britz, T.J., Sigge, G.O., Buys, E.M., Schmidt, S., Potgieter, N. & Taylor M.B. (2012). Baseline study on extent (types and quantities) of contamination found in irrigation water at selected sites (K5/1773). In: *Quantitative investigation into the link between irrigation water quality and food safety* (Volume 2). WRC Report no. K5/1773/4, Pretoria, South Africa.

Britz, T.J., Sigge, G.O., Huisamen, N., Kikine, T., Ackermann, A., Lötter, M., Lamprecht, C. & Kidd, M. (2013). Fluctuations of indicator and index microbes as indication of pollution over three years in the Plankenburg and Eerste Rivers, Western Cape, South Africa. *Water SA*, **39**(4), 457-465.

CDC (Centers for Disease Control and Prevention) (2010). Investigation update: multistate outbreak of human *E. coli* O145 infections linked to shredded romaine lettuce from a single

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processing facility. [Internet document]. URL http://www.cdc.gov/ecoli/2010/ecoli_o145/index.html. Accessed 26/03/2014.

CDC (Centers for Disease Control and Prevention) (2013). Multistate outbreak of *Salmonella* Saintpaul infections linked to imported cucumbers (final update). [Internet document]. URL http://www.cdc.gov/salmonella/saintpaul-04-13/. Accessed 08/06/2014.

DEAT (Department of Environmental Affairs and Tourism) (2011). Environmental Sustainability Indicators, Technical Report. Department of Environmental Affairs and Tourism, Pretoria. p. 185.

DWAF (Department of Water Affairs and Forestry) (1996). *South African Water Quality Guidelines*, 2nd ed. (edited by S. Holmes). *Volume 4: Agricultural Water Use: Irrigation*. Pp. 71-76. The Government Printer. Pretoria, South Africa.

DWAF (Department of Water Affairs and Forestry) (2009). Water Growth and Development Framework (Version 7). [Internet document]. URL http://www.dwa.gov.za/WFGD/documents/WFGD_Frameworkv7.pdf. Accessed 05/07/2015.

EFSA (European Food Safety Authority) (2011). *Shiga toxin-producing E. coli (STEC) O104:H4 2011 outbreaks in Europe: taking stock*. European Food Safety Authority, Parma. Pp. 1-22.

Friedberg, E.C., Walker, G.C., Siede, W., Wood, R.D., Schultz, R.A. & Ellenberger, T. (2006). DNA damage. In: *DNA Repair and Mutagenesis*, 2nd ed. Pp. 9-57. USA: ASM Press.

Gayán, E., Condón, S. & Álvarez, I. (2014). Biological aspects in food preservation by ultraviolet light: a review. *Food and Bioprocess Technology*, **7**, 1-20.

Guo, M., Hu, H., Bolton, J.R. & El-Din, M.G. (2009). Comparison of low- and medium-pressure ultraviolet lamps: photoreactivation of *Escherichia coli* and total coliforms in secondary effluents of municipal wastewater treatment plants. *Water Research*, **43**(3), 815-821.

Guo, M., Huang, J., Hu, H. & Liu, W. (2011). Growth and repair potential of three species of bacteria in reclaimed wastewater after UV disinfection. *Biomedical and Environmental Sciences*, **24**(4), 400-407.

Guo, M., Huang, J., Hu, H., Liu, W. & Yang, J. (2013). Quantitative characterisation and prediction modelling of photoreactivation of coliforms after ultraviolet disinfection of reclaimed municipal wastewater. *Water, Air, & Soil Pollution*, DOI: 10.1007/s11270-013-1774-z.

Hanjra, M.A. & Qureshi, M.E. (2010). Global water crisis and future food security in an era of climate change. *Food Policy*, **35**, 365-377.

Hijnen, W.A.M., Beerendonk, E.F. & Medema, G.J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Research*, **40**(1), 3-22.

Hu, J.Y., Chu, X.N., Quek, P.H., Feng, Y.Y. & Tan, X.L. (2005). Repair and regrowth of *Escherichia coli* after low- and medium-pressure ultraviolet disinfection. *Water Science and Technology: Water Supply*, **5**(5), 101-108.

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Hu, X., Geng, S., Wang, X. & Hu, C. (2012). Inactivation and photorepair of enteric pathogenic microorganisms with ultraviolet irradiation. *Environmental Engineering Science*, **29**(6), 549-553.

Ijabadeniyi, O.A. & Buys, E.M. (2012). Irrigation water and microbiological safety of fresh produce; South Africa as a case study: a review. *African Journal of Agricultural Research*, **7**(35), 4848-4857.

Jones, L.A., Worobo, R.W. & Smart, C.D. (2014). UV light inactivation of human and plant pathogens in unfiltered surface irrigation water. *Applied and Environmental Microbiology*, **80**(3), 849-854.

Kirk, M.D., Fullerton, K. & Gregory, J. (2008). Fresh produce outbreaks in Australia 2001-2006. Board 21. In: *2008 International Conference on Emerging Infectious Diseases Program and Abstracts*. Pp. 49-50. Atlanta: Centers for Disease Control and Prevention.

Kowalski, W. (2009). Introduction. In: *Ultraviolet Germicidal Irradiation Handbook*. Pp. 1-13. New York: Springer.

Kollu, K. & Örmeci, B. (2015). Regrowth potential of bacteria after ultraviolet disinfection in the absence of light and dark repair. *Journal of Environmental Engineering*, DOI: 10.1061/(ASCE)EE.1943-7870.0000905.

Le Roux, W.J., Schaefer, L.M. & Genthe, B. (2012). Microbial water quality in the upper Olifants River catchment: implications for health. *African Journal of Microbiology Research*, **6**(36), 6580-6588.

Liu, W., Andrews, S.A., Bolton, J.R., Linden, K.G., Sharpless, C. & Stefan, M. (2002). Comparison of disinfection by product (DBP) formation from different UV technologies at bench scale. *Water Supply*, **2**(5-6), 515-521.

Lötter, M. (2010). *Assessment of microbial loads present in two Western Cape rivers used for irrigation of vegetables*. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Lynch, M.F., Tauxe, R.V. & Hedberg, C.W. (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology and Infection*, **137**, 307-315.

Michino, H., Araki, K., Minami, S., Tayaka, S., Sakai, N., Ono, A. & Yanagawa, H. (1999). Massive outbreak of *Escherichia coli* O157:H7 infection in school children in Sakai City, Japan, associated with consumption of white radish sprouts. *American Journal of Epidemiology*, **150**, 787-796.

Mody, R.K., Greene, S.A., Gaul, L., Sever, A., Pichette, S., Zambrana, I., Dang, T., Gass, A., Wood, R., Herman, K., Cantwell, L.B., Falkenhorst, G., Wannemuehler, K., Hoekstra, R.M., McCullum, I., Cone, A., Franklin, L., Austin, J., Delea, K., Behravesh, C.B., Sodha, S.V., Yee, J.C., Emanuel, B., Al-Khaldi, S.F., Jefferson, V., Williams, I.T., Griffin, P.M. & Swerdlow, D.L. (2011). National outbreak of *Salmonella* serotype Saintpaul infections:

Stellenbosch University https://scholar.sun.ac.za

Importance of Texas restaurant investigations in implicating jalapeño peppers. *PLoS ONE*, **6**(2), 1-7.

Moussa, S.H. & Massengale, R.D. (2008). Identification of the sources of *Escherichia coli* in a watershed using carbon-utilization patterns and composite data sets. *Journal of Water and Health*, **6**(2), 197-207.

Norton-Brandäo, D., Scherrenberg, S.M. & Van Lier, J.B. (2013). Reclamation of used urban waters for irrigation purposes – a review of treatment technologies. *Journal of Environmental Management*, **122**, 85-98.

Odonkor, S.T. & Ampofo, J.K. (2013). *Escherichia coli* as an indicator of bacteriological quality of water: an overview. *Microbiology Research*, **4**(2), 5-10.

Olaimat, A.N. & Holley, R.A. (2012). Factors influencing the microbial safety of fresh produce: a review. *Food Microbiology*, **32**, 1-19.

Pachepsky, Y., Shelton, D.R., McLain, J.E.T., Patel, J. & Mandrell, D.E. (2011). Irrigation waters as a source of pathogenic microorganisms in produce: a review. *Advances in Agronomy*, **113**, 73-138.

Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R., Angulo, F.J. & Griffin, P.M. (2013). Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerging Infectious Diseases*, **19**(3), 407-415.

Paulse, A.N., Jackson, V.A. & Khan. W. (2009). Comparison of microbial contamination at various sites along the Plankenburg- and Diep Rivers, Western Cape, South Africa. *Water SA*, **35**(4), 469-478.

Poepping, C., Beck, S., Wright, H. & Linden K.G. (2014). Evaluation of DNA damage reversal during medium-pressure UV disinfection. *Water Research*, **56**, 181-189.

Quek, P.H. & Hu, J. (2008). Indicators for photoreactivation and dark repair studies following ultraviolet disinfection. *Journal of Industrial Microbiology and Biotechnology*, **35**(6), 533-541.

Rastogi, R.P., Richa, K.A., Tyagi, M.B. & Sinha, R.P. (2010). Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *Journal of Nucleic Acids*, **2010**, 1-32.

Raudales, R.E., Parke, J.L., Guy, C.L. & Fisher, P.R. (2014). Control of waterborne microbes in irrigation: a review. *Agricultural Water Management*, **143**, 9-28.

Renner, J. (2012). Global irrigated area at record levels, but expansion slowing. [Internet document]. URL http://www.worldwatch.org/global-irrigated-area-record-levels-expansion-slowing-0. Accessed 26/08/2014.

Taft, H.L. (2015). Chapter 16: Water Scarcity: Global Challenges for Agriculture. In: *Food, Energy, and Water: The Chemistry Connection* (edited by S. Ahuja). Pp. 395-428. USA: Elsevier.

Stellenbosch University https://scholar.sun.ac.za

Teksoy, A., Alkan, U., Eleren, S.Ç., Topaç, B.Ş., Şağban, F.O.T. & Başkaya, H.S. (2011). Comparison of indicator bacteria inactivation by the ultraviolet and the ultraviolet/hydrogen peroxide disinfection processes in humic waters. *Journal of Water and Health*, **9**(4), 659-669.

Turtoi, M. (2013). Ultraviolet light potential for wastewater disinfection. *Annals. Food Science and Technology*, **14**(1), 153-164.

Van Vuuren, L. (2009). New water framework counts every drop. *The Water Wheel*, **8**(3), 28-30.

Velázquez, L.C., Barbini, N.B., Escudero, M.E., Estrada, C.L. & Stefanini de Guzmán, A.M. (2009). Evaluation of chlorine, benzalkonium chloride and lactic acid as sanitizers for reducing *Escherichia coli* O157:H7 and *Yersinia enterocolitica* on fresh vegetables. *Food Control*, **20**(3), 262-268.

Vélez-Colmenares, J.J., Acevedo, A. & Nebot, E. (2011). Effect of recirculation and initial concentration of microorganisms on the disinfection kinetics of *Escherichia coli*. *Desalination*, **280**, 20-26.

Warriner, K., Huber, A., Namvar, A., Fan, W. & Dunfield, K. (2009). Recent advances in the microbial safety of fresh fruits and vegetables. *Advances in Food and Nutrition Research*, **57**, 155-208.

WHO (World Health Organisation) (1989). *Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture.* WHO, Technical Report Series no. 778. Geneva, Switzerland.

Zimmer, J.L. & Slawson, R.M. (2002). Potential repair of *Escherichia coli* following exposure to UV radiation from both medium- and low-pressure UV sources used in drinking water treatment. *Applied and Environmental Microbiology*, **68**(7), 3293-3299.

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Chapter 2
LITERATURE REVIEW

2.1. BACKGROUND

In the agricultural field, water as natural resource is of critical importance for the production of nutritious, safe and readily available fresh produce. Globally, approximately 19% of cropland accounts for irrigated agriculture and supplies 40% of the food demand (Molden *et al*., 2010). Such irrigation contributes 70% of water withdrawals from river systems, and plays a significant part in the maintenance of global food security (Molden *et al*., 2007). Food security, however, is now threatened as urbanisation, industrialisation and other non-agricultural water requirements continue to overshadow the importance of water used for irrigation (Hanjra & Qureshi, 2010). Adding to this, addressing environmental concerns such as changed water flows and erosion may redirect the water supply away from irrigated agriculture (Hanjra & Qureshi, 2010). Economic growth in developing countries such as South Africa may further influence water management policies. These should always be aligned in relation to observed sectorial growth, in order to ensure sensible water abstraction. Moreover, it has been reported that South African infrastructure for wastewater management is in urgent need of restoration, as a lack of maintenance has led to the establishment of an ineffective system (Ijabadeniyi *et al*., 2011). In effect, this will help relieve the increased water demand generated by economic growth and the requirement of food security. Climatic changes observed in many arid regions of the world add to the declining availability of water for agricultural irrigation. South Africa's mean annual rainfall of approximately 450 mm, is almost half the world average (DEAT, 2006). Of this, only 9% is accessible as surface water (UNEP FI, 2009). Since South Africa is classified as a semi-arid country, care should be taken to manage inland water resources properly, thereby ensuring a continual supply of fresh fruit, vegetables and other agricultural products (DEAT, 2006).

Since fruit and vegetables yield significant levels of vitamins, minerals, fibre and phytochemicals, high intakes thereof are internationally recommended and associated with health-promoting benefits (Slavin & Lloyd, 2012). In 2006, it was reported that an increase of 29% per capita in the consumption of fresh and minimally processed foods (MPFs) was observed in the United States between 1980 and 2000 (Matthews, 2006). This trend was expected to continue as health organisations constantly promote the intake of such foods. Globally, however, trends towards decreased physical activity and increasingly sedentary lifestyles have been reinforced by the development of westernised diets. In 2008, it was estimated that approximately 1.5 billion adults could be classified as being overweight or obese and were consequently subjected to life-threatening conditions such as diabetes, hypertension and cardiovascular disease (Popkin *et al*., 2011). Thus, public health actions have been implemented in various countries in an effort to promote the benefits associated with fruit and vegetable consumption (Dallongeville *et*

al., 2010). It is, therefore, critically important that the global agricultural sector provides the population with wholesome, safe and readily available produce.

Of growing concern, however, is the fact that gastrointestinal illness is increasingly associated with the consumption of MPFs (Pachepsky *et al*., 2011). While there may be many contributing factors, declining irrigation water quality and the consequent increase in the prevalence and ingestion of bacteria associated with common MPFs, have often been reported (Paulse *et al*., 2009; Pachepsky *et al*., 2011; Ijabadeniyi & Buys, 2012). The scope of pathogenic microorganisms associated with irrigated fresh produce has also been thoroughly investigated. Enterohemorrhagic *Escherichia coli* (e.g., *E. coli* O157:H7), *Listeria monocytogenes*, *Cryptosporidium* spp., *Giardia* spp. and viruses such as enteroviruses and noroviruses contribute to an extensive list of problematic pathogens (Pachepsky *et al*., 2011). Both developing and developed countries are impacted by these and other produce related microorganisms. In the United States alone, an estimated $39 billion is spent annually in an effort to combat fresh produce related foodborne illnesses (Scharff, 2010).

Regarding the sources of fruit and vegetable contamination, one has to consider both pre- and post-harvest factors. While there are many problem areas, the use of irrigation water of poor microbiological quality has been identified as the leading source of contamination (Duffy *et al*., 2005; Johnston *et al*., 2006). This is worrying since South African rivers, which are commonly utilised for agricultural irrigation, reportedly carry extremely high pathogenic loads (Paulse *et al*., 2009). The levels of faecal indicator and index organisms in South African rivers often exceed the guidelines set by the Department of Water Affairs (DWA) and the World Health Organisation (WHO). For the irrigation of fresh produce, the DWA and WHO allow a maximum of 1 000 faecal coliforms per 100 mL of water (WHO, 1989; DWAF, 1996). In the Western Cape, water used for irrigation has reached *E. coli* counts of more than 500 000 colony forming units (cfu) per 100 mL of water (Paulse *et al*., 2009). Faecal coliform and *E. coli* counts of up to 17.4 x 10^6 coliforms per 100 mL and 12.99 x 10^6 *E. coli* per 100 mL, respectively, have been detected in the Plankenburg River (Barnes & Taylor, 2004). More recently, Britz *et al.* (2013) reported that coliform and faecal coliform counts of up to 4.897 MPN.100 mL^{-1} were detected in the Eerste River. Increasing water scarcity and contamination of water resources call for immediate action to combat the prevalence of food safety risks.

The solution to the current problem is not as simple as using irrigation water of high quality. Such resources are becoming scarce and alternative interventions are required to enhance the quality of the available waters. In effect, pollution has to be prevented at source or alternatively at the point of use. Apart from using good quality water, other factors such as crop type and the type of irrigation system used should also be taken into consideration (Stine *et al*., 2005). These play an important role in the rate of pathogen transfer from water to crop.

Since an array of political, financial, social and other factors complicate the prevention of water contamination, disinfection of irrigation water receives much attention. Disinfection methods

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are typically divided into chemical and mechanical techniques, but alternative treatments such as ultrasound and ultraviolet (UV) light are also used. Treatment techniques should always be assessed in terms of financial and practical viability as well as technical feasibility prior to their implementation or recommendation. Nevertheless, research confirms that specific carry-over of pathogens frequently occurs between irrigation water and irrigated produce. The consumption of fruit and vegetables may, therefore, pose significant risks to the health of consumers (Britz *et al.*, 2012). In mentioning this, emphasis is placed on the prevention of pre-harvest contamination of MPFs by implementing novel strategies (Lynch *et al.*, 2009).

2.2. THE CURRENT STATE OF GLOBAL WATER RESOURCES

Water forms part of what is known as the water-energy-food security nexus which implies that complex relations between these resources exist (Gulati *et al.*, 2013). Accordingly, 70% of global water use is attributed to the requirements of irrigated agriculture (Renner, 2012). However, water scarcity, climate change and the energy crisis affect food security as a global water crisis emerges (Hanjra & Qureshi, 2010). Moreover, constant growth in population and income, increase the demand for water in irrigation, domestic and industrial applications (Hanjra & Qureshi, 2010). According to the United Nations, water scarcity rather than shortages in agricultural land, will hinder the need for increased food production in the near future (UNDP, 2006). In Australia for instance, the production of cereal and rice in the Murray-Darling Basin (MDB), decreased by 40% early in the 20th century (ABS, 2010). By 2050, a projected increase of 65% in global cereal demand will put enormous pressure on the already limited global water resources (De Fraiture *et al.*, 2007). To worsen matters, urbanisation and industrialisation contribute to over-exploitation of water resources, which further results in an increase of foodborne diseases related to irrigated crops as water quality is inevitably diminished.

In recent decades, investment in water infrastructure alleviated the demand for food by a growing population at the cost of the environment and hundreds of millions of people who still lack food security. In many developing countries, for instance, water may either be unavailable or inaccessible due to the lack of infrastructure. Data on water availability and demand is distressing: by 2050, the required volume of water for crop production may increase with 70% to 110% if productivity is not increased (De Fraiture *et al.*, 2007). Furthermore, aquifers are emptied at rates which exceed the natural supply, and approximately 50% of the world's rivers are polluted (Hanjra & Qureshi, 2010).

In the light of continual water scarcity, various sectors will be in competition for the available water and may force water use away from agriculture (Molden, 2007). There may also be an increase in the occurrence of water-related, foodborne diseases as water quality steadily declines. These and other factors contribute to the vast challenge of maintaining agricultural production and

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global food security. Effective water resource management for food security requires novel initiatives as population growth and income increase drastically.

2.3. THE STATE OF WATER RESOURCES IN SOUTH AFRICA

Rainfall and climatic variability, surface flow characteristics as well as groundwater replenishment and quality, contribute to what is known as the hydrological cycle and require extensive management to ensure effective, sensible water use (DEAT, 2006). In the South African context, 19 Water Management Areas (WMAs) have been established for this purpose, specifically. Nevertheless, inappropriate management practices and various other challenges threaten water security (SADC, 2008). Rivers and dams, which are surface water resources, provide for most of the urban water requirements, while groundwater is primarily used by rural communities and in arid areas (DEAT, 2006).

2.3.1. Available water per capita

According to calculations, water availability in South Africa is estimated as 1 100 m^3 per person per annum from the available freshwater and groundwater resources (DEAT, 2006). In relation to the estimated minimum requirement of 1 000 m^3 per person per annum, as recommended by the United Nations, South Africa is classified as one of the 20 most water scarce countries globally (DEAT, 2011). Considering population growth only, water availability in 2030 is projected as 1 186 m^3 per person per annum (DEAT, 2011). However, changes in the total amount of water resources were not considered and this estimation may not be accurate (DEAT, 2011). A more comprehensive projection by the National Water Research Strategy (NWRS) suggests that an insufficiency of water will be reached by 2025 when water requirements are calculated with respect to different scenarios of economic growth (DWAF, 2004).

Rainfall in South Africa is low, approximately 450 – 500 millilitres per year ($mL.yr^{-1}$) (DEAT, 2006). Moreover, as a result of the local climate ranging from desert to sub-humid, the spatial distribution of rainfall is also highly variable (DWA, 2013). The total mean runoff in the country amounts to 49 000 million cubic meters per annum ($m^3.yr^{-1}$), with only 8.6% of the yearly rainfall being utilised (DEAT, 2006; DWA, 2013). Although rivers and dams are extensively developed across South Africa, various sources of pollution contribute to a compromised usable yield of surface waters (DEAT, 2006). These may include urban and mining drainage and irrigation return flows. South African dams, nonetheless, represent a capacity to the order of 66% of the annual runoff and predominantly supply the water requirements of the country (DWAF, 2004).

In dry and rural areas, especially in the eastern and north-eastern parts of South Africa, groundwater is often utilised as an alternative to surface water and contributes approximately 10 000 – 16 000 million $m^3.yr^{-1}$ on average, but only 7 000 million $m^3.yr^{-1}$ in times of drought. As a result of several geological factors the use of such water is rather limited (DEAT, 2006). Water that

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is used non-consumptively in certain sectors also contributes to the total water availability as return flow. The value of return flows are immense as it resembles volumes much greater than that provided by groundwater resources (Table 1). However, it is important, to ensure that such water complies with the required quality parameters concerned with its intended use (DEAT, 2006).

Table 1 Average available water yield (million $m^3.yr^{-1}$) calculated for 19 South African WMAs in the year 2000 (DEAT, 2006)

Source of water		**Available water yield (million $m^3.yr^{-1}$)**
Natural	Surface (1)	10 240
	Ground (2)	1 088
Return flow	Irrigation	675
	Urban	970
	Mining and bulk industrial	254
Total		**13 227**

(1) From river-run-of and existing storage after consideration of losses to urban run-off, alien vegetation, rain-fed sugar cane, the ecological component of the rivers and reserves
(2) From existing springs and boreholes

2.3.2. Current and future water requirements

Sectorial water requirements vary with regard to assurance of supply as well as quality, quantity and temporal distribution (DEAT, 2006). Agricultural irrigation represents a strong seasonality factor in water requirement while the domestic, industrial and mining sectors require a more constant supply (DWAF, 2004). With reference to all of the 19 WMAs in South Africa, the average sectorial water requirements are shown as percentages of a total of 12 871 million $m^3.yr^{-1}$ in the year 2000 (Fig. 1) (DEAT, 2006). The fact that agricultural irrigation contributes the majority of fresh water use, is noteworthy. Ideally, sectors consuming high volumes of water should also contribute strongly to the South African economy.

Data shows that water use in South Africa is predominantly consumptive. When considering water requirements and the usable return flows from the irrigation, urban and mining, and bulk industrial sectors, respectively, yields are calculated as 9, 33 and 34% (DEAT, 2006). Power generation, irrigation and rural activities are the major consumptive water users, while return flows from the other sectors are often poorly managed and carelessly discharged (DWAF, 2004).

Considering the relationship between economic growth and water requirement, the NWRS has estimated water requirement based on expected growth in gross domestic product (GDP). A base scenario of 1.5% GDP growth and a high scenario of 4.0% GDP growth up to 2025 implies that local water requirements will increase to 14 230 million $m^3.yr^{-1}$ and 16 814 million $m^3.yr^{-1}$, respectively. For the two scenarios, water availability was calculated as 14 166 million $m^3.yr^{-1}$ and 14 940 million $m^3.yr^{-1}$, respectively, by 2025, resulting in deficits of 234 million $m^3.yr^{-1}$ and 2 044 million $m^3.yr^{-1}$ (DWAF, 2004).

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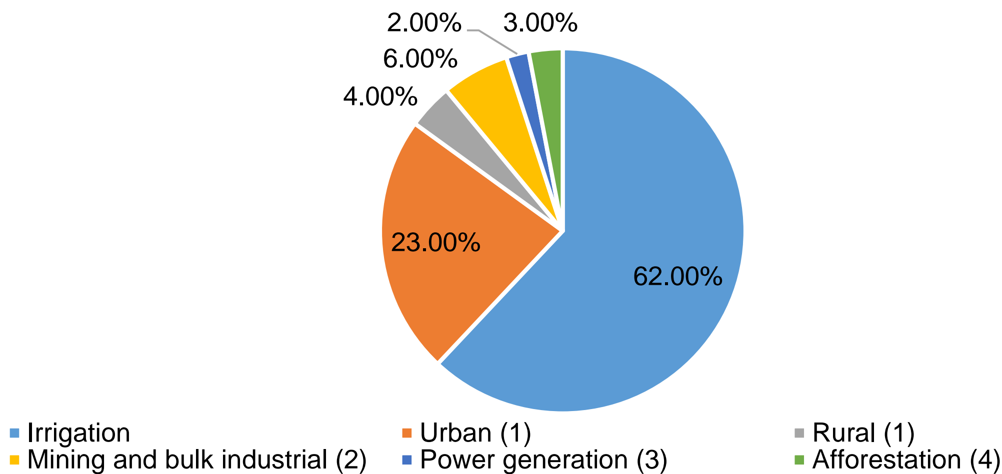

Figure 1 Percentage sectorial water requirement for the 19 WMAs of South Africa in the year 2000 (DEAT, 2006).

(1) Includes basic human needs estimated at 25 litres per person daily
(2) Those excluded from urban structures
(3) Water used to generate thermal power only
(4) Values only refer to impact on yield

Increases in urban domestic requirements, accompanied by general population growth, define the base scenario. However, in this instance, commercial, communal and industrial water use is expected to increase in congruence with domestic requirements (DWAF, 2004). Unprecedented socio-economic growth, again accompanied by population growth, defines the above-mentioned upper scenario in which communal, industrial and commercial water use increases in relation to GDP growth (DWAF, 2004).

2.3.3. Microbiological state of South African (Western Cape) rivers

It has been shown by many that the microbiological quality of South African river water has become a cause for concern (Barnes & Taylor, 2004; Paulse *et al.*, 2009). Insufficient sanitation facilities and inadequate sewage treatment works throughout the country are often referred to as primary sources of pollution. Untreated sewage is frequently released into South African surface waters as urbanisation and population growth surpass the rate at which sewage disposal systems are developed or maintained (Van Vuuren, 2009; Ijabadeniyi & Buys, 2012). Consequently, faecal contamination of irrigation water frequently occurs.

In 2007, the South African Water Research Commission (WRC) initiated a scoping study in order to understand the extent of the problem better. Contamination levels of South African river water were compared to guidelines set by the South African Department of Water Affairs (DWA) and the World Health Organisation (WHO). For technical and practical reasons, *Escherichia coli* was selected as an indicator of the level of faecal pollution of river water. Coliform bacteria naturally occur in the intestines of mammals and therefor significant numbers of *E. coli* in river water may imply that faecal waste such as untreated sewage, as well as additional pathogens, may be present (Britz *et al.*, 2012; Britz *et al.*, 2013). With regard to *E. coli* contamination, the

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WHO and DWA have suggested a guideline limit of ≤ 1 000 faecal coliforms (*E. coli*) per 100 mL if water is intended to be used for the irrigation of fresh produce (WHO, 1989; DWAF, 1996).

Short-term research preceding the mentioned WRC study, found that South African river water often exceeds these limits. Western Cape Rivers are of particular concern with regard to faecal contamination and subsequent contamination of irrigated produce. It was reported in 2004 that counts of 1.74 x 10^7 faecal coliforms per 100 mL and 1.29 x 10^7 *E. coli* per 100 mL of water were detected in the Plankenburg River near Stellenbosch (Barnes & Taylor, 2004). This research was conducted over a five year period and indicated that the highest levels of contamination occurred during the summer months (Van Blommestein, 2012). Another study concluded that counts of up to 3.5 x 10^6 faecal coliforms and *E. coli* per 100 mL were detected in the same river (Paulse *et al*., 2009). Poor sanitation and waterworks in the Kayamandi informal settlement, together with neighbouring industrial and agricultural areas downstream of Kayamandi, were regarded as sources of contamination. Samples from the Diep River represented maximum counts of 1.6 x 10^6 faecal coliforms and *E. coli* per 100 mL, probably being contaminated by industrial effluent from local establishments (Paulse *et al*., 2009). Paulse *et al.* (2007) also reported that samples from the Berg River represented *E. coli* counts of 1.7 x 10^6 per 100 mL resulting from spills of untreated sewage and effluent running into the river from the surrounding informal settlements.

The WRC study concluded that neither the Plankenburg nor the Eerste River met the guidelines set by the WHO and DWA (Britz *et al*., 2013). An array of microbiological and physico-chemical parameters were considered in this regard. Using the Multiple Tube Fermentation Method (MTF) and Most Probable Number (MPN) tables, faecal coliform counts varied from nought to 6.845 MPN.100 mL^{-1} for the three sampling sites of the study (Britz *et al*., 2013). The maximum *E. coli* count was also concluded to be 6.845 MPN.100 mL^{-1} (Britz *et al*., 2013). The Eerste River represented a maximum of 4.897 MPN.100 mL^{-1} for coliform and faecal coliform counts. This lower number may be attributed to the absence of neighbouring industrial areas and informal settlements. Nevertheless, approximately one third of the Eerste River samples exceeded the guideline of ≤ 1 000 faecal coliforms (*E. coli*) per 100 mL. It was concluded that neither the Plankenburg, nor the Eerste River, were suitable for the irrigation of minimally processed fresh produce.

In support of these results, Lötter (2010) reported that faecal coliform counts of up to 160 000 and 460 000 cfu.100 mL^{-1} were recorded in the Plankenburg and Mosselbank Rivers, respectively, while Ackerman (2010) recorded values of up to 1 700 000 cfu.100 mL^{-1} in samples from the Eerste and Berg Rivers. Huisamen (2012) recorded maximum faecal coliform and *E. coli* concentrations of 7 x 10^6 cfu.100 mL^{-1} in both the Plankenburg and Eerste Rivers. The high level of *E. coli* detected in these rivers is a cause for concern, since the possibility of disease outbreaks supress its use for irrigation in both commercial and subsistence food production.

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2.4. WATER USE AND ECONOMIC IMPORTANCE OF IRRIGATED AGRICULTURE IN SOUTH AFRICA AND THE WESTERN CAPE

Approximately 13% of South Africa's area is classified as being fit for the production of crops (Britz *et al.*, 2012). Less than a quarter of this represents high production potential with water availability being the primary constraint. Furthermore, South African land suitable for irrigation shows great inter-provincial variation (Fig. 2) and amounts to roughly 1 498 000 hectares (ha) (FAO, 2005). Nevertheless, the agricultural economy of South Africa is defined by one sector being focussed on commercial production of agricultural products and another being driven purely for the purpose of subsistence (Britz *et al.*, 2012). These sectors contribute to a combined area of over 1.3 million ha being irrigated in South Africa (Backeberg *et al.*, 1996; Perret, 2002).

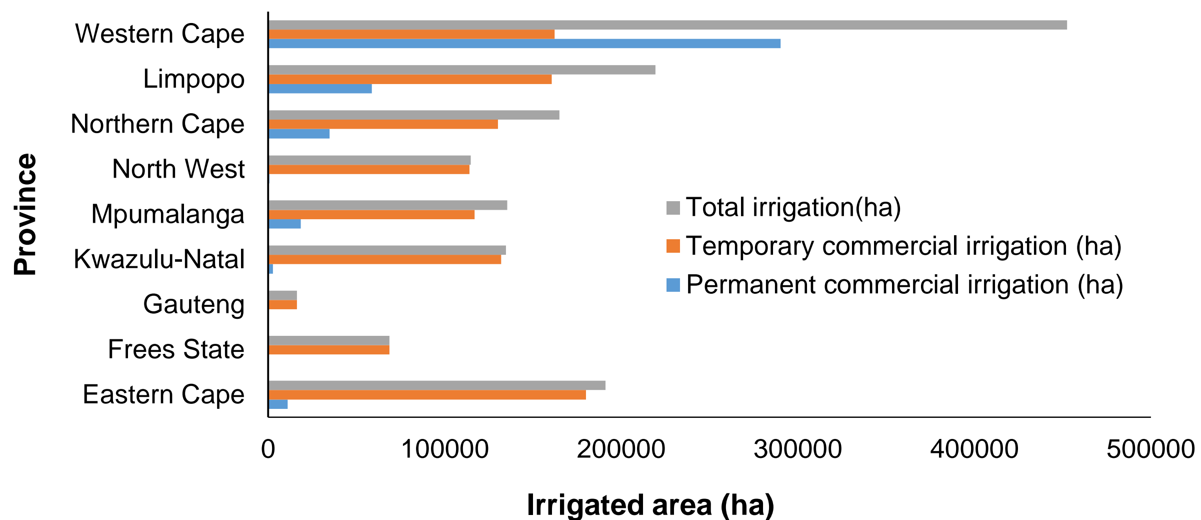

Figure 2 Inter-provincial variation in irrigated land area in South Africa (FAO, 2005).

Given that agricultural irrigation contributes more than 60% of the South African water requirement, it is expected that primary agriculture contributes strongly to the South African economy (DWAF, 2004). It is estimated that 10% and 30% of maize and wheat production, respectively, originates from irrigated agriculture. In addition, 90% of grape, deciduous fruit, citrus and vegetable production relies on irrigation (Backeberg, 2006). These are all important contributors to local and export markets. According to Ndiame & Jaffee (2005) a total of 73% of vegetables and fresh fruit exported from Africa to the USA is produced in South Africa. The country is also known as the primary third world exporter of horticultural products to the European Union (EU), holding 31% of the EU's share for imported vegetables and fruit (Ndiame & Jaffee, 2005). As a matter of fact, 60% of all fruit cultivated in the country is exported. Of the remaining 40%, half is consumed while the other half is locally processed into juice and/or fruit concentrate for retail in supermarkets (WESGRO, 2006). With regard to vegetables, only three Sub-Saharan Africa countries represent nearly 90% of exports and again South Africa is the dominant exporter (Ndiame & Jaffee, 2005).

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As reported by the Department of Agriculture, Forestry and Fisheries (DAFF), the gross farming income for the year ended in mid-June 2012 amounted to R178 050 million (DAFF, 2013). With reference to previous years, income from field crops, horticultural products and animal products increased by 7.3, 11.3 and 11.2%, respectively (DAFF, 2013). For the 2012/13 financial year the gross value of primary agriculture amounted to R180 360 million, with an increase of 10.2% from the previous year (DAFF, 2013). The contribution of horticultural products to the gross value during this period was estimated at 25.0%. Nonetheless, growth in the total economy exceeded growth of the primary agricultural sector in recent decades and agriculture's contribution to the gross domestic product (GDP) decreased to 1.9% in 2012 (DAFF, 2013). Furthermore, according to DWAF (2004) approximately 25 – 30% of the contribution of agriculture to the GDP originates directly from irrigated agriculture. This implies that irrigation consumes a great deal of South African water while contributing less than 0.6% to the GDP (DWAF, 2004).

While water usage may seem high in relation to the low economic output of irrigated agriculture, one must be aware of the economic linkages of this sector with others of the South African economy (DWAF, 2004). This implies that irrigated agriculture influences the economy on levels other than solely generating returns from local sales and exports. Factoring in the contribution of agriculture to employment, transport, and earning foreign exchange, its contribution to the GDP may actually be in the region of 20 – 30% (Lötter, 2010).

In the Western Cape, approximately 270 000 ha of the cultivated 2.5 million ha is currently under irrigation, and produces the bulk of fruit and vegetables in South Africa (EADP, 2013). As a matter of fact, the Western Cape annually contributes approximately 21% to commercial agriculture in South Africa (WESGRO, 2012). Agriculture in the Western Cape acts as a key employer in addition to the value it contributes to the South African economy. It is estimated that at least 1.5 million dependants are supported by the 11 000 commercial and development farmers and their 220 000 employees (WESGRO, 2006).

In accordance with the afore-mentioned figures, the Western Cape produces 70% of all fruit in South Africa, 15 – 20% of South African citrus and 55 – 60% of the total exported produce (WESGRO, 2012). These figures result from a combination of ideal climatic conditions, the fair availability of water and intra-provincial geographical variation, all of which allow for the cultivation of a variety of produce. While pears and apples, for example, are produced primarily in the Elgin and Ceres areas, a variety of stone fruit is cultivated in the Small Karoo (Britz *et al.*, 2012). Agriculture in the province also yields 12% of the vegetables cultivated in South Africa (WESGRO, 2003). Not surprising thus, is the fact that the Western Cape represents the swiftest development and growth in the produce sector in South Africa. The province is the leading cultivator of fresh produce in the country and exports amount to approximately R7 billion per year (Britz *et al.*, 2012). The multiplicity of agricultural activities and opportunities in the Western Cape contributes significantly to the economic and social stability of South Africa. Since

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this sector is primarily dependant on irrigation, the quality of water in the Western Cape is immensely important.

2.5. FOODBORNE DISEASE OUTBREAKS RELATED TO IRRIGATED PRODUCE

An increase in the number of foodborne outbreaks related to fresh produce is currently observed in industrialised and developing countries alike (Lynch *et al*., 2009). Such outbreaks have increased from < 1% in the 1970s to approximately 12% in the 1990s in the USA, while 4% of all outbreaks in Australia resulted from contaminated fresh produce from 2001 until 2005 (Kirk *et al*., 2008; Lynch *et al*., 2009). In addition to increased consumption of MPFs, enhanced food safety systems, epidemiological surveillance, and growing public awareness have contributed to this situation (Lynch *et al*., 2009). More recent data show that 14.8% of illness outbreaks in the USA resulted from fresh produce and accounted for 22.8% of foodborne illnesses from 1998 – 2007 (CSPI, 2009). Furthermore, it was reported by De Waal & Bhuiya (2009) that fresh produce in the United States resulted in 13% of outbreaks and a subsequent 21% of illness cases from 1990 until 2005.

In developing countries, especially, factors such as decreased water availability, an increase in high risk population size and unsound practices along the "farm-to-fork" continuum contribute to the growing number of outbreaks (Lynch *et al*., 2009; Britz *et al*., 2012). Unfortunately, due to improper surveillance and documentation systems, data regarding produce related foodborne outbreaks in South Africa is scarce (Niehaus *et al*., 2011; Huisamen, 2012). However, it is expected that enhanced identification and tracking methods, as well as the occurrence of pathogens with low infective doses, will continue to contribute to an increase in the number of outbreaks observed across the globe.

Waterborne pathogens contaminating irrigated produce are often identified as being either viruses, protozoa or bacteria (Beuchat, 2002; Aruscavage *et al*., 2006). Amongst these, researchers have reported that *E. coli* and *Salmonella* are most frequently associated with produce-related illness outbreaks (Warriner *et al*., 2009). Many factors, including rainfall patterns, socio-economic standing of nearby communities, seasonal factors, urbanisation and industrialisation may contribute to the level at which these microorganisms occur in irrigation water (Nasser, 2005). Once fresh produce has been contaminated, a set of extrinsic and intrinsic factors influence pathogen survival and growth. These include the availability of nutrients, temperature, pH, atmospheric composition and redox potential (Kotzé, 2015). Additionally, adherence of pathogens to fresh produce surfaces and the phenomenon of pathogen internalisation contribute to the ultimate level of contamination and subsequent consumer infection (Lynch *et al*., 2009).

2.5.1 Produce-associated disease outbreaks: pathogens of particular concern

Produce-related foodborne outbreaks is defined as "the occurrence of two or more cases of the same illness in which epidemiologic investigation implicated the same uncooked fruit, vegetable,

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salad or juice" (Sivapalasingam *et al.*, 2004). In the light of such outbreaks it should be noted that an extensive amount of variables are known to impact the likelihood of fruit and vegetable contamination (Harris *et al.*, 2003). Incidence studies provide useful information regarding contamination of specific products during specific times, but seldom discuss the sources of contamination. In the context of this study, which considers irrigation water as source of contamination, a positive correlation between pathogens resulting in produce-related illness outbreaks and commonly encountered waterborne pathogens is expected.

The significance of *E. coli* and *Salmonella* spp. in fresh produce associated outbreaks has been mentioned already. Brandl (2006) reported that from 1990 to 2004, *E. coli* O157:H7 and *S. enterica* were the predominant causes of produce related food poisoning in the US. During this period, *Escherichia coli* contamination resulted in 19% and 48% of the outbreaks related to fruits and leafy vegetables, respectively. *Salmonella enterica* was responsible for 76% and 30% of outbreaks related to the respective categories mentioned above (Brandl, 2006). Table 2 shows recent fresh produce related outbreaks (2005 to 2011) that resulted in subsequent illnesses and/or deaths (Olaimat & Holley, 2012). From here, the prevalence and impact of *Salmonella* and *E. coli* are visible.

In 1996, the largest *E. coli* O157:H7 outbreak yet, claimed 12 lives following 12 000 reported cases. These were linked to the consumption of raw radish sprouts in Japan (Michino *et al.*, 1999). *Escherichia coli* was also responsible for a devastating outbreak in the US in 2006 where severe illness resulted in three deaths (Olaimat & Holley, 2012). More recently, in 2010, *E. coli* O145 resulted in 26 confirmed infections across 5 states in the US following the consumption of romaine lettuce (CDC, 2010). In 2011, another tragic *E. coli* O104:H4 outbreak claimed the lives of 47 people (EFSA, 2011). Contaminated fenugreek seeds of Egyptian origin were identified as the causative carrier, which eventually led to approximately 4 000 confirmed infections in Germany. Four weeks later, the same sprouts resulted in 16 illnesses being reported in Bordeaux, France (Olaimat & Holley, 2012). In 2013, another *E. coli* O157:H7 outbreak associated with ready-to-eat salads infected 33 individuals, resulting in seven hospitalisations (CDC, 2013a). In addition to these examples, and many more that exist, *E. coli* has been associated with various other fresh produce sources and continues to be one of the most troublesome pathogens.

Foodborne outbreaks resulting from *Salmonella* species are most commonly associated with the consumption of improper poultry products (Greig & Ravel, 2009). Various outbreaks related to fresh produce have now also confirmed such foodstuffs as common carriers of the pathogen. Salmonellosis affected 1 442 individuals across Canada and the USA in 2008, while another outbreak resulted in 140 cases early in 2011 (CDC, 2011a; Mody *et al.*, 2011). These two outbreaks resulted from hot pepper and alfalfa sprout consumption, respectively. Early in 2013, the consumption of cucumbers, contaminated with *Salmonella* Saintpaul, resulted in 84 reported illnesses and 17 hospitalisations across 18 states in the US (CDC, 2013b).

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In addition to *E. coli* and *Salmonella,* various other pathogens have caused significant disease outbreaks following fruit and vegetable consumption. In 2011, *L. monocytogenes* resulted in 30 deaths following an outbreak related to contaminated cantaloupe in the USA (CDC, 2011b). *Shigella*, *Yersinia pseudotuberculosis* and the norovirus have been found on baby corn, lettuce and raspberries, respectively, indicating the vast amount of potential hazards (Nuorti *et al*., 2004; Hjertqvist *et al*., 2006).

Table 2 Global fresh produce related illness outbreaks documented from 2005 to 2011 (Olaimat & Holley, 2012)

Pathogen	Year	Country	Produce	Illnesses	Deaths
Salmonella	2005	Canada	Mung bean sprouts	592	-
Salmonella	2005	USA	Tomatoes	459	-
E. coli O157:H7	2006	USA	Spinach	199	3
Salmonella	2006	Australia	Alfalfa sprouts	125	-
Salmonella	2006	USA	Tomatoes	183	-
Salmonella	2006	Australia	Cantaloupe	115	-
Salmonella	2007	Europe	Baby spinach	354	-
Shigella sonei	2007	Australia, Europe	Baby carrots	230	-
Salmonella	2008	USA, Canada	Peppers	1 442	2
E. coli O157:H7	2008	USA, Canada	Lettuce	134	-
Salmonella	2008	USA, Canada	Peanut butter	714	9
Salmonella	2009	USA	Alfalfa sprouts	235	-
E. coli O145	2010	USA	Lettuce	26	-
Salmonella	2010	USA	Alfalfa sprouts	44	-
L. monocytogenes	2010	USA	Celery	10	5
Salmonella	2011	USA	Papaya	106	-
E. coli O104:H4	2011	Europe	Vegetable sprouts	3 911	47
L. monocytogenes	2011	USA	Cantaloupe	146	31
E. coli O157:H7	2011	USA	Strawberries	15	1

2.6. SOURCES OF FRESH PRODUCE CONTAMINATION

The contamination of fresh produce can be multi-dimensional and includes chemical and microbiological aspects (Ackerman, 2010). Although the two forms of contamination may differ with regard to source, regularity of occurrence and detection, they are equally important, often resulting in immediate and/or chronic health implications (Ackerman, 2010).

Microbiological contamination of fresh produce may occur at various points along the production continuum. Accordingly, contamination is classified as having either a pre or post-harvest origin (Beuchat & Ryu, 1997). This implies that production, harvesting, processing,

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storage, transportation, retailing and consumer handling may all pose risks if hygienically compromised practices are the norm. Commonly encountered pre-harvest contamination sources include faeces, soil, irrigation water and water used to apply chemicals, green manure, dust, animals and human handling (Beuchat, 2006; Johnston *et al.*, 2006). Faeces, handling, processing equipment, containers for transport and storage, animals, dust and water used for washing and rinsing are well known post-harvest sources. Regardless of the possible sources of contamination, effective handling and sanitising practices in the production of MPFs must be maintained in order to minimise the risk of infections and disease outbreaks.

2.6.1 Soil as contaminant

Providing ample conditions of nutrients, pH, temperature and organic material, soil often favours the survival and reproduction of pathogenic microorganisms. Clostridial spores and spores from *Bacillus cereus* commonly occur in soil and may pose a health risk to consumers if carried over to MPFs (Beuchat & Ryu, 1997). Other pathogens that naturally occur in agricultural land include *Listeria monocytogenes* and *Aeromonas* (Olaimat & Holley, 2012). *Listeria monocytogenes* is the most common pathogenic microorganism found in soil and prefers to exist as saprophyte in a variety of decomposing plant sources (Beuchat, 1996; Beuchat & Ryu, 1997).

Upon exposure to animal waste, the profile of microbes within soil may be widened (Whipps *et al.*, 2008). This is problematic since cultivated land may have been previously used for animal farming purposes, thereby exposing the soil to faeces. In addition, fertilizing farmland with animal manure or slurry may add to creating a reservoir of pathogens (Table 3) (Ackerman, 2010). As a matter of fact, sewage and the manure of cattle and sheep are the primary contributors of *E. coli* O157:H7 and *Salmonella* in soil (Olaimat & Holley, 2012). These enteric pathogens are likely to occur in land treated with manure since they may survive in soil for years (Doyle & Erickson, 2008). Faecal contamination from cattle, poultry and pigs may further introduce *Campylobacter jejuni* to land intended for agricultural cultivation (Warriner *et al.*, 2009). Soil can also be contaminated with pathogenic microorganisms during times of heavy rain, as flooding may carry such microbes from contaminated upstream sources (Ackerman, 2010). The mentioned zoonotic pathogens, as well as *Salmonella*, survive optimally in clay-based soil of low temperature when manure is still present (Holley *et al.*, 2006).

Table 3 Levels of common microbial pathogens found in faecal based substances (Olaimat & Holley, 2012)

	Source		
Pathogen	**Faeces (cfu.g^{-1})**	**Slurry (cfu.g^{-1})**	**Manure (cfu.g^{-1})**
E. coli	$10^2 – 10^5$	$10 – 10^4$	-
Salmonella spp.	$10^2 – 10^7$	-	$10^2 – 10^7$
Yersinia spp.	-	$10 – 10^4$	-

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2.6.2 Irrigation water as contaminant

Globally, surface water resources are extensively utilised for irrigational purposes. This, too, is the case in South Africa where commercial and subsistence farmers draw water from rivers to irrigate a variety of produce (DEAT, 2006). In this regard, researchers have identified microbiologically unsound irrigation water as the predominant pre-harvest contaminant of fresh produce (Warriner *et al.*, 2009; Pachepsky *et al.*, 2011). Contamination of such water may result from either point-source or non-point-source origins (DEAT, 2006; Stewart *et al.*, 2008). A point-source problem is easily identified, quantified and resolved and may include sewage and/or industrial discharge practices (DEAT, 2006). Non-point-source factors are commonly not identifiable or quantifiable.

A variety of bacteria, viruses and protozoa have been isolated from irrigation water and subsequently reported to contaminate cultivated produce (Warriner *et al.*, 2009). However, as was the case for soil, microbiological pathogens in water are predominantly of faecal origin or often even transferred directly from faecal matter. The use of manure as a fertilising practise may easily result in the contamination of irrigation water. Untreated manure may introduce *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* into cultivated soil, but may further contaminate irrigation water if contact should occur. By means of manipulating feed rations, or treating manure prior to use, the risk of microbiological contamination of irrigation water as well as soil may be reduced (Jamieson *et al.*, 2004).

In developing countries such as South Africa, irrigation water contaminated with human faecal matter is a growing cause for concern. This may result from improper sewage treatment systems, the nonchalant release of untreated waste and the establishment of informal settlements close to rivers (Barnes & Taylor, 2004). In the South African context, sanitation facilities in rural areas are often inadequate in relation to the amount of residents present (Barnes & Taylor, 2004). Consequently, rivers have become receivers of waste and waste water while still being used for agricultural irrigation (Matthews, 2006). In addition to the effect of rural areas established close to rivers, various other sources contribute to the pollution of South African rivers. Industrial operations such as wineries and food producing factories for instance, have been reported to discharge manufacturing effluents into local rivers (Barnes, 2003). Such activities may significantly influence river water quality as it was shown that effluent from a yeast producing company represented a variety of disease-causing microorganisms. These include *E. coli* O157:H7 as well as species of *Clostridium* and *Staphylococcus* (Van der Merwe & Britz, 1994a & b).

In addition to the quality of the water, the type of irrigation system used may also influence the extent of microbiological carry-over from water to crop (Warriner *et al.*, 2009). It was found that 90% of lettuce samples that were spray-irrigated with *E. coli* invested water were contaminated in comparison to 19% being contaminated following surface irrigation with the same water sample (Solomon *et al.*, 2002). Spray and flood irrigation represent the most significant risk of carry-over since these techniques transfer water directly onto the edible parts of fresh produce (FDA, 1998).

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2.6.3 Indicator microorganisms for determining irrigation water quality

Research has found a positive correlation between irrigation water of poor microbiological quality and the subsequent level of contamination of irrigated produce (Warriner *et al*., 2009; Pachepsky *et al*., 2011). It is, therefore, important to evaluate the microbiological quality of water sources prior to its utilisation for irrigation. Since microbiological inhabitants in such water may be represented by a variety of pathogens in varying concentrations, it would be impractical to analyse water samples with respect to the entire population. Adding to this is the fact that identification methods are often costly, complex and time consuming (Campos, 2008; Britz *et al*., 2012). The exercise of monitoring the presence of "indicator" and "index" microorganisms has, therefore, become an internationally accepted method of determining water quality (Britz *et al*., 2012).

Analysing and quantifying the presence of indicator organisms provides an accurate indication of the presence and numbers of additional pathogens in a sample. The FDA has defined an indicator as "a microorganism or a group of them that indicate that food or water has been exposed to conditions that pose to increase the risk of it becoming contaminated with a pathogen if held under conditions conducive for pathogenic growth" (James, 2006). Indicators are thus generally very useful in providing information regarding the type of microbiological contamination of water. They are often used to evaluate water for faecal contamination and to estimate the efficacy of water treatment processes (Campos, 2008). *Escherichia coli*, total and faecal coliforms as well as faecal enterococci for instance, serve as indicators of faecal contamination and the possible presence of additional enteric pathogens (Savichtcheva & Okabe, 2006). Index organisms on the other hand are groups, species or strains used to predict and quantify the presence of specific additional pathogens (Campos, 2008; Parajuli *et al*., 2009). These index microorganisms function in manners similar to the ones they are associated with and thus provide behavioural models for particular contaminants (WHO, 2001).

If indicators are to be representative of pathogens they should comply with the following basic criteria: it should possess broad survival criteria regarding water type and other aspects; its quantity in the analysed substance should exceed that of the pathogens; it should not be able to multiply in an aquatic environment; it must represent a fairly long survival time; its quantity must correlate well with the degree of water pollution; it must be absent when pollution is absent and it should be safe to work with under laboratory conditions (DWAF, 1996). Some drawbacks associated with the use of indicator microorganisms for the detection of faecal pollution include their capability to proliferate outside of the gastro-intestinal tract, their sensitivity to common disinfectants, their quick die-off rates, the fact that they do not provide information regarding the source of contamination, the fact that they are often difficult to cultivate and their potential of originating from non-faecal sources (Savichtcheva & Okabe, 2006).

2.6.3.1. *Escherichia coli* as indicator of faecal contamination in irrigation water

Globally*, E. coli* is commonly used as indicator of the level of faecal contamination of water as well as the effectiveness of disinfection procedures (Figueras & Borrego, 2010; Britz *et al*., 2012). Such monitoring activities are very important since bacteria of faecal origin include *Campylobacter*, *Shigella*, *Yersinia,* as well as viruses and protozoa which all represent significant risks to the health of humans (Savichtcheva & Okabe, 2006).

Since *E. coli* occur naturally in the gut of mammals, its presence in irrigation water may point to faecal pollution as the bacteria are frequently released together with faeces (Jagals *et al*., 2006; Odonkor & Ampofo, 2013). It is important, however, to note that no single organism will comply with all of the requirements of an indicator. *Escherichia coli* for instance, is able to proliferate in soil under warmer conditions which may influence its accuracy as indicator of the level of original faecal contamination. Britz *et al.* (2012), however, suggest that *E. coli* complies with most of the criteria of a good indicator and is, therefore, regarded as the most commonly used indicator microorganism. Most strains are not pathogenic to humans and generally occur in concentrations exceeding that of the pathogens it represents (Warriner, 2011; Britz *et al*., 2012). Even though the use of alternative indicators of faecal pollution, such as *Enterococcus* spp., *Clostridium perfringens* and bacteriophages, has been suggested (Scott *et al*., 2002; Harwood *et al*., 2005), *E. coli* remains the principal internationally recognised indicator of such contamination (Yan & Sadowsky, 2007).

Detection of *E. coli* in irrigation water is based on the ability of β-glucuronidase to hydrolyse 4-methyl-umbelliferone glucuronide (MUG), yielding a fluorescing product. This test is not specifically designed to detect pathogenic *E. coli,* but is well suited since such organisms function very similarly to non-pathogenic *E. coli* strains (Nwachuku & Gerba, 2008). Once *E. coli* has been detected, faecal contamination can be confirmed by means of a positive indole test (Britz *et al*., 2012).

2.6.3.2. *Escherichia coli* characteristics

Escherichia coli is classified as a faecal coliform which naturally occurs in the gastro-intestinal tract of humans and other warm-blooded animals (Warriner & Namvar, 2010). It, therefore, naturally occurs in faeces and is, with very little exception, always present in faecally contaminated water (Britz *et al*., 2012). A variety of strains have been isolated, characterised and classified as members of the family called the *Enterbacteriaceae* (Todar, 2012). Warriner (2011) reported that most of the *E. coli* strains are non-pathogenic in nature and positively impact human health. These microorganisms may be required for the production of vitamin K, while also providing additional probiotic advantages (Warriner, 2011). They are referred to as commensal *E. coli* strains. Nonetheless, a small group, known as the enteropathogenic strains, are capable of causing mild to adverse diarrheal symptoms when ingested (Bridle, 2014).

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Phenotypically, *E. coli* strains are classified as being facultative anaerobes capable of producing gas from carbohydrate fermentation (Percival *et al*., 2004). The organisms are rod shaped, motile, gram-negative and not capable of forming spores. Phylogenetically, *E. coli* strains belong to one of four groups namely A, B1, B2 or D (Gordon *et al*., 2008; Carlos *et al*., 2010). Each group of strains represents distinctive genetic characteristics and is based on differences in preferred environmental niches and the propensity to cause infection (Gordon *et al*., 2008; Carlos *et al*., 2010). This implies that these sub-categories represent organisms with different phylogenetic properties with regard to antibiotic resistance, the size of their genomes and their manner of carbohydrate metabolism.

Commensal *E. coli* lack the presence of virulence genes and are, therefore, unable to cause infection (Ingerson-Mahar & Reid, 2011; Bridle, 2014). They occur in the intestines of mammals where they promote general health. In contrast, intestinal pathogenic *E. coli* are capable of causing adverse diarrhoea and other symptoms in the intestines of humans and have been categorised according to virulence (Pu, 2009; Warriner, 2011; Todar, 2012). The following six groups have been identified: enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), diffusely adherent *E. coli* (DAEC) and enteroaggregative *E. coli* (EAEC) (Kaper *et al*., 2004; Bridle, 2014). Strains representing the different groups, differ with regard to their mode of pathogenic action and infective dose (Table 4), but may all lead to detrimental health effects in humans. An example of a very well-known and well-documented EHEC strain is *E. coli* O157:H7, which commonly occurs in the intestines, and thus faeces, of humans, cattle, goats and sheep (Odonkor & Ampofo, 2013; Bridle, 2014). When ingested, symptoms such as extremely bloody and watery diarrhoea, haemorrhagic colitis and haemolytic uremic syndrome (HUS) may occur (Percival *et al*., 2004; Kämpfer *et al*., 2008). The latter two conditions may be fatal and are especially threatening to the health of young children (Bridle, 2014). Other pathogenic strains may also have severe impacts on human health. In 2011, 47 deaths were recorded in Europe following an outbreak of verocytotoxigenic *E. coli* that was associated with contaminated sprouts. The source of contamination was suggested to be irrigation water of poor microbiological quality (Bridle, 2014).

Three pathogenic *E. coli* sub-groups namely, uropathogenic *E. coli* (UPEC), neonatal meningitis associated *E. coli* (NEMEC) and sepsis associated *E. coli* (SEPEC) are now collectively referred to as extraintestinal pathogenic *E. coli* (ExPEC) and occur in the gastro-intestinal tract (Russo & Johnson, 2003; Russo & Johnson, 2009). These however, will not result in gastroenteritis and related symptoms in humans. The ExPEC strains are rather known to access the extraintestinal areas of the human body where they cause infection. Known targets for these pathogens include the lungs, the abdomen, the central nervous system as well as pelvic sites and the urinary tract (Russo & Johnson, 2003).

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Table 4 Infective dose and associated symptoms represented by the respective groups of Intestinal pathogenic *E. coli*

Pathogenic group	Infective dose	Common symptoms	Reference
EPEC	$10^{8} – 10^{10}$ organisms	Diarrhoea	Percival *et al.* (2004)
EHEC	> 100 organisms	Watery and bloody diarrhoea, bloody vomiting, haemorrhagic colitis, HUS	Kämpfer *et al.* (2008), Percival *et al.* (2004)
EIEC	$10^{6} – 10^{10}$ organisms	Bloody and watery mucoid diarrhoea	Bhunia (2008), Percival *et al.* (2004)
ETEC	$10^{6} – 10^{9}$ organisms	Watery diarrhoea, vomiting, abdominal pain, fever	Percival *et al.* (2004)
DAEC	Not known	Diarrhoea	Percival *et al.* (2004), Scaletsky *et al.* (2002)
EAEC	Not known	Watery, inflammatory diarrhoea	Okeke & Nataro (2001), Percival *et al.* (2004)

2.7. GENERAL KEYS TO PREVENTION OF FRESH PRODUCE CONTAMINATION

Food products referred to as "ready-to-eat" are typically consumed without the prior application of a cooking or heating process and often in its raw state (Lynch *et al*., 2009; León *et al*., 2013). Since microbiological contamination of such products cannot be sufficiently reduced by washing procedures, attention should be given to the prevention of early contamination (Lynch *et al*., 2009). Accordingly, various strategies for managing the risk of produce contamination have been suggested. Minimising pathogen transfer from direct sources and/or reservoirs, treating water at various stages of the irrigation cycle and the use of alternative irrigation methods may all reduce the extent of irrigated fresh produce contamination (Pachepsky *et al*., 2011).

Guidelines developed by the FDA consider the same strategies and were developed in view of the following important principles: preventing contamination is easier than applying corrective procedures; good agricultural practices (GAP) and management is required; contamination can occur at any stage throughout production; produce contamination from improper water sources should be prevented; the use of manure-based fertilisers should be properly managed and adequate sanitation and human hygiene is of critical importance to ensure food product safety (Olaimat & Holley, 2012). With reference to the Hazard Analysis and Critical Control Point (HACCP) system, no current control points exist that will reduce the microbial hazard of contaminated produce following harvesting or prior to service (Tauxe *et al*., 1997). The importance of preventing fresh produce contamination is thus again highlighted and is in this context regarded as a critical control point.

Recently, the WHO and the Food and Agriculture Organisation (FAO) of the United States made several recommendations as to increase fresh produce safety by means of controlling

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foodborne pathogens. These recommendations cover an extensive list of factors and include the following: protecting natural water sources from contact with sewage, manufacturing effluent, wildlife activity and animal waste; making use of good manufacturing practices (GMPs) and GAPs, including the use of sanitary agricultural equipment and the provision of facilities promoting personnel hygiene and health habits; and the application of standard operating procedure (SOPs) in order to maintain good hygiene practices throughout production and processing (Olaimat & Holley, 2012). The importance of strict regulations regarding the quality of irrigation water and the use of fertilisers have also been highlighted when considering the safety of fresh produce (Krtinic *et al.*, 2010). Guidance documents produced by the FDA further emphasise the importance of maintaining GAP and GMP within the fresh produce production and processing sectors, respectively (Lynch *et al.*, 2009). These however, lack regulatory prescriptions and compulsory processing steps for pathogen reduction.

Regulatory bodies and the fresh produce industry will have to work closely together in order to minimise the occurrence of fresh produce-related foodborne outbreaks. All role players are thus required to understand the implications of certain practices within the industry, thereby enabling intervention. Furthermore, when outbreaks do occur, assessment of the case should happen swiftly and accurately, ensuring identification of contamination sources. Variables relating to methods of irrigation and harvesting, as well as the effect of field surroundings, can then provide information as to what preventative measures will be most effective (Lynch *et al.*, 2009).

Coming back to the importance of water quality, the versatility of this resource in the production of fresh produce must be emphasised. Pre-harvest pesticide application and post-harvest processing and cooling for instance, require the use of water from which pathogens may be transferred to the produce (Lynch *et al.*, 2009). Of greater relevance however, is the use of microbiologically contaminated water for irrigational purposes. The introduction of pathogens into irrigation water is multi-factorial and the prevention thereof is an extremely difficult task. Informal settlements developing next to rivers, failing sewage treatment plants and careless waste-water disposal contribute to South African rivers showing high levels of microbiological contamination (Britz *et al.*, 2013). In addition to water quality, however, crop type and the method of irrigation used significantly impacts the transfer of pathogens from irrigation water to produce (Ijabadeniyi & Buys, 2012). Even though these factors may be managed to an extent, the use of water of poor microbiological quality is not recommended. Accordingly, on-farm treatment of irrigation water is suggested as a means of minimising pathogen transfer to fresh produce.

2.8. ON-FARM TREATMENT OF CONTAMINATED IRRIGATION WATER

Owing to poor water quality in South Africa, the importance of water treatment prior to irrigation is immense. Several disinfection techniques have been established and are currently used as on-farm treatments. These are generally classified into one of three categories (Table 5) (Momba *et*

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al., 2008; Raudales *et al.*, 2014). The effectiveness of treatment techniques depend on parameters of water quality, including total dissolved solids (TDS), turbidity, pH, total suspended solids (TSS) and chemical oxygen demand (COD) (Huang *et al.*, 2011; Jones *et al.*, 2014). These parameters are highly variable, changing daily or seasonally as a result of natural or human influences. All of the disinfection methods are therefore not similarly appropriate for the treatment of surface water (Jones *et al.*, 2014).

Table 5 Commonly applied methods for the disinfection of water (Momba *et al.*, 2008)

Chemical	Mechanical/physical removal	Physical/photochemical disinfection
Chlorine based	Ultrafiltration	Ultrasound
Bromine	Sand filtration	Ultraviolet light
Hydrogen peroxide		
Peracetic acid		
Ozone		

The methods listed in Table 5 differ with regard to various aspects of the process and selection of the most appropriate technique may be a difficult task. Factors to consider include toxigenicity of the disinfecting substance, the formation of hazardous disinfection by-products (DBPs), the influence of water quality on disinfection efficacy and the sanitising capability of the technique in relation to the associated capital and operational costs (Lazarova & Bahri, 2005).

2.8.1 Chemical treatment of irrigation water

With reference to the use of chemical substances, irrigation water can be effectively disinfected only if pathogenic microorganisms are exposed to adequate doses and proper contact times (Forney, 2008). Commonly used chemical disinfectants rely on their oxidising capabilities to disrupt microbial cell wall components, ultimately resulting in cell death (Yiasoumi *et al.*, 2005). In the food processing industry, a wide variety of chemical substances are currently utilised for the purpose of disinfecting water. Identifying the most appropriate substance for a particular application is driven by a variety of factors and may become a tedious task (Forney, 2008). Of primary importance is the requirement that the substance should be reliable, consistent and effective against a variety of indicator microorganisms (Forney, 2008). Chemical disinfectants should also be easy to transport, store and apply, without posing significant safety hazards to the handlers thereof.

Chlorine

Introduction, mode of action and advantages

From its earliest use in the 1800s, chlorine has become one of the most commonly used chemical disinfectants to date (Freese & Nozaic, 2004). Its ease of application and general effectiveness

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have been thoroughly documented and despite some drawbacks related to its use, recreational, agricultural and drinking water are today sanitised by means of chlorination (Momba *et al*., 2008).

For the purpose of water disinfection, chlorine is utilised in one of the following forms: elemental chlorine (gaseous Cl_2); sodium hypochlorite (liquid NaOCL) or calcium hypochlorite (solid $Ca(ClO)_2$) (Momba *et al*., 2008; Raudales *et al*., 2014). With regard to cost, it has been reported that gaseous chlorine is most effective but it is often not considered the best option as gas leaks and other risk factors complicate its application in modern water treatment facilities (Freese & Nozaic, 2004). Calcium hypochlorite on the other hand, is the most expensive in terms of cost per mass of active chlorine but is often the preferred form because of its ease of application and overall safety (Freese & Nozaic, 2004). Essentially, the local availability of chemicals and dosing equipment determine the form of chlorine that will be applied (Momba *et al*., 2008).

In the presence of water, chlorine firstly reacts to form hypochlorous acid (HOCl) and hydrochloric acid (HCl). This is followed by the dissociation of HOCl to form hypochlorite (OCl^-) and a hydrogen (H^+) atom (LeChevallier & Au, 2004). The HOCl and OCl^- species are known as free chlorine and are solely responsible for the disinfection capabilities of the chemical (LeChevallier & Au, 2004; Momba *et al*., 2008). Between these two species, hypochlorous acid is much more reactive and acts as a stronger oxidant and thus disinfectant (Momba *et al*., 2008). Nevertheless, both forms of free chlorine show significant interaction with different components of the bacterial cell wall (Bitton, 2005). Bitton (2005) has reported that free chlorine disrupts and destroys bacterial cells through a three phase process. Firstly, the bacterial cell membrane may be damaged, which leads to the leakage of DNA and/or RNA and eventual cell death. Free chlorine is then further capable of disrupting enzymes and nucleic acids within the bacterial cell itself. In addition, the cells may be further subjected to insufficient levels of adenylate energy due to compromised transportation and respiration activities resulting from sulfhydryl oxidation by chlorine (Bitton, 2005). These cells will not be able to remain viable.

Several factors contribute to chlorine being one of the most widely used and popular chemical disinfectants. The initial cost of installing chlorine treatment infrastructure is fairly low and the substance is easily handled, measured and dosed. Contributing to its ease of use, is the high solubility (7 000 $mg.L^{-1}$) of chlorine. This adds to its effectiveness against a spectrum of pathogens and the fact that it represents good residual levels when applied to water (Momba *et al*., 2008). Doses in the order of 2 – 3 $mg.L^{-1}$ have been reported to result in microbiological reductions of up to 3 log, and consequently treatment costs are fairly low. Moreover, through oxidation reactions, chlorine decreases foul-tasting and smelling compounds that are either naturally present in water, or those of chemical nature, which enter water as pollutants (Momba *et al*., 2008). In conclusion, the versatility of chlorine has been said to be unsurpassed and it, therefore, remains one of the most commonly used chemical disinfectants, despite the availability of more effective alternatives.

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Bromine

Introduction, mode of action and advantages

Reports on the use of bromine as disinfectant are primarily concerned with its application in swimming pools and cooling towers. Its use in applications such as drinking water treatment is less common as a result of the foul odour and taste it may contribute following its application (Freese *et al*., 2003). Bromine, like chlorine, is classified as a halogen and disinfects water by means of similar reactions (Momba *et al*., 2008). It has been used in swimming pools in the United States since 1936 to reduce the levels of bacteria, algae and unpleasant odours (Lenntech, 2014). More recently, in the 1990s, bromine was used in combination with chlorine in wastewater treatment facilities in the USA (Freese *et al*., 2003).

Bromine is utilised in one of three forms: a highly corrosive liquid, less corrosive bromine chloride (BrCl) and sodium bromide (NaBr). The latter is used in conjunction with liquid chlorine which oxidises the salt to form bromine. More specifically, a common practice is to add NaBr to sodium hypochlorite as a means of forming hypobromous acid (HOBr) (Yiasoumi *et al*., 2005). It is this HOBr that is responsible for the disinfection action of bromine (which is similar to the action of hypochlorous acid that is formed when chlorine is used for disinfecting water) (Yiasoumi *et al*., 2005). It has been reported, however, that the presence of HOBr is less dependent on pH in comparison to hypochlorous acid (Momba *et al*., 2008). The destructive mechanism of HOBr is based on the fact that its oxidising potential is adequate for the alteration of the chemical structure of microbiological cell components (Newman, 2004; Punyani *et al*., 2006).

Water used for agricultural purposes often contains nitrogen-based compounds, such as ammonium, which will result in the formation of bromamines and chloramines when bromine and chlorine, respectively, are used for disinfection purposes (Momba *et al*., 2008). While bromamines still show significant disinfecting capabilities, chloramines do not and in this regard the use of bromine is more advantageous (Yiasoumi *et al*., 2005). Other advantages related to the use of bromine include greater biocidal activity than chlorine against enteric viruses, its lesser dependence on pH in comparison to that of chlorine and ease of handling, storage and transportation as it occurs in liquid form at room temperature (Momba *et al*., 2008).

Hydrogen peroxide

Introduction, mode of action and advantages

Since its discovery in 1818, hydrogen peroxide (H_2O_2) has been used in an extensive range of applications and is described as a safe and versatile oxidant. In addition to common uses such as the control of odours in water and bleaching of pulp in the paper industry, H_2O_2 is often utilised for its disinfection capabilities (Labas *et al*., 2008; Lenntech, 2014). It has been shown to be effective

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against viruses, bacteria and various other microorganisms when used as anti-microbial agent in irrigation water or on contaminated surfaces (Newman, 2004).

Hydrogen peroxide is predominantly applied by means of addition to water during which it decomposes into water and oxygen, not resulting in the formation of any toxic or unstable disinfection by-products (Momba *et al*., 2008; Joshi *et al*., 2013). As an oxidising agent with fairly high potential (Table 6), the disinfection capabilities of H_2O_2 results from this formation of highly reactive oxygen. By means of catalytic reactions, H_2O_2 can also be converted into superoxide radicals and hydroxyradicals (•OH) which show even greater oxidation potential (Raffellini *et al*., 2011). These species are capable of disrupting microbial DNA, proteins and lipids, consequently leading to disinfection (Halliwell & Gutteridge, 1984; Block, 2001). It has been suggested that sulfhydryl and double bonds of microbial cell components are particular targets of the radicals (Block, 1991). McDonnell & Russel (1999) suggest that hydroxyradicals specifically oxidise thiol groups in microbial enzymes and proteins, inhibits DNA synthesis and initiates breakage of existing DNA strands.

Table 6 Oxidation potential of commonly encountered disinfectants (Newman, 2004)

Oxidising agent	Oxidation potential (mV)	Oxidation potential relative to chlorine (mV)
Chlorine	1.360	1.000
Potassium permanganate	1.680	1.250
Hydrogen peroxide	1.780	1.310
Ozone	2.070	1.520
Hydroxyradicals	2.800	2.056
Fluorine	3.050	2.250

It has been reported that hydrogen peroxide shows greater activity against gram-positive than gram-negative bacteria (McDonnell & Russel, 1999). Peroxidases, such as catalase, in these organisms however, may allow for fairly high tolerance when the chemical is used in low concentrations. In order for hydrogen peroxide to show sporicidal activity, concentrations of 10% to 30% are required in combination with increased contact times (McDonnell & Russel, 1999). When using oxidising compounds for disinfection it should be noted that their activity is lost during the oxidation of microorganisms and also other organic substances within the water (Newman, 2004). It is, therefore, important to maintain high enough concentrations of the disinfectant to allow for effective decontamination.

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Ozone

Introduction, mode of action and advantages

Ozonation is a well-known and well-documented technique that has been used to disinfect water for more than a century (Voigt *et al*., 2013). In addition to occurring naturally as an activated form of oxygen, ozone is produced artificially by means of discharging high voltages in the presence of oxygen (Momba *et al*., 2008). This is known as corona discharge (Yousef *et al*., 2011). Since its first experimental application as water disinfectant in 1886, ozone has been used extensively and applied for the removal of taste, odour and colour compounds and to reduce the turbidity, total organic carbon and levels of disinfection by-product precursors in water (Burns, 2010). Of particular interest is the disinfection potential shown by ozonation against parasites and bacteria such as *Cryptosporidium*, *Giardia lamblia* and *Escherichia coli* (Burns, 2010). Today, thousands of global water treatment facilities employ ozone as chemical disinfectant and its numerous advantages sees the chemical being useful in an array of applications. It is Generally Recognized As Safe (GRAS) and is, therefore, often used in the food industry (MartÍnes *et al*., 2011).

When used as disinfectant in water treatment, ozone is required to be generated on-site owing to its instability and rapid rate of breakdown (Momba *et al*., 2008). Disinfection is achieved by diffusing the gas into fine bubbles, thereby mixing it with the polluted water in a contact chamber (Voigt *et al*., 2013). In water, ozone rapidly decomposes yielding free radicals responsible for its disinfection properties. As shown in Table 6, ozone represents an extremely high thermodynamic oxidation potential, consequently being a very effective disinfectant (Freese & Nozaic, 2004). This results from the oxidising capacity of the hydroperoxyl (HO_2) and hydroxyl (•OH) free radicals that spontaneously form from ozone in aqueous media (Gómez-López, 2012; Voigt *et al*., 2013). In addition to the action of the radicals, it has been suggested that molecular ozone (O_3) is capable of showing direct disinfection properties (Newman, 2004).

The disinfection mechanisms of ozone and its associated radicals are based on the lyses of pathogenic cell walls, as well as the disruption of microbiological genetic material (Burns, 2010). Bacterial cells are affected and destructed as a result of cell membrane oxidation by ozone itself and by the effect of the ozone related radicals on microbial nucleic acids (Voigt *et al*., 2013). Bacterial spores and viruses are inactivated when the inner spore membrane, and in the case of viruses the protein coat and nucleic acid core, are damaged (Bitton, 2005). Changes such as chromosomal destruction and the breakage of phosphate, hydrogen and nitrogen-carbon bonds, essentially result in the leakage of microbial cell constituents and the inhibition of enzymes (Freese *et al*., 2003; Lazarova & Bahri, 2005).

Even though the use of ozone is associated with some disadvantages, it has been praised worldwide due to its advantages over disinfectants such as chlorine. Its effectiveness against parasites and bacteria, and the absence of disinfectant by-products following its application, have

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been widely regarded as its key properties (Voigt *et al.*, 2013). Nevertheless, the antimicrobial activity of ozone is dependent on a variety of factors including temperature, pH and relative humidity, and precise care should be taken in calculating optimal dosages for effective disinfection (MartÍnes *et al.*, 2011).

Peracetic acid

Introduction, mode of action and advantages

Peracetic acid (PAA) has been widely used as disinfectant in the agronomical, medical and pulping industries and is now being utilised increasingly in water purification and treatment systems (Lenntech, 2014). Commercial production of peracetic acid is based on the reaction between hydrogen peroxide and acetic acid, but it may also be formed by oxidation of acetaldehyde (Lenntech, 2014). Commercially, PAA is available as a stabilised mixture of peracetic acid, hydrogen peroxide, acetic acid and water. At a concentration of 5% to 15% (w/w), PAA acid is regarded as the key active compound within this blend (Luukkonen *et al.*, 2014).

Similar to hydrogen peroxide, peracetic acid is described as a peroxygen and with regard to potency it is considered to be the more effective disinfectant (McDonnell & Russel, 1999). As a result of its oxidising capabilities exceeding that of chlorine and chlorine dioxide, much interest has been shown in the bactericidal, fungicidal, virucidal and sporicidal properties of PAA (Rossi *et al.*, 2007). In a study performed by Freese & Nozaic (2004) it was observed that PAA has disinfection capabilities similar to that shown by chlorine at corresponding mass concentrations. Accordingly, it was reported that peracetic acid was just as effective, sometimes more so, in destroying faecal and total coliforms as well as faecal streptococci (Freese & Nozaic, 2004).

It has been stated that the efficacy of peracetic acid as disinfectant is only marginally influenced by factors such as the presence of organic materials and the total suspended solids (TSS) concentration of water (Koivunen & Heinonen-Tanski, 2005a; Luukkonen *et al.*, 2014). However, Luukkonen *et al.* (2014) suggests that for optimal results, wastewater intended to be disinfected with PAA should be well processed since some chemical and physical factors of the effluent may influence or compromise its efficacy. Table 7 serves as a summary of the effect of some of these factors (parameters) on the activity of PAA.

As mentioned, the disinfection capabilities of PAA is based on its oxidising capacity. When applied for water treatment it breaks down to form acetic acid, hydrogen peroxide, water and oxygen, primarily. These lead to the oxidation of components of the outer cell membranes of microorganisms (Lenntech, 2014). Subsequent disruption of sulphur (S-S) and sulfhydryl (-SH) bonds will lead to increased cell wall permeability. In addition, enzymes and proteins may also be denatured (McDonnell & Russell, 1999). The disinfection mechanism of peracetic acid is thus comparable to that of hydrogen peroxide.

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Table 7 The effect of water quality on the efficacy of PAA disinfection

Water quality parameter	Influence of PAA efficacy	Reference
pH	Decreased efficacy above 9. Acidic conditions preferred.	Kitis (2004)
Temperature	Activity seen between 0 - 100°C. Efficacy increases with temperature.	Stampi *et al.* (2001)
Turbidity	Efficacy slightly decreases with increased turbidity.	Koivunen & Heinonen-Tanski (2005a)
Organic material	Slight decrease in efficacy in presence of very high organic loads. Less influenced than similar disinfectants.	Koivunen & Heinonen-Tanski (2005a)

When using chemicals for disinfection purposes, the formation of DBPs is often a topic of concern. In the case of peracetic acid disinfection, Dell´Erba *et al.* (2007) reported that no phenols, brominated or chlorinated, are formed during water treatment. The formation of aldehydes did occur, however, but these were reported to be further oxidised to carboxylic acids and subsequently carbon dioxide. By applying bacterial mutagenicity and plant genotoxicity tests, Crebelli *et al.* (2005) reported that no harmful DBPs were formed when secondary effluent was treated with PAA. Other advantages related to the use of PAA acid include the fact that it is not decomposed by peroxidases, the fact that when stored properly it is fairly stable and the fact that it is reported to show good residual activity (Freese & Nozaic, 2004).

General limitations related to the use of disinfecting chemicals

The effectiveness of chemical disinfectants are drastically influenced by a variety of water quality parameters. Inorganic and organic substances in water result in the rapid consumption of a portion of the dose, leaving a residual level available for disinfection (Forney, 2008). Important parameters influencing the activity of chemical substances in water include, chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS), turbidity, pH as well as conductivity (Acher *et al*., 1997; Yiasoumi *et al*., 2005). Considering the effect of these parameters, the efficacy of chemical disinfectants will thus be determined by the initial residual concentration of the substance rather than the applied dose (Forney, 2008).

Regardless of their wide application, the use of chemical disinfectants is associated with some additional, well-known limitations. Protozoan pathogens, especially *Cryptosporidium* and *Giardia*, as well as some viruses, have been reported to be resistant to chemical disinfection (Voigt *et al*., 2013). Furthermore, when pathogens occur within particles or flocks in the treated water, disinfection efficacy may be compromised significantly (Voigt *et al*., 2013). Critics often highlight the fact that chemical disinfectants are well-known for producing DBPs within the treated

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water (Momba *et al.*, 2008). These include carcinogenic and mutagenic substances such as trihalomethanes, which have resulted in great concern over the effect of chemical water disinfection on human health. A variety of factors contribute to the type of DBPs that may potentially form. Table 8 serves as an illustration of the array of possible DBPs occurring in water when using four common chemical disinfectants. In addition to these by-products, many more may be present in treated water. The health concerns related to DBP formation, however, are small in comparison to the effect of using microbiologically contaminated irrigation water and should never be used to justify compromised disinfection processes (Voigt *et al.*, 2013).

Table 8 Examples of the types of DBPs formed from four commonly used chemicals (Richardson *et al.*, 2010; Lenntech, 2014)

	Disinfection by-product		
Disinfectant	**Organohalogenic**	**Inorganic**	**Non-halogenic**
Chlorine	Trihalomethanes, halogenic acetic acids	Chlorate	Aldehydes
Ozone	Bromoform, monobromine acetic acid	Chlorate	Aldehydes
Chlorine dioxide	-	Chlorate	Unknown
Bromine	Bromoform	Benzeneacetonitile	Aldehydes

A great variety of chemicals have been used for the disinfection of contaminated water sources. For the purpose of this study, the most commonly used disinfectants and ones showing potential for sanitising irrigation water were discussed briefly. In addition to the mentioned limitations, some chemical substances represent an extensive list of additional concerns. Furthermore, chemical disinfection poses significant risks to human handlers as well as the environment during its production, distribution and storage (Yiasoumi *et al.*, 2005). A final mutual limitation to take note of is the fact that chemical disinfectants always require a contact time in order to effectively inactivate microorganisms. Considering these drawbacks, it is of great importance to evaluate the practicality and viability of alternative methods of water disinfection, subsequently comparing their use to that of chemical disinfectants.

2.8.2 Physical/Mechanical treatment of irrigation water

In addition to the use of chemical disinfectants, various physical or mechanical processes are applied for the decontamination of polluted water. Broadly categorised as either gravity separation processes or filtration processes, these are predominantly based on some form of physical retention of microorganisms (Yiasoumi *et al.*, 2005). For the purpose of disinfecting irrigation, waste and municipal water, filtration methods, which incorporate techniques such as straining, absorption and adsorption, are of particular importance (Yiasoumi *et al.*, 2005; Lenntech, 2014). In

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addition to microbiological pathogens, filtration processes are generally capable of removing suspended solids as well as unwanted taste compounds, odours and chemicals from water (Momba *et al*., 2008). Such disinfection treatments are often preceded by straining or settlement processes and accompanied by additional disinfection methods in order to increase their efficacy (Yiasoumi *et al*., 2005; Momba *et al*., 2008).

Slow bed sand filtration

Introduction, mode of action and advantages

Slow bed sand filtration as treatment technique can be described as a bio-filtration process which represents both biological and physical aspects of water treatment (Hendricks, 2006). Its application dates back to the early 1800s when the the first successful slow bed sand filter was installed in 1829 to treat the water supply of London (Hendricks, 2006). At the time, however, the process was applied as a means of reducing the suspended solids concentration and turbidity of water, while its disinfection capabilities were only realised later (Huisman & Wood, 1974). Bitton (2005) reported that the first slow sand filter installed in the United States was intended to specifically reduce *Salmonella typhi* levels in water.

Disinfection by means of slow sand filtration occurs when water slowly passes through a bed of porous material. As the name suggests, sand is predominantly used, but materials such as pumice have also been used successfully (Zheng & Dunets, 2014). Hendricks (2006) describes such a system as a bio-depth-filtration process occurring in a filter medium, aided by a straining process provided by a biologically active layer. Contaminated water is disinfected as it moves through the filter, allowing pathogens to be captured by pore spaces within the medium. Adding to this, microbial pathogens are further diseased by biofilms that slowly form on the surfaces of the filtration grains during normal operation of the filter (Zheng & Dunets, 2014). Microorganisms residing in the biofilm compete with water pathogens for resources or act by direct attacks. This active biofilm is known as the schmutzdecke and consists of inorganic and organic particulate, as well as a variety of fungi, nematodes, protozoa and bacteria (Steward-Wade, 2011). Although the schmutzdecke is regarded as the predominant biological control measure provided by slow sand filtration, the sand bed self should be biologically mature as well (Hendricks, 2006). This means that in addition to the physical filtration provided by the sand, biofilm formation deep within the bed aids in disinfection by means of antagonistic effects and direct competition (Hendricks, 2006; Zheng & Dunets, 2014).

The typical construction of a slow bed sand filter is shown in Figure 3. A water layer of approximately 0.9 m deep should be maintained in order to protect the schmutzdeke from moisture and temperature fluctuations while providing sufficient pressure for movement through the sand (Steward-Wade, 2011; Zheng & Dunets, 2014). The sand, or medium layer, should be at least 80 cm deep and is supported by a gravel layer with a depth of approximately 15 cm (Zheng &

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Dunets, 2014). The latter prevents sand from flowing to the outlet tank while allowing water to move freely. Since the development of the schmutzdecke results in losses of maximum headwater, it is constantly monitored and removed once an established criterion for maximum head-loss is reached (Hendricks, 2006).

Slow bed sand filtration has been reported to be effective in removing a great variety of pathogenic microorganisms (Zheng & Dunets, 2014). According to Hendricks (2006), a biologically mature sand filter is generally capable of delivering 2 to 4 log reductions of bacteria, cysts, viruses, oocysts, algae and parasite and nematode eggs. Zheng & Dunets (2014) reported, however, that some nematodes and viruses, as well as *Fusarium,* can only be partially removed from water and only at slow flow rates. Adding to this, Hugo & Malan (2006) stated that the filtration process is ineffective in removing nematodes as a result of the large pore size of the sand bed. *Phytopthora* and *Pythium* species are reported to be very easily removed (Zheng & Dunets, 2014). In conclusion, it should be mentioned that slow bed sand filtration is a versatile water disinfection process. While operating costs are low, the use of chemicals and its associated risks are also eliminated when such a process is used (Langenbach *et al*., 2009). Its effectiveness against disease causing microorganisms, including *Giardia* and *Cryptosporidium* (Hijnen *et al*., 2007), lastly highlights its potential for the disinfection of irrigation water.

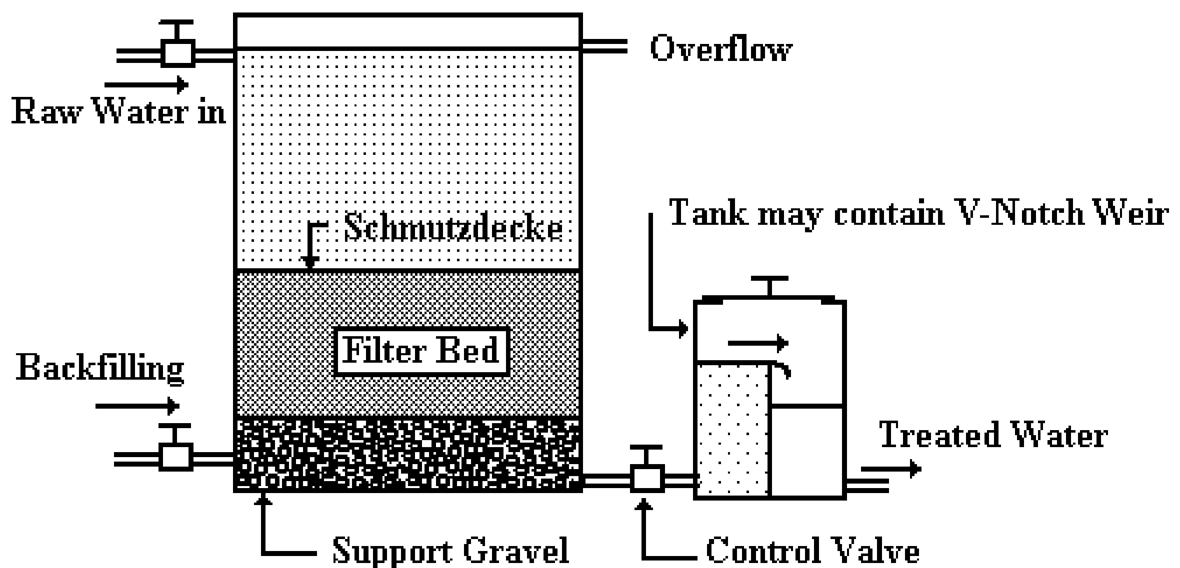

Figure 3 Graphic representation of a typical slow bed sand filter (Ludwig, 2014).

Ultrafiltration

Introduction, mode of action and advantages

Being physical in nature, ultrafiltration is described as a membrane process and offers an appealing alternative for the disinfection of water (Momba *et al*., 2008). Since its development in the early 1900s, the technology has been widely applied for the production of pure water in the

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biopharmaceutical, food and beverage and biochemical industries, just to name a few (Nath, 2006). Today, however, ultrafiltration is also utilised for the production of microbiologically safe drinking water (Vickers, 2005). It has been reported that, during recent years, the process has been increasingly applied for the removal of particulate and organic material as well as a wide spectrum of unwanted microorganisms from water (Arnal *et al.*, 2009; Konieczny *et al.*, 2009).

Membrane processes in general can be driven by differences in osmotic pressure and temperature and, in the case of nanofiltration (NF), reverse osmosis (RO), microfiltration (MF) and ultrafiltration (UF), by differences in pressure (Fane *et al.*, 2008; Peter-Varbanets *et al.*, 2009). Ultrafiltration is thus described as a separation process, driven by low pressure, during which water and substances with low molecular weight move through a porous membrane to produce what is known as permeate or ultrafiltrate (Nath, 2006). Larger particles, macromolecules and colloidal substances do not pass through the membrane and are retained as what is known as retenate (a concentrated solution) (Nath, 2006). Even though substances are primarily retained because of size, factors such as the surface chemistry of the membrane and particulate substances, as well as its electrical charge, may also be influential (Nath, 2006). With regard to the mentioned membrane processes, one can say that the variance in the size of the substances they retain is the only fundamental difference (Nath, 2006). Table 9 provides a summary of the operational differences observed between four membrane processes.

Table 9 Operational and technical differences observed between membrane processes used for water disinfection (Van der Bruggen *et al.*, 2003; Baker, 2004; Ozaki, 2004; Fane *et al.*, 2008)

Specifics	Microfiltration	Ultrafiltration	Nanofiltration	Reverse osmosis
Size of pores	10 nm – 1 µm	3 – 10 nm	2 – 5 nm	N/D
Particulates retained (MW)	> 300 000	1 000 – 300 000	> 150	< 350
Pressure exerted (MPa)	0.005 – 0.20	0.01 – 0.30	0.30 – 1.50	1.00 – 10.00

N/D = Not detectable

With reference to the information in Table 9, substances in the size range of 1 000 to 500 000 Dalton (Da) will permeate the typical membrane used in ultrafiltration, retaining only particles of high molecular weight (Nath, 2006). This implies that the osmotic pressure difference across the membrane is very small and that fairly low pressures are adequate for establishing high flux rates (Table 9). Compared to microfiltration, however, larger pressures are required by ultrafiltration as a result of the smaller pore size it represents (Table 9). Typically, peristaltic pump systems or compressed nitrogen are used to generate the pressure required by the membrane separation system. Figure 4 represents an illustration of an ultrafiltration system.

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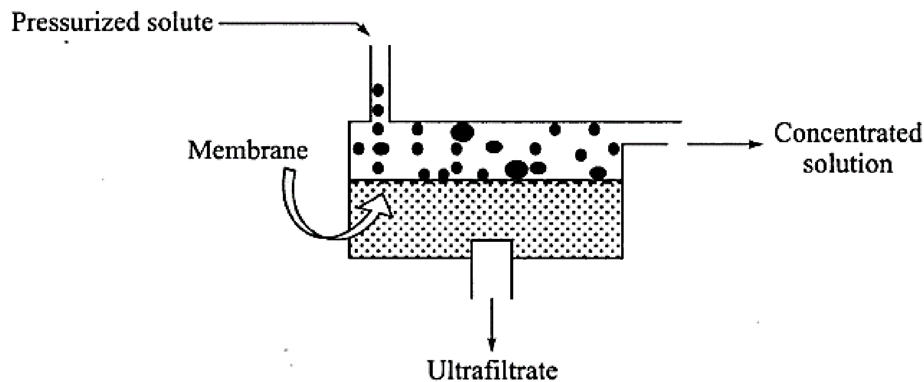

Figure 4 Graphic representation of a typical ultrafiltration membrane setup (Nath, 2006).

Ultrafiltration has become a widely used disinfection method in addition to its primary application of removing organic materials and other substances from water. It has been reported to be sufficient in removing various viral and bacterial species, as well as protozoan cysts such as *Cryptosporidium* and *Giardia,* from water (Konieczny, *et al.*, 2009; Kajitvichyanukul *et al.*, 2011). As a result of this versatility, it has been stated that UF is one of the most commonly applied water treatment techniques. The process is advantageous in various senses. It serves as effective pre-treatment of wastewater, is capable of recovering biologically valuable by-products, it does not require the use of chemicals and does not lead to the formation of disinfection by-products (Jacangelo & Noack, 2005; Momba *et al.*, 2008; Konieczny, *et al.*, 2009). These are just to name a few in addition to the capability of UF to reduce the microbiological load of contaminated water. Various limitations, however, are also associated with the process and should be carefully considered prior to its selection and installation as disinfection technology.

General limitations related to the use of physical water treatment processes

Despite its widely acclaimed versatility and benefits, the use of physical disinfection processes are also associated with some drawbacks. With regard to filtration, the size of the unwanted microorganisms for instance play a significant part in the efficacy of the treatment (Momba *et al.*, 2008). Viruses typically range between 20 – 100 nm in size and are very difficult to remove by means of filtration (Momba *et al.*, 2008). Slow bed sand filtration also requires a fair amount of maintenance as the thickening schmutzdecke is required to be removed from time-to-time to maintain sufficient flow of water in the system (Hendricks, 2006). This implies that a new top layer of sand is added to the filter. Following this, a conditioning period of at least 24 h is required before again using the filter. Thereafter, its effectiveness may be slightly impaired for a few days (Bitton, 2005). Generally, it is recommended that slow sand filtration is used in combination with additional filtration techniques as a means of effectively reducing pathogen and particulate levels in irrigation water (Hugo & Malan, 2006; Zheng & Dunets, 2014). This is important since high levels of turbidity may result in rapid clogging of the small pores within the

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filter medium. In addition to these disadvantages, slow bed sand filters occupy large surface areas and are associated with high installation expenses (Zheng & Dunets, 2014). Lastly, it should be mentioned that the slow flow rates required to properly disinfect water, makes slow bed sand filtration an unlikely option for the treatment of large volumes of irrigation water (Zheng & Dunets, 2014).

Ultrafiltration is a complex process represented by high capital costs and the requirement of expertise for its operation (Momba *et al.*, 2008). It has also been reported that, in spite of the continual cross-flow cleaning procedure, ultrafiltration membranes are at risk of clogging and that water pre-treatment is often required (Momba *et al.*, 2008). Another disadvantage of ultrafiltration is the fact that pathogenic microorganisms, especially some viruses, may pass through the pores of the membrane to remain in the treated water (Davey & Schäfer, 2009). This implies that, in addition to treating the concentrated mass retained by the membrane, further disinfection processes are required to ensure water safety (Van der Bruggen *et al.*, 2003). Such treatment significantly contributes to the high operating costs associated with ultrafiltration. The concept of membrane fouling, in which water gradually permeate the membrane at slower rates when pressure remains constant, has been identified as a major limitation related to UF (Nath, 2006). Membrane integrity failure and membrane corrosion further contribute to the negative aspects associated with ultrafiltration (Childress *et al.*, 2005). To conclude, it should be noted that one cycle of ultrafiltration runs for up to 20 h, thus making this process less attractive for the disinfection of irrigation water (Cheremisinoff, 2002).

2.8.3 Ultraviolet light irradiation as treatment option for irrigation water

Introduction to the use of ultraviolet light as method of water disinfection

Ultraviolet (UV) light forms part of the electromagnetic spectrum and represents wavelengths ranging from 100 – 400 nm (Dai *et al.*, 2012). Thus, UV light is that part of the electromagnetic spectrum with wavelengths longer than x-rays and shorter than visible light (Fig. 5) (Dai *et al.*, 2012). Four spectral areas namely, vacuum UV, UVC, UVB and UVA have been identified and characterise UV light according to wavelengths of 100 – 200 nm, 200 – 280 nm, 280 – 315 nm and 315 – 400 nm, respectively. The sub-categories differ considerably with regard to their application with UVC, UVB and UVA being most commonly encountered in literature concerned with disinfection.

The discovery of UV light dates back to the early 1800s when the destructive effect of invisible rays on silver chloride was reported (Bolton & Cotton, 2008a). The germicidal properties of UV were discovered soon thereafter and during the following century the technology was gradually investigated and developed. It was in 1910, in France, that UV light was first applied as disinfection treatment for drinking water. Its use, however, was suppressed by the high cost and maintenance and the poor reliability associated with the quartz tubes and mercury vapour lamps

used at the time (Henry *et al.*, 1910; Wolfe, 1990; Hoyer, 2004). The benefits associated with chlorination as disinfection treatment further hampered the general utilisation of UV irradiation. Today, however, this technique is used increasingly on a global scale for the disinfection of work surfaces, air and contaminated sources of water (Hallmich & Gehr, 2010; Gayán *et al.*, 2014).

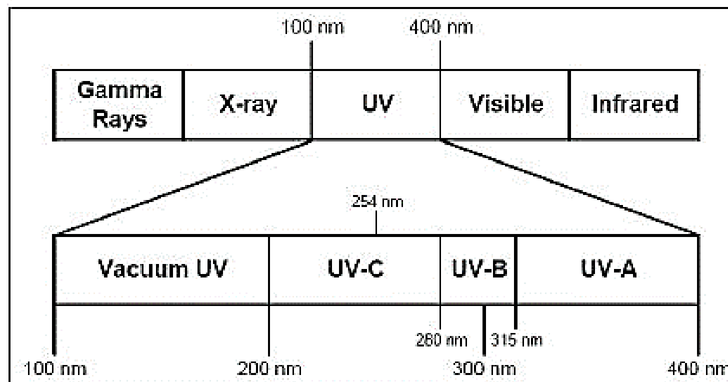

Figure 5 Ultraviolet light as part of the electromagnetic spectrum (USEPA, 2006).

The use of UV light as disinfection method is associated with various advantages and it is reported to be effective against a wide range of pathogenic and spoilage microorganisms (Koivunen & Heinonen-Tanski, 2005b; Hijnen *et al.*, 2006; Gayán *et al.*, 2014). Particular interest in the treatment has been shown as a result of its capability of terminating *Giardia* and *Cryptosporidium,* which are waterborne pathogens well-known for showing resistance to other disinfection techniques (Craik *et al.*, 2000; Craik *et al.*, 2001; Hijnen *et al.*, 2006). In practice, however, the complexity of irrigation water quality and resistance shown by certain pathogenic strains may hinder the effectiveness of UV light irradiation. These factors should be thoroughly considered when evaluating disinfection efficacy.

Disinfection mechanisms of UV irradiation

Introduction and basic principles

The destructive mechanism of UV irradiation is based on the fact that microbial cell components absorb UV light in a complex, photochemical process (Bolton & Cotton, 2008b). In order to establish an in-depth understanding of the mechanism of UV disinfection, the principles of photochemistry and photobiology can be extensively studied. Nonetheless, even though proteins and other cellular components may be damaged by UV absorption, the treatment is primarily recognised for its effect on genetic materials (Quek & Hu, 2008a; Quek & Hu, 2008b; Gayán *et al.*, 2014).

As a form of electromagnetic energy, light is transferred as photons. When these are absorbed by molecules an excited state is established, providing adequate energy for the occurrence of chemical reactions within the molecule of concern (Bolton & Cotton, 2008b). This is

referred to as photochemistry and acts as the principle on which UV disinfection is based. The spectral range in which photochemistry is of importance ranges from 100 – 1 000 nm and accounts for various reactions that occur in nature. Of particular interest is the UV range (100 – 400 nm) which is subdivided into the aforementioned categories of vacuum UV, UVC, UVB and UVA. Each of these induce different chemical reactions within the human skin for instance, with UVC (200 – 280 nm) being absorbed by proteins, DNA and RNA, resulting in cellular mutations and even cell death (Table 10) (Bolton & Cotton, 2008b). Accordingly, it has been reported that UVC irradiation is capable of destroying a wide range of pathogenic microorganisms and is, therefore, known as the germicidal region of the UV spectrum (Gayán *et al.*, 2011; Werschkun *et al.*, 2012).

Table 10 Photochemical effects of UV light on human skin as example (Bolton & Cotton, 2008b)

UV range	Photochemical effects in humans
Vacuum UV (100 – 200 nm)	Absorbed by almost all substances; only transferred in vacuum
UVC (200 – 280 nm)	Absorbed by DNA/RNA, proteins; leads to mutations/cancer; cataract formation
UVB (280 – 315 nm)	Causes sun burning and may initiate cancer
UVA (315 – 400 nm)	Sun tanning of human skin

Since microbial deactivation by means of UV occurs as a result of light absorption, a complex combination of factors related to UV emission, transmission and absorption may potentially impact treatment efficacy. It has been reported that UV disinfection is most effective at wavelengths of 260 – 265 nm with DNA/RNA and proteins being the primary microbial constituents that absorb the light (Bolton & Cotton, 2008c; Koutchma *et al.*, 2009a). However, while proteins are the predominant light absorbing molecules below 230 nm rather, DNA dominates absorption at higher levels (Fig. 6).

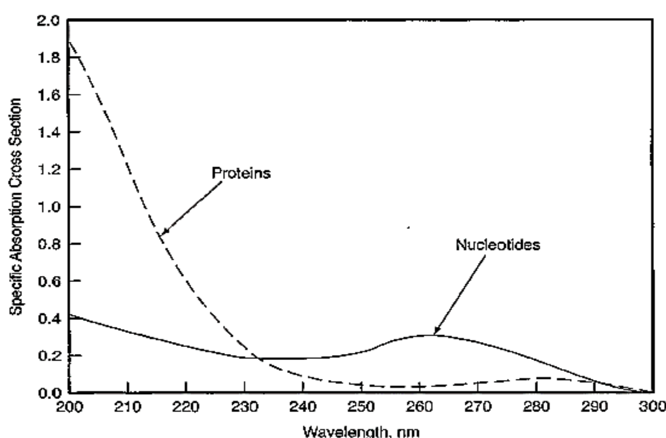

Figure 6 Absorption of UV light by proteins and nucleotides, respectively (Bolton & Cotton, 2008c).

Importantly, since water is capable of absorbing UV light below 230 nm, high doses will be required for the inactivation of pathogens should these lower wavelengths be utilised for disinfection. This

problem does not occur at wavelengths higher than 230 nm and, therefore, it is not surprising that the maximum effectiveness of UV disinfection occurs at the corresponding wavelength of maximal absorption by the nucleotides of microbial DNA (Kowalski, 2009). Figure 7 demonstrates that all of the respective DNA nucleotides absorb light within the UVC region. Understanding the photochemistry related to absorption of UV light by nucleotides especially, is key to understanding the disinfection mechanism of the treatment.

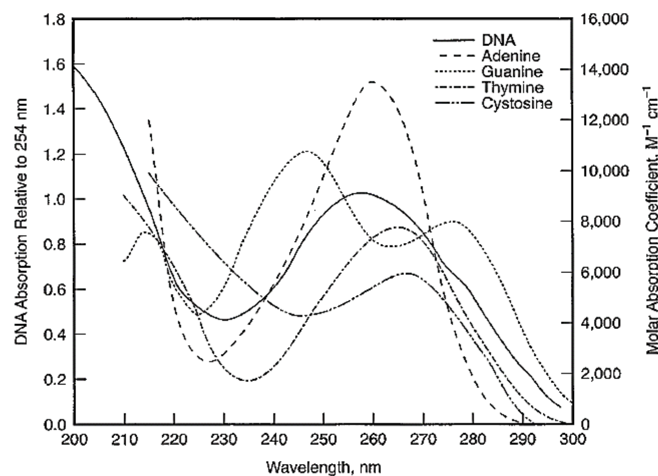

Figure 7 Schematic representation of the absorption spectra of DNA nucleotides (Bolton & Cotton, 2008c).

Effect of UV irradiation on the physiology of microorganisms

As mentioned above, photochemical reactions in both microbial proteins as well as DNA and RNA contribute to the deactivation of microorganisms by UV light (Kalisvaart, 2011; Gayán *et al.*, 2014). As secondary mechanism of disinfection, proteins predominantly absorb UV light below 230 nm. Tryptophan, tyrosine and phenylalanine are the only amino acids capable of absorbing UV light above 280 nm, consequently yielding a small absorption peak as shown in Figure 6. At very high UV doses microbial death may result from protoplasmic substances leaking from affected cells due to damage done to the outer cell membrane when proteins absorb UV light (Bolton & Cotton, 2008c).

Of greater importance is the destructive effect of UV absorption by DNA and RNA. In this instance, the light is absorbed at low doses compared to absorption by proteins. Subsequently, the affected microorganism's ability to replicate is impaired and so too its ability to cause disease when ingested (Bolton & Cotton, 2008c; Rodríguez *et al.*, 2013). Since phosphate and sugar molecules do not significantly absorb UV light above 210 nm, it should again be noted that DNA/RNA disruption results from UV absorption by nucleotides (Kalisvaart, 2011). When these absorb light from the UVC region, cyclobutane pyrimidine dimers (CPDs), pyrimidine 6-4 pyrimidones (6-4PPs) and other photoproducts may be formed as a result of photon absorption by purine and pyrimidine nucleic acid bases (Gayán *et al.*, 2014; Poepping *et al.*, 2014). Purine

nucleic acids include adenine and guanine while thymine and cytosine are described as pyrimidines. Pyrimidine derived photoproducts are of primary concern since these nucleic acids absorb UV light 10 times stronger than purines do (Kowalski, 2009; Gayán *et al*., 2014). After inhibiting the processes of transcription and replication, photoproducts eventually lead to cell death as a result of mutagenesis (Friedberg *et al*., 2006).

Ring structures can be formed when the number five and six-carbon atoms of adjacent pyrimidines are saturated by UV absorption (Fig. 8) (Rastogi *et al*., 2010; Douki, 2013; Gayán *et al*., 2014). This results from a covalent bond that forms when one of the nucleic acid bases absorbs a photon. Although these CPDs may be formed between any of the pyrimidines, thymine-thymine dimers are most frequently encountered (Rastogi *et al*., 2010). Bolton & Cotton (2008c) suggest that if a critical number of dimers are formed, DNA replication will not be able to occur within microbial cells. This then is the primary disinfection mechanism of UV light. Figure 8 shows the formation of a ring structure between two thymine bases. Figure 9 demonstrates the effect within the actual DNA molecule.

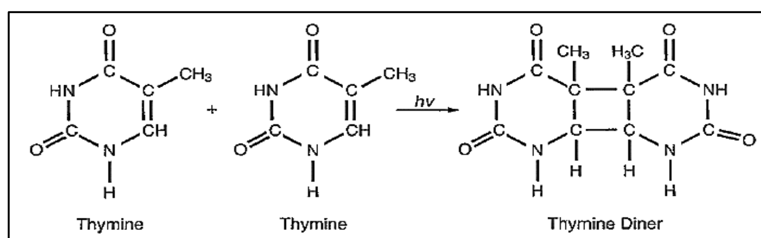

Figure 8 The formation of a thymine-dimer resulting from UV irradiation (Bolton & Cotton, 2008c).

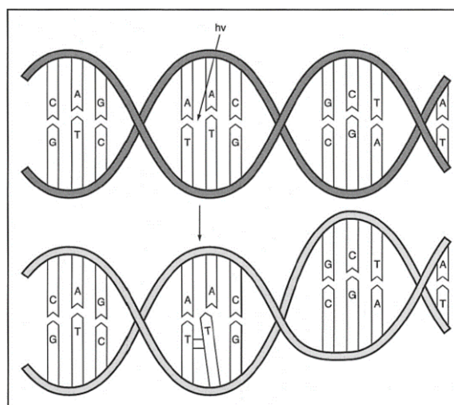

Figure 9 Representation of a thymine-dimer within microbial DNA (Bolton & Cotton, 2008c).

The 6-4PPs are formed in a comparable manner, with the additional formation of adducts. In a multi-step process, oxetane and azetidine are formed which are then rearranged to yield 6-4PPs. In this case, cytosine-cytosine (CC) and tyrosine-cytosine (TC) sequences are more commonly associated with 6-4PPs than tyrosine-tyrosine (TT) and cytosine-tyrosine (CT) sequences are (Gayán *et al*., 2014). During UV irradiation, approximately 25% of DNA damage can be attributed to the effect of 6-4PPs (Sinha & Hader, 2002). Nevertheless, both CPDs and

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6-4PPs initiate cell death by complicating transcription and replication within irradiated microorganisms (Friedberg *et al*., 2006).

In addition to CPDs and 6-4PPs, spore photoproducts (SPs) may form when bacterial spores are irradiated with UVC light (Douki *et al*., 2005; Moeller *et al*., 2007). This occurs when the methyl group of one thymine residue attaches to carbon number five of an adjacent thymine residue to yield 5-thyminyl-5, 6-dihydrothymine (Fig. 10) (Rastogi *et al*., 2010). Again, these photoproducts lead to mutagenesis and cell death by means of hindering transcription and replication processes during mitosis (Gayán *et al*., 2014).

Considering the importance of DNA and RNA for the existence and multiplication of microbial pathogens, the mechanism of UV disinfection is now well understood. With RNA differing from DNA only in having uracil as nucleotide rather than thymine, disruption of these materials occurs through the same basic mechanisms (Bolton & Cotton, 2008c).

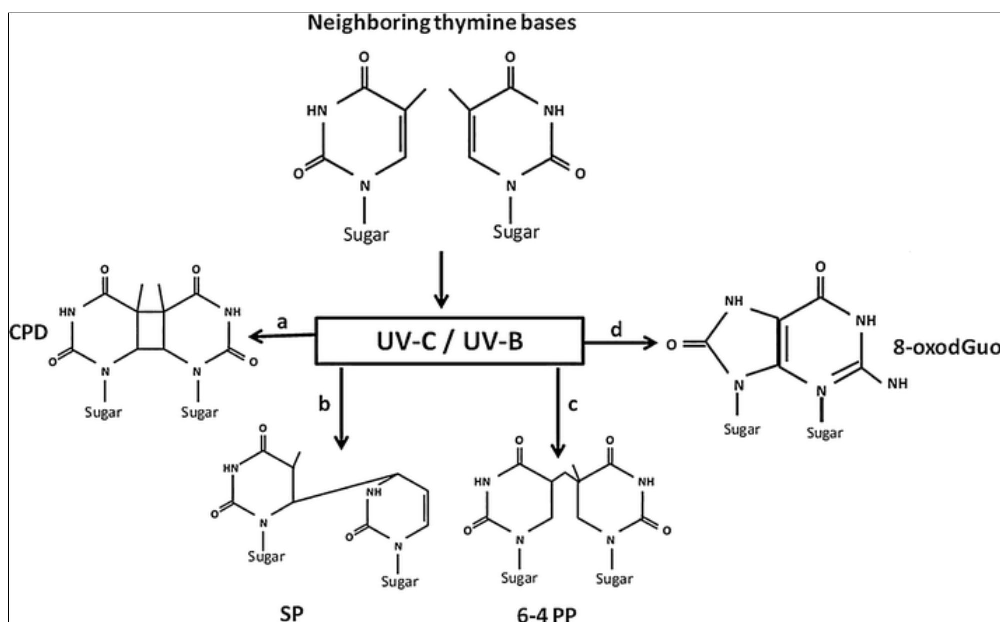

Figure 10 Key DNA photoproducts resulting from UV irradiation (Gayán *et al*., 2014).

Mechanisms of DNA repair and microbial reactivation

Despite the destructive mechanisms of UV irradiation, microorganisms (in particular bacteria) have developed several mechanisms for repairing the damage done to their genetic material (Hallmich & Gehr, 2010; Kalisvaart, 2011; Gayán *et al*., 2014). Adding to this, some viruses are capable of being reactivated by means of employing reactivation enzymes within their hosts (Bolton & Cotton, 2008c). These defence mechanisms, broadly categorised as dark and light (photoreactivation) repair, may have developed naturally since pathogenic microorganisms in the environment are constantly exposed to UV light from the sun (Hijnen *et al*., 2006; Bolton & Cotton, 2008c).

Gayán *et al.* (2014) identifies three key routes of DNA repair within microorganisms. Firstly, reverse-damage-repair results in the restoration of microbial DNA prior to replication. This is

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achieved by the *in situ* recovery of the damaged molecule, resulting from the activity of DNA lyases (Gayán *et al*., 2014). DNA lyases can either be CPD lyases or SP lyases (Benjdia, 2012). The former are responsible for the process of photoreactivation (photo-repair) and are often referred to as photolyases (Gayán *et al*., 2014). These enzymes harness the energy of visible light, with wavelengths ranging from 350 – 450 nm, and act specifically to repair the damage done by CPDs (Bolton & Cotton, 2008c; Gayán *et al*., 2014). In short, photolyases employ the energy of visible light to split pyrimidine dimers and repair the DNA string of microorganisms exposed to UVC irradiation (Kalisvaart, 2011). It is fairly obvious that inadequately low UV dosages (implying less microbial damage) and prolonged exposure of treated water to sunlight would enhance the ability of microorganisms to become viable again and infectious following disinfection (Bolton & Cotton, 2008c). In contrast, SP lyases do not require visible light for the repair of SPs during spore germination and can be reinforced by RecA-mediated pathways (Setlow, 2006; Moeller *et al*., 2007).

Secondly, excision-repair mechanisms recover damaged DNA by resynthesizing and replacing the affected nucleotides within microbial DNA (Bolton & Cotton, 2008c; Gayán *et al*., 2014). Since DNA polymerase 1 is responsible for this recovery, it is expected to be free from errors as this enzyme works from the parental DNA strand as prototype (Gayán *et al*., 2014). Base-excision-repair (BER) is performed by DNA glycosylases which act by first removing damaged bases after recognizing specific lesions. In contrast, nucleotide-excision-repair (NER) mechanisms directly remove lesion-containing nucleotides through the action of the UvrABC exinuclease. This process is referred to as “dark-repair” since damaged microbial cells can make use thereof in the absence of light (Rastogi *et al*., 2010). Lastly, tolerating-damage-pathways are repair mechanisms which are initiated after replication has been activated or completed. These mechanisms include RecA-mediated-excision-repair (RAMER), post-replication-repair (PRR) and translesion DNA synthesis (TLS) (Bichara *et al*., 2011). In contrast to excision repair mechanisms, which preserve genetic stability, processes such as mutagenic TLS may result in harmful effects in microorganisms and are therefore employed only as last resort (Bichara *et al*., 2011).

It is observed that many mechanisms of DNA repair are employed by irradiated microorganisms. These can then affect microbial resistance to UV disinfection and the parameters related to its occurrence should, therefore, be thoroughly evaluated prior to applying UV light for disinfection purposes (Gayán *et al*., 2014).

UV treatment plant-design and commonly used equipment

Equipment used for UV disinfection is either described as closed-pipe or open-channel systems and generally make use of high intensity, medium-pressure (MP) or low intensity, low-pressure (LP) mercury vapour lamps (Bolton & Cotton, 2008d; Koutchma *et al*., 2009b; Howe *et al*., 2012). Even though other technologies have been developed, these systems are predominantly used.

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In open-channel systems, water is irradiated by lamps that are positioned perpendicular or parallel to the gravity driven flow in an open, rectangular channel. When MP lamps are used in such a system, water is often passed through channels that are octagonal or circular in shape and fitted with a quartz sleeve in addition to a UV lamp. Open-channel systems are primarily employed for the disinfection of wastewater rather than drinking water (Bolton & Cotton, 2008d).

Closed-pipe systems consist of units containing UV lamps, UV sensors and usually an apparatus intended for cleaning the quartz sleeves found on the outsides of the lamps. These units are placed directly into a water carrying pipe and are usually employed for the disinfection of drinking water (Bolton & Cotton, 2008d). Different types of closed-pipe systems have been developed of which the most commonly used ones are indicated in Table 11.

Table 11 Characteristics of three typical closed-pipe UV systems (Bolton & Cotton, 2008d)

Reactor type	Example of Application	Lamp type
One-lamp annular	Water treatment at home	Low-pressure
Multiple lamps parallel to flow	Water treatment of small community	Low-pressure
Multiple lamps perpendicular to flow	Medium/large water treatment plants	Medium-pressure

Review of factors influencing the disinfection capability of UV irradiation

When translating the effectiveness of UV disinfection against laboratory-grown microorganisms (determined using collimated beam (CB) testing) to the effectiveness of UV disinfection on a large-scale (against environmental pathogens), various factors should be taken into account (Hijnen *et al*., 2006; AWWA, 2014; Gayán *et al*., 2014; Walters *et al*., 2014). A summary of the key aspects is presented in Table 12.

Table 12 Factors influencing the effectiveness of UV disinfection during large-scale water treatment operations (Hijnen *et al*., 2006; AWWA, 2014; Gayán *et al*., 2014; Walters *et al*., 2014)

Intensity related parameters		
Equipment design	**Water quality**	**Microbiological properties**
Lamp spacing	UV Transmission (UVT%)	Species, strain variation
Lamp age	Turbidity	Repair potential
Sleeve fouling	Total suspended solids (TSS)	Particle association
	Particle size distribution	Physiological state
	Chemical oxygen demand (COD)	Environmental conditions
	Total Dissolved Solids (TDS)	
Time related parameters		
Flow rate		
Reactor design		

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Most importantly, the inactivation capability of UV essentially relates to, and is dependent on, the amount (dose) of UV energy absorbed by pathogens (Oguma *et al.*, 2004; Eischeid & Linden, 2007). Ultraviolet dose is expressed as $mJ.cm^{-2}$ and is defined as the product of exposure time (s) (influenced by flow rate and retention time) and UV intensity ($mW.cm^{-2}$). Ultraviolet intensity is defined by the impact of water quality and equipment design parameters (Table 12) (AWWA, 2014).

UV dose as sole determinant of UV inactivation efficacy

Differences in microbial sensitivity toward UV disinfection may be attributed to both extrinsic and intrinsic aspects (Gayán *et al.*, 2014). Cell size, genome size, intrinsic repair efficacy, growth phase, the absence or presence of proteins that absorb UV light, differences in cell-wall characteristics and differences in the actual structure of microbial nucleic acids are a few factors which may contribute to variations in UV disinfection efficacy amongst different microorganisms (Child *et al.*, 2002; Koutchma *et al.*, 2009a; Oteiza *et al.*, 2010; Müller *et al.*, 2011). A direct correlation between the thymine content of bacterial DNA and UV sensitivity is also observed. Furthermore, gram-negative bacteria are more sensitive to UV light in comparison to spore-formers and gram-positive bacteria (Koutchma *et al.*, 2009a).

Due to variances in UV sensitivity, different UV doses are required to achieve predetermined log reductions of different microorganisms. Therefore, UV dose-response curves are constructed with data from collimated beam tests and used as a means of investigating microbial sensitivity (Bolton & Cotton, 2008c; Howe *et al.*, 2012). Bacteria and protozoa are reported to be the most sensitive, followed by most viruses, bacterial spores, the adenovirus and algae (Bolton & Cotton, 2008c). Table 13 serves as a summary of the available literature and show the estimated dose ranges required to achieve a 1 log (D_{10}) reduction of different groups of microorganisms.

Table 13 UV dose required for a 1 log reduction of different microbial groups at 253.7 nm (Koutchma *et al.*, 2009a)

Group of microorganisms	Required UV dose ($mJ.cm^{-2}$)
Enteral bacteria	2.0 – 8.0
Cocci and micrococci	1.5 – 20.0
Spore formers	4.0 – 30.0
Enteric viruses	5.0 – 30.0
Yeast	2.3 – 8.0
Fungi	30.0 – 300.0
Protozoa	60.0 – 120.0
Algae	300.0 – 600.0

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Review of dose requirements for UV disinfection of microorganisms

In 1985, Chang *et al.* investigated the dose requirements for inactivating a range of bacteria in secondary effluent from a wastewater treatment plant. It was reported that a 3 log reduction of *E. coli*, *S. typhi*, *S. sonnei* and *S. aureus* was achieved following exposure to doses of 5 – 15 $mJ.cm^{-2}$ (Chang *et al.*, 1985). He further found that doses of 28 – 42 $mJ.cm^{-2}$ were required to achieve a 3 log reduction of the polio and rotaviruses. Siddiqui (2005) states that UV doses in the range of 3 – 30 and 30 – 100 $mJ.cm^{-2}$ are sufficient for the disinfection of bacteria and viruses, respectively. Koivunen & Heinonen-Tanski (2005b) reported that UV doses of 6 – 18 and 22 – 38 $mJ.cm^{-2}$, respectively, resulted in reductions of 1 – 3 log and 1 – 1.5 log of enteric bacteria and the coliphage MS2 virus. Tests were performed using a collimated beam device, fitted with a LP mercury vapour lamp, and peptone water as test medium (Koivunen & Heinonen-Tanski, 2005b).

A study of UV inactivation of bacteria in a small-scale sequential batch reactor (SBR) showed that a dose of 40 $mJ.cm^{-2}$, produced by a LP lamp, was sufficient for reducing total coliforms and faecal coliforms to 14.6 $cfu.100\ mL^{-1}$ and 8.7 $cfu.100\ mL^{-1}$, respectively (Melidis *et al.*, 2009). Wang *et al.* (2011) found that a very low dose (< 2 $mJ.cm^{-2}$) resulted in 4.5 – 4.9 log reductions during the first phase of inactivation of *E. coli* DH5α in phosphate buffered saline and two wastewater samples. It was further reported that the quality of wastewater did not significantly impact the inactivation kinetics of the tested microorganism (Wang *et al.*, 2011). Mezzanotte *et al.* (2007) also evaluated the UV inactivation of bacteria found in municipal wastewater on pilot-scale. It was found that low UV doses (10 – 20 $mJ.cm^{-2}$) were effective in completely removing *E. coli* strains from the treated municipal water (Mezzanotte *et al.*, 2007).

In comparison to bacteria, viruses have been reported to be more resistant to UV disinfection (Bolton & Cotton, 2008c). Nevertheless, Gerba *et al.* (2002) states that > 4 log inactivation of most pathogenic viruses is readily achieved at UV doses applied for water disinfection. Hijnen *et al.* (2006) reported maximum microbial inactivation credit (MIC_{max}) of 5.4 log for poliovirus type 1 at UV dose ≥ 50 $mJ.cm^{-2}$. For the Rotavirus SA-11 Malley *et al.* (2004) reported MIC_{max} of 4.6 log at UV doses of 5 – 30 $mJ.cm^{-2}$ when a polychromatic UV source was used. Adenovirus, on the contrary, is regarded as the most resistant waterborne pathogen currently threatening public health. It is reported to require a UV dose of approximately 186 $mJ.cm^{-2}$ for a 4 log reduction (USEPA, 2006; Linden *et al.*, 2007). Baxter *et al.* (2007) found that a UV dose of 160 – 170 $mJ.cm^{-2}$ was required for 4 log inactivation of adenovirus type 5 (Ad5), which was comparable to that required by Ad2 and other types. Other studies report even higher resistance and have shown that UV doses of 109 $mJ.cm^{-2}$ and 120 $mJ.cm^{-2}$ resulted in 2 log reductions of Ad40 and Ad41, respectively (Thurston-Enriquez *et al.*, 2003; Baxter *et al.*, 2007). Results were obtained using LP mercury vapour lamps as source of UV light. Linden *et al.* (2007) reported that UV doses of 30 $mJ.cm^{-2}$ and 40 $mJ.cm^{-2}$, respectively, were required to achieve 3 log

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and 4 log inactivation of Ad40 when a MP lamp was used. Tests were performed in both filtered drinking water and buffered laboratory water.

Hijnen *et al.* (2006) reviewed the literature with regard to the effectiveness of UV disinfection against bacteria, viruses and protozoa in water. While the adenovirus, the protozoan *Acantamoeba* and bacterial spores were reported to be the most resistant, pathogenic bacteria and (oo)cysts of *Giardia* and *Cryptosporidium* were reported to be very sensitive (Hijnen *et al.*, 2006). For the latter protozoan species, an average dose of < 20 $mJ.cm^{-2}$ was required to achieve a MIC of 3 log. Hijnen *et al.* (2006) determined that UV doses in the range of 0.5 – 6.1 $mJ.cm^{-2}$ achieved this result when polychromatic light was applied to reduce *Cryptosporidium parvum* in water samples. The use of monochromatic light at doses of 0.9 – 13.1 $mJ.cm^{-2}$ also resulted in a MIC_{max} of 3 log. Furthermore, *G. muris* and *G. lamblia* exposed to monochromatic UV light, was reported to be reduced by 2.4 and 2.5 log at doses in the range of 1.5 – 11 $mJ.cm^{-2}$ and 0.05 – 1.5 $mJ.cm^{-2}$, respectively (Craik *et al.*, 2000; Linden *et al.*, 2002). This is significant since conventional disinfectants, chlorine and ozone specifically, have been shown to have limited inactivation capabilities against these protozoan (oo)cysts (Hijnen *et al.*, 2006).

Again, it is observed that different groups of pathogenic microorganisms differ with regard to their responses to UV light. Based on published data from previous studies, Chevrefils *et al.* (2006) presented a summary of UV doses required to achieve multiple log reductions of bacteria, viruses and protozoa. Inactivation, however, is influenced by microbial characteristics as well as water quality and plant design parameters. Thus, in the absence of standardised protocols, such data should be very carefully used for the purpose of validating UV disinfection.

The influence of equipment design-related parameters on UV disinfection

Specific design parameters

In UV equipment, factors such as lamp type and the number used, as well as how they are spaced, strongly influence UV intensity (AWWA, 2014). Low-pressure and MP mercury vapour lamps are usually used for water disinfection purposes and differ significantly with regard to the light they emit (Gayán *et al.*, 2014). In the case of LP lamps, 85% of their emission occurs in the form of a narrow band of quasi-monochromatic light at 253.7 nm (Guo *et al.*, 2009; Gayán *et al.*, 2014). This is close to where microbial DNA maximally absorb UV light. In MP mercury vapour lamps, higher vapour pressure and temperature results in the production of polychromatic light at 200 – 600 nm (Kowalski, 2009). Of this, only 15 – 23% is emitted at 253.7 nm. Medium-pressure lamps are thus expected to disrupt not only genetic materials, but also other cell components (AWWA, 2014). Compared to LP lamps, which emit no more than 40% of their power input, MP lamps represent much higher outputs and radiation of much higher intensity (Freese & Nozaic, 2004). Systems employing MP lamps thus typically require less lamps to deliver specific UV doses in water.

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Lamps are always spaced in a manner that will provide the highest average intensity within the apparatus.

In the literature, studies comparing low and medium-pressure disinfection efficacy are primarily based on the extent of photoreactivation observed following the treatments. Results of these experiments are often found to be contradictory. Photo-repair has been observed in *E. coli* treated with doses < 10 mJ.cm^{-2} using LP UV lamps, while no repair was reported following exposure to MP UV at the same dose (Zimmer & Slawson, 2002; Kalisvaart, 2004; Hu *et al*., 2005). Quek & Hu (2008a) also reported that less photoreactivation of *E. coli* was observed following treatment with MP UV in comparison to LP UV.

On the contrary, *Legionella pneumophila* showed no difference in photoreactivation following low and medium-pressure treatment (Oguma *et al*., 2004). The same effect was observed for *Mycobacterium terrae* (Bohrerova & Linden, 2006). In addition, *Cryptosporidium parvum* showed no degree of photoreactivation following either LP or MP UV treatment (Zimmer *et al*., 2003). However, these studies were all performed using pure cultured microorganisms and the difference in the efficacy of LP and MP UV for treating water containing mixed cultures has not been investigated profoundly (Guo *et al*., 2009).

Guo *et al.* (2009) investigated the degree of photoreactivation of cultured *E. coli* as well as total coliforms in saline solution and wastewater, respectively, following LP and MP UV treatment. For *E. coli* treated in saline solution, photoreactivation of 50% and 20%, respectively, was achieved when a UV dose of 5 mJ.cm^{-2} was applied using LP and MP lamps (Guo *et al*., 2009). At a higher dose of 15 mJ.cm^{-2}, however, MP UV light was no longer advantageous since no photoreactivation occurred following either of the treatments. Similar results were obtained when wastewater was treated with no significant difference being observed between LP and MP treatments at any of the tested doses (Guo *et al*., 2009). At a dose of 40 mJ.cm^{-2}, photoreactivation did not exceed a level of 1% for either of the treatments.

In a well-known study, Oguma *et al.* (2002) investigated photoreactivation of the *E. coli* K-12 strain following inactivation by LP, MP and filtered MP lamps. The number of pyrimidine dimers that were formed during irradiation was determined using an endonuclease sensitive site (ESS) assay. The colony-forming ability (CFA) of the bacteria was determined using a conventional method (Oguma *et al*., 2002). It was found that 84.2%, < 0% and 83.1% of dimers formed during UV treatment were repaired following the use of LP, MP and filtered MP lamps, respectively. The CFA ratios calculated from the different treatments were estimated at 2.09, 0.61 and 1.02 log, following an initial 3.0 log reduction. It is clear that MP lamps were more effective in inactivating *E. coli*. Experimental data showed that the 220 – 300 nm wavelength light (emitted by the MP lamp) disrupted endogenous photolyase, thereby reducing the ability to repair pyrimidine dimers. Light in the 300 – 580 nm wavelength range influenced the CFA ratio as a result of effects not related to pyrimidine dimers. With reference to this study, it is concluded that the use of UV light at multiple wavelengths (MP) may be advantageous compared to the use of monochromatic light

sources (LP) considering its inhibitory effect on photoreactivation and regrowth following disinfection (Oguma *et al.*, 2002).

Maintenance factors

Different factors related to equipment maintenance may influence the UV intensity delivered by mercury vapour lamps. With increasing lamp age, UV intensity progressively decreases (Schmalwieser *et al.*, 2014). When designing UV systems this should be kept in mind in order to ensure that the required UV dose is delivered up until lamp replacement. For both LP and MP lamps, replacement is suggested following approximately 5 000 h of usage. However, this period is influenced by the regularity of switching the lamp on and off (AWWA, 2014).

A phenomenon known as sleeve fouling is characterised by decreased UV intensity due to the absorption of UV light by materials that build-up on the quartz sleeves of UV lamps (Wait & Blatchley, 2010; AWWA, 2014). These may include organic and inorganic particles, algae and biofilms. High levels of calcium, magnesium and iron may increase the rate of fouling. In conservative low-pressure systems, a fouling factor is unified with the design to ensure that the required UV intensity is consistently delivered (AWWA, 2014).

The influence of water quality parameters on UV disinfection

Various researchers have reported that the efficacy of UV disinfection is greatly influenced by parameters of water quality (Salgot *et al.*, 2002; Selma *et al.*, 2008; Howe *et al.*, 2012). Those parameters related to the optical aspects of UV transmission in water (UVT%, turbidity, TSS, COD, particle size and TDS) are of primary concern as it may influence the maximum number of photons, thus UV intensity, available for destroying microorganisms (Koutchma *et al.*, 2009a; Brahmi *et al.*, 2010). Bolton & Cotton (2008e) describes UV transmittance (UVT%) in treated water as the most important water quality parameter as it may significantly affect plant size, design and operating costs. Thus, it is this parameter that is often used in the design and monitoring of UV systems developed for the inactivation of microorganisms (Howe *et al.*, 2012). In essence, UVT% indicates the amount of UV light absorbed by water and its different components and is defined as a function of the absorption coefficient (α) of the treated substance ($UVT = 100 \times 10^{-\alpha}$). Low UVT% values thus indicate high absorption and results in the delivery of lower UV doses.

Bolton & Cotton (2008e) identify humic and alginic acids, which contribute to total organic carbon (TOC), and substances such as phenols as major contributors to the absorption coefficient of water. Since inorganic compounds (such as dissolved iron), together with organic compounds, are capable of absorbing UV light in water, it is expected that chemical oxygen demand (COD) and total dissolved solids (TDS) will be positively correlated with decreased UVT%. Chemical oxygen demand serves as an indicator of the level of organic pollution in water and is defined as "the amount of oxygen equivalents consumed in oxidising the organic compounds of samples by strong oxidising agents such as dichromate or permanganate" (Wu *et al.*, 2011). Most of these pollutants

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show peaks of absorption in the UV region as a result of their molecular characteristics (Wu *et al.*, 2011). Chemical oxygen demand is, therefor, expected to significantly impact UVT% in water. Nevertheless, Hijnen *et al.* (2006) suggests that UV dose is easily corrected in accordance with the UVT% represented by water samples.

The correlation between UVT% and the efficacy of UV disinfection has been investigated by many (Koutchma *et al.*, 2009a; Gayán *et al.*, 2011; Gayán *et al.*, 2014). Gayán *et al.* (2011) found that when α was increased by 15.9 cm^{-1}, the inactivation rate of *E. coli* was decreased with a factor of 10. He identified a linear relationship between the absorption coefficient and log-inactivation of *E. coli* (Gayán *et al.*, 2011). Hijnen *et al.* (2006) compared results of wastewater studies to that of drinking water studies to demonstrate the effect of water quality on UV disinfection. One of his findings was that the inactivation rate constant was lowered with a factor 1.6 for the poliovirus in wastewater, with lower UVT% and higher turbidity, in comparison to drinking water (Hijnen *et al.*, 2006).

The efficiency of UV disinfection has also been evaluated with respect to additional water quality parameters. Particulate material may influence disinfection efficacy by means of absorbing, scattering and blocking UV light. Particularly, Howe *et al.* (2012) refers to shading and encasement as mechanisms of interference. This implies that properties such as turbidity, TSS and particle size are of importance. Christensen & Linden (2003) investigated the effect of particulate material on UV disinfection of drinking water. They found that a 1 – 10 nephelometric turbidity units (NTU) increase in turbidity was accompanied by a 5 – 33% decrease in the delivered UV dose (Christensen & Linden, 2003). In practice, increased turbidity may thus compromise UV disinfection efficacy. Jones *et al.* (2014) evaluated the effect of turbidity on UV inactivation of a five-strain cocktail of each of six pathogenic microbial species in water obtained from a creek and pond. Inactivation in phosphate-buffered saline (PBS) was also investigated as a means of having a low turbidity representative. For the *E. coli* strains, specifically, log reductions of approximately 10.0, 9.5 and 7.3 were achieved in PBS, creek water and pond water, respectively, following LP UV disinfection at a dose of 14.2 $mJ.cm^{-2}$ (Jones *et al.*, 2014). These samples represented respective turbidity values of 0.1 NTU, 3.0 – 4.4 NTU and 15.8 – 22.7 NTU. Thus, even though inactivation of 99.9% or higher was achieved in all instances, UV disinfection decreased as turbidity increased (Jones *et al.*, 2014).

Pathogens in water often associate with particles (suspended solids) and so become more difficult to inactivate due to absorption and scattering of UV light. Higher TSS levels would then be expected to increase the UV demand for water disinfection. Walters *et al.* (2014) examined the influence of TSS concentration as well as particle association and size on UV disinfection of wastewater containing *E. coli* and enterococci. Results of the study show that those microorganisms associating with particles with diameter (d_p) ≤ 12 µm were destroyed two times and 1.7 times faster than those associated with larger particles (12 – 63 µm d_p) for *E. coli* and

enterococci, respectively. Regarding TSS concentration, for both particle sizes, with $d_p \leq 12$ µm and $12 < d_p \leq 63$ µm, increased TSS concentration resulted in decreased removal rate coefficients (*k*) (Walters *et al.*, 2014). As the TSS concentration increased from 51 mg.L^{-1} to 130 mg.L^{-1}, *k*-values decreased from 1.0 to 0.7 and 1.2 to 0.8 for *E. coli* and enterococci, respectively. Cantwell & Hofmann (2008) examined the potential of small, suspended particulate materials ($d_p < 11$ µm) to associate with and protect coliform bacteria against UV inactivation in unfiltered surface/river water. It was found that a UV dose of 20 mJ.cm^{-2} resulted in a 2 – 2.5 log inactivation of coliform bacteria, while > 3.4 log inactivation was achieved in filtered water at the same dose. From their research it was concluded that even very small particles (11 µm) show potential for protecting microorganisms against UV disinfection (Cantwell & Hofmann, 2008).

The influence of microorganism-related factors on UV disinfection

Influence of DNA repair mechanisms

With reference to the literature, photoreactivation as repair mechanism has been most widely studied (Guo *et al.*, 2012). Guo *et al.* (2011) investigated the effect of UV inactivation and the potential for damage-repair represented by *E. coli* CGMCC, *B. subtilis* CGMCC 1.73 and the G215 faecal coliform strain in reclaimed wastewater. Photoreactivating light, with an intensity of 20 µW.cm^{-2} at 365 nm, resulted in *E. coli* levels of approximately 10^5 cfu.mL^{-1} being reached following an eight hour treatment period (Guo *et al.*, 2011). The highest levels of repair observed for *E. coli* and the faecal coliform were 28.73% and 14.37%, respectively, following initial inactivation at a dose of 5 mJ.cm^{-2} (Guo *et al.*, 2011). *Bacillus subtilis* did not show significant potential for photoreactivation. The same study reported that only the *E. coli* strain showed potential for the mechanism of dark-repair. Following a 4 h incubation period in the absence of light, its levels increased from 2 x 10^2 cfu.mL^{-1} to 10^3 cfu.mL^{-1} (Guo *et al.*, 2011).

Guo *et al.* (2012) evaluated photoreactivation potential and bacterial characteristics following repair of two *E. coli* strains. These contained plasmids of ampicillin-resistance and fluorescence, respectively. Following a 4 h treatment with photoreactivating light, the ampicillin-resistant and fluorescing strain showed maximum recovery of 1% and 46%, respectively, after being inactivated with a dose of 5 mJ.cm^{-2}. In addition, the bacteria maintained their characteristics of antibiotic-resistance and fluorescence. Thus, it is important to consider that, following UV disinfection, pathogenicity of bacterial strains may possibly be revived in instances where photoreactivation may occur. In both of the mentioned studies, negligible photoreactivation occurred when UV doses > 5 mJ.cm^{-2} were applied. This indicates that photoreactivation potential greatly depends on the extent of DNA damage caused by UV disinfection (Guo *et al.*, 2011).

Wang *et al.* (2011) also studied the potential of light and dark-repair of bacterial strains. It was reported that *E. coli* DH5α was greatly reactivated following UV disinfection at a dose of 8 mJ.cm^{-2} and subsequent dark treatment. After four days of incubation, *E. coli* levels of

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10^5 cfu.mL^{-1} were reached from an initial concentration of less than 10^2 cfu.mL^{-1} (Wang *et al.*, 2011). Photoreactivation levels of 62.84% and 55.32% were reported for *E. coli* DH5α and *S. dysenteriae* CGMCC 1.2428, respectively, following UV disinfection at a dose of 0.8 mJ.cm^{-2}. In the case of *E. coli* DH5α, photoreactivation was observed up to a UV dose of 60 mJ.cm^{-2}, while *S. dysenteriae* showed no reactivation at a dosage of 23 mJ.cm^{-2} (Wang *et al.*, 2011).

These studies clearly show that different bacterial species show differences in damage-repair potential. As supportive conclusion, Cheigh *et al.* (2012) attributed the greater UV sensitivity of *E. coli* O157:H7 to a lesser capability of reversing the formation of CPDs in comparison to *L. monocytogenes.* Similarly, in comparison to *Enterococcus faecium*, *P. aeruginosa* showed much greater levels of DNA repair (Suss *et al.*, 2009). Differences in photoreactivation capabilities have also been observed between strains of the same species.

Quek & Hu (2008a) evaluated dark-repair and photoreactivation of seven *E. coli* strains (Table 14). The bacteria were exposed to maximum UV doses, based on the requirement of a 5 log reduction, of approximately 16 mJ.cm^{-2} and 12 mJ.cm^{-2} during disinfection with LP and MP lamps, respectively.

Table 14 Characteristics of seven *E. coli* strains used to evaluate photoreactivation capability (Quek & Hu, 2008a)

Strain		**Distinctive characteristic**
ATCC	11775	Type strain for *E. coli*
	11229	Often used as indicator in disinfection studies
	15597	Host to bacteriophages; Derived from K-12 *E. coli* strain
	700891	Host to bacteriophages; Possesses plasmid (Famp)
NCIMB	9481	Acts as host for the lambda phage
	10083	Found in human faecal matter (wild-type strain)
CCUG	29188	Mitigated strain forming part of the *E. coli* O157:H7 serotype

Photoreactivation to a maximum of 85% was reported, which was in line with the 84% reactivation of *E. coli* following LP UV disinfection as reported by Oguma *et al.* (2002). Following MP UV irradiation, five of the seven strains (Table 14) were reactivated to levels of 70 – 80% when they were exposed to light with an intensity of 11.5 kLux for four hours. The NCIMB 9481 and 10083 strains were reactivated to lower levels of roughly 15% and 50%, respectively. These two strains also represented the lowest level of reactivation following LP UV treatment. Quek & Hu (2008a) identified lower levels of photoreactivation enzymes and less productive mechanisms of repair as possible causes of slower reactivation. For the seven strains, the individual rates of photoreactivation were also determined. The ATCC 15597 and 700891 strain dominated in both LP and MP experiments and showed repair at rates of 3.00 log.h^{-1} and 3.25 log.h^{-1}, respectively,

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following LP UV treatment. These two strains were also found to be the most resistant during UV disinfection experiments. Interestingly, the ATCC 700891 strain carries the Famp plasmid responsible for conferring microbial resistance to streptomycin and ampicillin. According to Quek & Hu (2008a), the presence of the plasmid might have some contribution to the UV resistance and high repair rate shown by the microorganism.

Regarding photoreactivation in irrigation water, it is important to understand practical implications such as the contribution of actual sunlight to the phenomenon. A study performed by Guo *et al.* (2009) utilised a lamp (Philips F20T12, 20 W) with a relative emission spectrum representing that of sunlight. It was found that *E. coli CGMCC 1.3373* showed 50% and 20% photoreactivation after initial inactivation at a dose of 5 $mJ.cm^{-2}$ using LP and MP lamps, respectively. This indicates that sunlight may result in significant photoreactivation when low UV doses are applied. Furthermore, it should be noted that MP UV light resulted in a lesser degree of reactivation compared to LP UV at the same dose (Guo *et al*., 2009). This is in accordance with the findings of Quek & Hu (2008b) who suggest that MP UV, in comparison to LP UV, more effectively reduces the photolyase activity of *E. coli*. The same lamp was used in the photoreactivation study performed by Guo *et al.* (2012). As discussed earlier, they reported that a low UV dose resulted in 1% and 46% photoreactivation of two respective *E. coli* strains. Again, the capability of sunlight to initiate photoreactivation was clear.

Quek & Hu (2008b) compared photoreactivation of two *E. coli* strains (ATCC 11229 and ATCC 15597) following exposure to both fluorescent light and sunlight. Under low intensity sunlight, < 12 kLux for LP UV and < 5 kLux for MP UV, bacterial counts for both strains rapidly increased within the first hour of exposure. The percentage log-recovery was reported as 80.7% and 65.4%, respectively, following LP and MP UV irradiation of the ATCC 11229 strain. Corresponding values for the ATCC 15597 strain were found to be 75.4% and 58.9%. Under high intensity sunlight, 12 – 80 kLux for LP UV and 5 – 70 kLux for MP UV, bacterial counts again increased significantly in the first hour of exposure, but was followed by a decrease during the next. This indicates that sunlight represents a germicidal effect which resulted in inactivation rather than reactivation at higher intensities. The percentage log-recovery was reported as 64.8% and 49.6%, respectively, following LP and MP UV irradiation of the ATCC 11229 strain. Corresponding values for the ATCC 15597 strain were found to be 61.7% and 49.5%. Quek & Hu (2008b) concluded that photoreactivation may be of great concern in areas where sunlight is plentiful and temperatures fairly high (23 – 37°C).

The influence of photoreactivation on UV dose requirements for achieving a certain degree of inactivation is clearly observed when considering disinfection kinetics (Hijnen *et al*., 2006). It is expected that increased UV doses will result in subsequent increases in the UV dose required to compensate for photoreactivation (*K*-values decrease). In 1985 already, it was reported that UV dose requirement increased with a factor of 2.8 – 4.6 to obtain 1 – 3 log inactivation of *Legionella pneumophila* (Knudson, 1985). In this case, the *K*-value decreased with a factor of 3.2.

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For *E. coli*, UV dose enhancement by a factor of 3.5 was required for 4 log inactivation taking into account the effect of photoreactivation (Hoyer, 1998). With regard to photoreactivation then, lower UV doses and greater exposure to visible light may enhance microbial repair (Bolton & Cotton, 2008c; Kalisvaart, 2011). This implies that design parameters of treatment plants may significantly influence the microbial safety of treated water and should be carefully considered prior to disinfection. Lastly, since various factors may influence photolyase-catalysed repair, standardised protocols should be developed to accurately compare results of different photoreactivation experiments.

General advantages and disadvantages related to UV disinfection

Due to the discussed limitations related to the use of conservative chemical and/or physical disinfection methods, UV irradiation has been thoroughly investigated as alternative for the treatment of water. Its general effectiveness against waterborne pathogens including bacteria, viruses and protozoa has been reported to be accompanied by several additional advantages.

First, being a physical process, the need of handling, transporting and storing harmful chemical substances is eliminated (Bolton & Cotton, 2008a; Turtoi, 2013). This implies obvious advantages for operators and all involved in water disinfection since risks such as gas leaks are abolished. The formation of disinfection by-products, including harmful mutagenic, carcinogenic and toxic substances, is also eliminated when employing UV irradiation as disinfection procedure (Bolton & Cotton, 2008a; Guo *et al.*, 2009; Turtoi, 2013). Adding to this, UV irradiation does not affect water quality with regard to parameters such as total organic carbon (TOC), turbidity, pH and corrosivity (Bolton & Cotton, 2008a). It is also reported that UV disinfection requires a contact time in the order of 20 – 30 seconds when LP lamps are used (Turtoi, 2013). This is considerably shorter than that required by most other treatments. Similarly, MP lamps are associated with much shorter residence times in comparison to alternative disinfection techniques (Howe *et al.*, 2012). The effectiveness of UV against the likes of the resistant *Cryptosporidium* and *Giardia* at low doses has been regarded as one of the key advantages of the process (Hijnen *et al.*, 2006; Bolton & Cotton, 2008a).

With regard to practical and economical aspects, UV disinfection again represents several advantages. Space requirement is minimal, allowing the system to be easily incorporated into existing water treatment works; capital costs are fairly low compared to those represented by ozone treatments and filtration (operating costs are also low compared to these methods) and operating UV systems with regard to the variability of dose requirements related to changes in water quality etc. is fairly easy (Bitton, 2005; Bolton & Cotton, 2008a; USEPA, 2011).

Despite the mentioned advantages, UV irradiation is also associated with some negative aspects. As opposed to some chemicals, UV light does not show any residual activity in water following the initial application and, therefore, allows for possible re-contamination if care is not

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taken (Freese & Nozaic, 2004; Bolton & Cotton, 2008a; USEPA, 2011). As discussed, the effect of water quality parameters may also significantly influence the effectiveness of UV disinfection. It has been suggested, and proven, that the UVT%, turbidity and TSS concentration significantly impacts UV disinfection efficacy since it is directly linked to the delivered UV dose (Koutchma *et al.*, 2009a; Gayán *et al.*, 2011; Gayán *et al.*, 2014; Jones *et al.*, 2014). If sub-lethal doses are applied, some of the more resistant pathogens may be inadequately deactivated. Furthermore, secondary measurements such as UV transmittance and sensor readings are often utilised in UV applications since technology that is capable of continuously measuring UV dose is currently not readily available (Bolton & Cotton, 2008a). This is problematic since applying the correct UV dose is of critical importance for effective disinfection. With regard to microbiological aspects, the phenomenon of DNA repair mechanisms, which include dark and photoreactivation, may significantly influence disinfection efficacy. Various researchers have reported that photoreactivation specifically, is of great concern (Guo *et al.*, 2011; Wang *et al.*, 2011; Guo *et al.*, 2012). In addition, microorganisms may associate with particles suspended in water, subsequently being shielded from the damaging effects of UV rays (Walters *et al.*, 2014). Other, less common, disadvantages of UV disinfection include: mercury hazards in water should the mercury containing lamps break; compromised disinfection resulting from interruptions in power supply and inadequate disinfection resulting from the phenomenon of lamp warm-up (Bolton & Cotton, 2008a).

Ultraviolet based Advanced Oxidation Processes (AOPs)

As for any other method, UV irradiation cannot be regarded as a flawless, unsurpassable technique with respect to water disinfection. While each of the individual technologies are associated with their own limitations, research indicates that the application of combination treatments often result in considerable benefits (Lotierzo *et al.*, 2003; Zoutman *et al.*, 2011; Tawabini *et al.*, 2013).

Advanced Oxidation Processes (AOPs) are increasingly utilised in the different sectors of the water industry including the production of drinking water and the treatment of water containing waste (Ijpelaar *et al.*, 2010; Tawabini *et al.*, 2013; Sherchan *et al.*, 2014). The technology of AOPs is based on the combination of UV light with secondary oxidants, such as ozone and hydrogen peroxide, in order to destruct disinfection by-products, microorganisms and other organic micro pollutants present in water (Wols & Hofman-Caris, 2012; Tawabini *et al.*, 2013). This occurs through the formation of high concentrations of hydroxyl radicals ($OH^{\bullet}$) which are very reactive, non-selective oxidising agents (Timchak & Gitis, 2012; Tawabini *et al.*, 2013).

Various studies have reported on the bactericidal effect of H_2O_2 (Ksibi, 2006; Labas *et al.*, 2008; Rizvi *et al.*, 2013) but as a result of fairly low efficacy and disinfection rates the chemical is not commonly applied as primary disinfectant in water treatment (Koivunen & Heinonen-Tanski, 2005b). However, the combination of UV light and H_2O_2 as an AOP promotes disinfection and has

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been proven to be capable of destroying not only a variety of pollutants, but also spores and vegetative cells (Alkan *et al*., 2007; Mamane *et al*., 2007). This particular combination is often encountered in literature and utilises UV light for direct disinfection, but also for the generation of hydroxyl radicals in water (Timchak & Gitis, 2012). Such treatment thus combines the effects of direct photolysis and advanced oxidation (via indirect photolysis) in order to destroy organic substances in contaminated water (Pereira *et al*., 2007).

Within the literature various studies comparing the disinfection efficacy of UV irradiation versus that of UV/H_2O_2 combination treatments can be found. Teksoy *et al.* (2011) investigated the disinfection potential of UV and UV/H_2O_2 combination treatments against *E. coli* (ATCC 25922), *B. subtilis* (ATCC 6633) and *P. aeruginosa* (15542) in humic waters. For each of the respective treatments, a 3 log reduction was more rapidly achieved for *E. coli*. When the effect of the UV/H_2O_2 treatment was evaluated in bottled water with fulvic acid concentration of 2 mg.L^{-1} a 3 log reduction was achieved after 55 s and 33 s at H_2O_2 concentrations of 0 mg.L^{-1} and 50 mg.L^{-1}, respectively. Samples were exposed to UV light emitted at an intensity of 40 µW.cm^{-2}. When H_2O_2 was applied at these concentrations in the absence of UV light, no significant inactivation occurred. Tawabini *et al*. (2013) evaluated the effect of an UV/H_2O_2 treatment using two UV sources, a 15 W LP and 150 W medium-pressure (MP) lamp, in combination with H_2O_2 at concentrations of 20, 50 and 100 mg.L^{-1}. In comparison to UV treatment alone, the combination of LP UV irradiation and 20, 50 and 100 mg.L^{-1} H_2O_2 resulted in a 33% average decrease in the time required to achieve a 8 log reduction of the *E. coli* K12 wild-type strain MG 1655. It was also reported that the combination of MP UV and H_2O_2 resulted in increased rates of inactivation in comparison to the use of MP UV and H_2O_2 alone (Tawabini *et al*., 2013). Rizvi *et al*. (2013) investigated the disinfection potential of chemical oxidants and several AOPs for the treatment of municipal wastewater with regard to levels of total coliforms, faecal coliforms and *E. coli*. It was found that 60 s of UV irradiation (in combination with H_2O_2 at a concentration of 112 mg.L^{-1}) resulted in a 87 – 93% decrease in the time required to achieve 99.9% reduction of the mentioned pathogens (Rizvi *et al*., 2013). A LP mercury vapour lamp emitting light at an intensity of 5 mW.cm^{-2} was used to perform the disinfection experiments. The researchers suggested that UV light was absorbed by an adequate dose of H_2O_2, resulting in the formation of hydroxyl radicals.

Referring to these studies, one can conclude that the combination of UV/H_2O_2 as AOP shows potential as effective disinfection treatment of microbiologically contaminated water. Furthermore, it has been proven that such treatments often result in improved disinfection in comparison to the use of UV light alone. It may, therefore, be valuable to assess the potential of UV/H_2O_2 combination treatments for the disinfection of river water at laboratory-scale. Such experiments would consider the influence of different water quality parameters and will provide insight as to whether or not the technique could present increased disinfection of river water on a larger scale in comparison to UV light alone.

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2.9. CONCLUDING REMARKS

As is indicated by several researchers, South African rivers, and particularly those in the Western Cape, are extensively polluted with faecal matter and carry high microbial loads (Paulse *et al.*, 2009; Gemmell & Schmidt, 2010; Huisamen, 2012; Britz *et al.*, 2013). Consequently, these waters are often reported to be unsuitable for the purpose of fresh produce irrigation, as limits proposed by DWAF (1996) and WHO (1989) are frequently exceeded. Should contaminated surface water be used for agricultural irrigation, however, significant risks of produce contamination and subsequent foodborne disease outbreaks become a reality. As a matter of fact, microbiologically polluted irrigation water has been identified as key source of fresh produce contamination (Warriner *et al.*, 2009; Pachepsky *et al.*, 2011). In this regard, decontamination, or disinfection, of river water prior to its use for agricultural irrigation is fundamental in minimising the risk of disease outbreaks following the consumption of such products. Thus, it is relevant to investigate the use of different disinfection techniques in order to be able to make recommendations as to which processes are capable of effectively disinfecting river water.

Traditional methods of surface water treatment include chemical disinfection (chlorine, ozone etc.) as well as physical processes such as filtration. However, due to the complexity and variability of surface water properties not all methods are equally suitable for disinfection purposes (Jones *et al.*, 2014). Water properties including pH, dissolved solids content, turbidity and colour, for instance, may influence disinfection efficiency and are subject to change on account of human interference or environmental events. Furthermore, disadvantages such as the formation of harmful disinfection by-products (DBPs) and the extreme resistance of some microorganisms toward certain chemicals have been reported (Momba *et al.*, 2008; Voigt *et al.*, 2013). Also, when chemical disinfectants are used additional risks to handlers thereof, as well as the environment, are introduced (Yiasoumi *et al.*, 2005).

As an alternative method of water disinfection, UV light irradiation is now a well-established and frequently encountered technique. Its benefits are well-documented too and include convenience factors, small requirements of space, general safety and the absence of chemical odours (Xu *et al.*, 2015). Regarding the operation and efficiency of UV systems, short contact times and its effectiveness against resistant microorganism such as *Cryptosporidium* and *Giardia* are advantageous (Hijnen *et al.*, 2006; Bolton & Cotton, 2008a). Nevertheless, the increased popularity of UV disinfection and technological progression within the industry constantly encourage research regarding challenges and improvement of the process.

Several researchers have investigated UV disinfection and damage-repair mechanisms, most often using laboratory-cultured, reference *E. coli* strains and sterile water or saline solutions. However, microorganisms occurring in the environment may present increased resistance to disinfection methods such as UV irradiation. While laboratory-scale experiments are important for the establishment of baseline process parameters, it is of critical importance to investigate UV

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disinfection on larger scale against a naturally occurring microbial population. As the process has also been shown to be influenced by water quality, it is particularly important to suggest process parameters based on studies performed in samples representative of the water to be disinfected. Adding to this, damage-repair should be considered following irradiation of the same samples as photo and dark-repair potential may differ with water quality and will vary between different microorganisms present in the water.

Globally, UV irradiation is often used for the disinfection of pre-treated wastewater, whereas in South Africa its application is fairly rare. Thus, although literature on the efficiency of wastewater disinfection is available, the lethality of UV irradiation may be different in river water used for irrigation. Adequate control and process measures can only be suggested once all of the above mentioned influential factors were taken into account in UV disinfection investigations. Conclusively, the absence of standardised protocols for UV disinfection and photoreactivation experiments contributes great variation in results found in literature. Therefore, the current study was performed to be able to make accurate and trustworthy recommendations regarding the use of UV light for the disinfection of South African river water.

REFERENCES

ABS (Australian Bureau of Statistics) (2010). *Water account, Australia, 2008-09*. Australian Bureau of Statistics, Canberra. Pp. 1-103.

Ackerman, A. (2010). *Assessment of microbial loads of the Plankenburg and Berg Rivers and the survival of Escherichia coli on raw vegetables under laboratory conditions*. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Acher, A., Fischer, E., Turnheim, R. & Manor Y. (1997). Ecologically friendly wastewater disinfection techniques. *Water Research*, **31**(6), 1398-1404.

Alkan, U., Teksoy, A., Atesli, A. & Başkaya, H.S. (2007). Efficiency of the UV/H_2O_2 process for the disinfection of humic surface waters. *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering*, **42**(4), 497-506.

Arnal, J.M., Garcia-Fayos, B., Verdu, G. & Lora, J. (2009). Ultrafiltration as an alternative membrane technology to obtain safe drinking water from surface water: 10 years of experience on the scope of the AUQAPOT project. *Desalination*, **248**, 34-41.

Aruscavage, D., Lee, K., Miller, S. & LeJeune, J.T. (2006). Interactions affecting the proliferation and control of human pathogens on edible plants. *Journal of Food Science*, **71**, 89-99.

AWWA (American Water Works Association) (2014). Overview of water disinfection. [Internet document]. URL https://awwoa.ab.ca/pdfs/UVDisinfection.pdf. Accessed 02/06/2014.

Backeberg, G.R., Bembridge, T.J., Bennie, A.T.P., Groenewald, J.A., Hammes, P.S., Pullen, R.A. & Thompson, H. (1996). *Policy proposal for irrigated agriculture in South Africa*. WRC Report no. KV96/96, Pretoria, South Africa.

Backeberg, G.R. (2006). Reform of user charges, market pricing and management of water: problem or opportunity for irrigated agriculture? *Irrigation and Drainage*, **55**, 1-12.

Baker, R.W. (2004). Overview of membrane science and technology. In: *Membrane Technology and Applications*, 2nd ed. Pp. 1-14. England: John Wiley and Sons.

Barnes, J.M. (2003). *The impact of water pollution from formal and informal urban developments along the Plankenburg River on water quality and health risk*. PhD Thesis in Food Science, University of Stellenbosch, South Africa.

Barnes, J.M. & Taylor, M.B. (2004). *Health risks assessment in connection with the use of microbiologically contaminated source waters for irrigation*. WRC Report no. 1226/1/04, Pretoria, South Africa.

Baxter, C.S., Hoffman, R., Templeton, M.R., Brown, M. & Andrews, R. (2007). Inactivation of adenovirus 2, 5, and 41 in drinking water by UV light, free chlorine, and monochloramine. *Journal of Environmental Engineering*, **133**(1), 95-103.

Benjdia, A. (2012). DNA photolyases and SP lyase: structure and mechanism of light-dependent and independent DNA lyases. *Current Opinion in Structural Biology*, **22**(6), 711-720.

Beuchat, L.R. (1996). Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection*, **59**, 204-206.

Beuchat, L.R. (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes and Infection*, **4**, 413-423.

Beuchat, L.R. (2006). Vectors and conditions for pre-harvest contamination of fruit and vegetables with pathogens capable of causing enteric diseases. *British Food Journal*, **108**, 38-53.

Beuchat, L.R. & Ryu, J. (1997). Produce handling and processing practices. *Emerging Infectious Diseases*, **3**, 1-9.

Bhunia, A.K. (2008). Chapter 10: *Escherichia coli*. In: *Foodborne Microbial Pathogens: Mechanisms and Pathogenesis*. Pp. 183-200. New York: Springer Science+Business Media.

Bichara, M., Meier M., Wagner, J., Cordonnier, A. & Lambert, I.B. (2011). Postreplication repair mechanisms in the presence of DNA adducts in *Escherichia coli*. *Mutation Research/Reviews in Mutation Research*, **727**(3), 104-122.

Bitton, G. (2005). Public health microbiology. In: *Wastewater Microbiology,* 3rd ed. (edited by R. Mitchell). Pp. 107-210. Hoboken: John Wiley & Sons.

Block, S.S. (1991). Peroxygen compounds. In: *Disinfection, Sterilisation and Preservation*, 4th ed. (edited by S.S. Block). Pp. 167-181. Philadelphia: Lea & Febiger.

Block, S.S. (2001). Peroxygen compounds. In: *Disinfection, Sterilisation and Preservation*, 5th ed. Pp. 185-187. Philadelphia: Lippincott Williams & Wilkins.

Bohrerova, Z. & Linden, K.G. (2006). Assessment of DNA damage and repair in *Mycobacterium terrae* after exposure to UV irradiation. *Journal of Applied Microbiology*, **101**(5), 995-1001.

Stellenbosch University https://scholar.sun.ac.za

Bolton, J.R. & Cotton, C.A. (2008a). Introduction. In: *The Ultraviolet Disinfection Handbook* (edited by M. Valentine). Pp. 1-9. USA: American Water Works Association (AWWA).

Bolton, J.R. & Cotton, C.A. (2008b). Fundamentals of UV Light and Photochemistry. In: *The Ultraviolet Disinfection Handbook* (edited by M. Valentine). Pp. 11-23. USA: American Water Works Association (AWWA).

Bolton, J.R. & Cotton, C.A. (2008c). Mechanism of UV Disinfection. In: *The Ultraviolet Disinfection Handbook* (edited by M. Valentine). Pp. 25-40. USA: American Water Works Association (AWWA).

Bolton, J.R. & Cotton, C.A. (2008d). UV Disinfection Equipment. In: *The Ultraviolet Disinfection Handbook* (edited by M. Valentine). Pp. 49-62. USA: American Water Works Association (AWWA).

Bolton, J.R. & Cotton, C.A. (2008e). Effects of water quality on UV disinfection. In: *The Ultraviolet Disinfection Handbook* (edited by M. Valentine). Pp. 77-86. USA: American Water Works Association (AWWA).

Brahmi, M., Belhadi, N.H., Hamdi, H. & Hassen, A. (2010). Modeling of secondary treated wastewater disinfection by UV irradiation: effects of suspended solids content. *Journal of Environmental Sciences,* **22**(8), 1218-1224.

Brandl, M.T. (2006). Fitness of human enteric pathogens on plants and implications for food safety. *Annual Review of Phytopathology*, **44**, 367-392.

Bridle, H. (2014). Overview of waterborne pathogens. In: *Waterborne Pathogens: Detection Methods and Applications* (edited by H. Bridle). Pp. 9-38. London: Academic Press.

Britz, T.J., Sigge, G.O., Buys, E.M., Schmidt, S., Potgieter, N. & Taylor M.B. (2012). Baseline study on extent (types and quantities) of contamination found in irrigation water at selected sites (K5/1773). In: *Quantitative investigation into the link between irrigation water quality and food safety* (Volume 2). WRC Report no. K5/1773/4, Pretoria, South Africa.

Britz, T.J., Sigge, G.O., Huisamen, N., Kikine, T., Ackermann, A., Lötter, M., Lamprecht, C. & Kidd, M. (2013). Fluctuations of indicator and index microbes as indication of pollution over three years in the Plankenburg and Eerste Rivers, Western Cape, South Africa. *Water SA*, **39**(4), 457-465.

Burns, N.L. (2010). Chapter 15: Ozone. In: *White's Handbook of Chlorination and Alternative Disinfectants*, 5th ed. (edited by C. Wallis-Lage). Pp. 767-848. USA: John Wiley & Sons.

Campos, C. (2008). New perspectives on microbiological water control for wastewater reuse. *Desalination*, **218**, 34-42.

Cantwell, R.E. & Hoffman, R. (2008). Inactivation of indigenous coliform bacteria in unfiltered surface water by ultraviolet light. *Water Research*, **42**(10-11), 2729-2735.

Carlos, C., Pires, M.M., Stoppe, N.C., Hachich, E.M., Sato, M.I.Z., Gomes, T.A.T., Amaral, L.A. & Ottoboni, L.M.M. (2010). *Escherichia coli* phylogenetic group determination and its

Stellenbosch University https://scholar.sun.ac.za

application in the identification of the major animal source of fecal contamination. *BioMed Central Microbiology*, **10**(161), 1471-2180.

CDC (Centers for Disease Control and Prevention) (2010). Investigation update: multistate outbreak of human *E. coli* O145 infections linked to shredded romaine lettuce from a single processing facility. [Internet document]. URL http://www.cdc.gov/ecoli /2010/ecoli_o145/index.html. Accessed 26/03/2014.

CDC (Centers for Disease Control and Prevention) (2011a). Investigation update: multistate outbreak of human *Salmonella* I 4, [5], 12:i:- infections linked to alfalfa sprouts. [Internet document]. URL http://www.cdc.gov/salmonella/i4512i-/021011/index.html. Accessed 26/03/2014.

CDC (Centers for Disease Control and Prevention) (2011b). Multistate outbreak of Listeriosis linked to whole cantaloupes from Jensen farms, Colorado. [Internet document]. URL http://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/120811/index.html. Accessed 26/03/2014.

CDC (Centers for Disease Control and Prevention) (2013a). Multistate outbreak of shiga toxin-producing *Escherichia coli* O157:H7 infections linked to ready-to-eat salads (final update). [Internet document]. URL http://www.cdc.gov/ecoli/2013/o157h7-11-13/index.html. Accessed 08/06/2014.

CDC (Centers for Disease Control and Prevention) (2013b). Multistate outbreak of *Salmonella* Saintpaul infections linked to imported cucumbers (final update). [Internet document]. URL http://www.cdc.gov/salmonella/saintpaul-04-13/. Accessed 08/06/2014.

Chang, J.C.H., Ossof, S.F., Lobe, D.C., Dorfman, M.H., Dumais, C.M., Qualls, R.G. & Johnson, J.D. (1985). UV inactivation of pathogenic and indicator micro-organisms. *Applied and Environmental Microbiology*, **49**(6), 1361-1365.

Cheigh, C-I., Park, M-H., Chung, M-S., Shin, J.K. & Park, Y-S. (2012). Comparison of intense pulsed light- and ultraviolet (UVC)-induced cell damage in *Listeria monocytogenes* and *Escherichia coli* O157:H7. *Food Control*, **25**(2), 654-659.

Cheremisinoff, N.P. (2002). Membrane separation technologies. In: *Handbook of Water and Wastewater Treatment Technologies*. Pp. 335-371. USA: Butterworth-Heinemann.

Chevrefils, G., Caron, É., Wright, H. & Sakamoto, G. (2006). UV dose required to achieve incremental log inactivation of bacteria, protozoa and viruses. *IUVA News*, **8**(1), 38-45.

Child, M., Strike, P., Pickup, R. & Edwards, C. (2002). *Salmonella typhimurium* displays cyclical patterns of sensitivity to UV-C killing during prolonged incubation in the stationery phase of growth. *FEMS Microbiology Letters,* **213**(1), 81-85.

Childress, A.E., Le-clech, P., Daugherty, J.L., Chen, C. & Leslie, G.L. (2005). Mechanical analysis of hollow fiber membrane integrity in water reuse applications. *Desalination*, **80**, 5-14.

Christensen, J. & Linden, K.G. (2003). How particles affect UV light in the UV disinfection of unfiltered drinking water. *Journal American Water Works Association*, **95**(4), 179-189.

Stellenbosch University https://scholar.sun.ac.za

Craik, S.A., Finch, G.R., Bolton, J.R. & Belosevic, M. (2000). Inactivation of *Giardia muris* cysts using medium-pressure ultraviolet radiation in filtered drinking water. *Water Research*, **34**(18), 4325-4332.

Craik, S.A., Weldon, D., Finch, G.R., Bolton, J.R. & Belosevic, M. (2001). Inactivation of *Cryptosporidium parvum* oocysts using medium- and low-pressure ultraviolet radiation. *Water Research*, **35**(6), 1387-1398.

Crebelli, R., Conti, L., Monarca, S., Feretti, D., Zerbini, I., Zani, C., Veschetti, E., Cutilli, D. & Ottaviani, M. (2005). Genotoxicity of the disinfection by-products resulting from peracetic acid- or hypochlorite-disinfected sewage wastewater. *Water Research*, **39**(6), 1105-1113.

CSPI (Center for Science in the Public Interest) (2009). *Outbreak Alert. Analysing foodborne outbreaks 1998 to 2007*. Center for Science in the Public Interest, Washington, DC. Pp. 1-23.

DAFF (Department of Agriculture, Forestry and Fisheries) (2013). *Economic Review of the South African Agriculture 2012/13*. The Government Printer. Pretoria, South Africa.

Dai, T., Vrahas, M.S., Murray, C.K. & Hamblin, M.R. (2012). Ultraviolet C irradiation: an alternative antimicrobial approach to localized infections? *Expert Review of Anti-infective Therapy*, **10**(2), 185-195.

Dallongeville, J., Dauchet, L., de Mouzon, O., Réquillart, V. & Soler, LG. (2010). Increasing fruit and vegetable consumption: a cost-effective analysis of public policies. *European Journal of Public Health*, **21**(1), 69-73.

Davey, J. & Schäfer, A.I. (2009). Ultrafiltration to supply drinking water in international development: a review of opportunities. In: *Appropriate Technologies for Environmental Protection in the Developing World* (edited by E.K. Yanful). Pp. 151-168. Dordrecht: Springer.

De Fraiture, C., Wichelns, D., Rockström, J., Kemp-Benedict, E., Eriyagama, N., Gordon, L.J., Hanjra, M.A., Hoogeveen, J., Huber-Lee, A. & Karlberg, L. (2007). Looking ahead to 2050: scenarios of alternative investment approaches. In: *Water for Food, Water for Life: A Comprehensive Assessment of Water Management in Agriculture* (edited by D. Molden). Pp. 91-145. Colombo: International Water Management Institute.

De Waal, C.S. & Bhuiya, F. (2009). Outbreaks by the numbers: fruits and vegetables 1990-2005. [Internet document]. URL http://www.cspinet.org/foodsafety/IAFPPoster.pdf. Accessed 23/03/2014.

DEAT (Department of Environmental Affairs and Tourism) (2006). *South Africa Environment Outlook. A report on the state of the environment*. Department of Environmental Affairs and Tourism, Pretoria. p. 371.

DEAT (Department of Environmental Affairs and Tourism) (2011). *Environmental Sustainability Indicators, Technical Report*. Department of Environmental Affairs and Tourism, Pretoria. p. 185.

Stellenbosch University https://scholar.sun.ac.za

Dell´Erba, A., Falsanisi, D., Liberti, L., Notarnicola, M. & Santoro, D. (2007). Disinfection by-products formation during wastewater disinfection with peracetic acid. *Desalination*, **215**(1-3), 177-186.

Doyle, M.P. & Erickson, M.C. (2008). Summer meeting 2007 - the problems with fresh produce: an overview. *Journal of Applied Microbiology*, **105**(2), 317-330.

Douki, T., Setlow, B. & Setlow, P. (2005). Effects of the binding of alpha/beta-type small, acid-soluble spore proteins on the photochemistry of DNA in spores of *Bacillus subtilis* and in vitro. *Photochemistry and Photobiology,* **81**(1), 163-169

Douki, T. (2013). The variety of UV-induced pyrimidine dimeric photoproducts in DNA as shown by chromatographic quantification methods. *Photochemical and Photobiological Sciences,* **12**(8), 1286-1302.

Duffy, E.A., Lucia, L.M., Kells, J.M., Castillo, A., Pillai, S.D. & Acuff, G.R. (2005). Concentration of *E. coli* and genetic diversity and antibiotic resistance profiling of *Salmonella* isolated from irrigation water, packing shed equipment, and fresh produce in Texas. *Journal of Food Protection*, **68**(1), 70-79.

DWAF (Department of Water Affairs and Forestry) (1996). *South African Water Quality Guidelines*, 2nd ed. (edited by S. Holmes). *Volume 4: Agricultural Water Use: Irrigation*. Pp. 71-76. The Government Printer. Pretoria, South Africa.

DWAF (Department of Water Affairs and Forestry) (2004). *National Water Resource Strategy*, 1st ed. Department of Water Affairs and Forestry, Pretoria.

DWA (Department of Water Affairs) (2013). *National Water Resource Strategy*, 2nd ed. Department of Water Affairs, Pretoria.

EADP (Department of Environmental Affairs and Development Planning) (2013). *State of Environment Outlook Report for the Western Cape Province. Inland Water Chapter - For Public Comment*. Western Cape Government, Environmental Affairs and Development Planning, Cape Town. p. 2.

EFSA (European Food Safety Authority) (2011). *Shiga toxin-producing E. coli (STEC) O104:H4 2011 outbreaks in Europe: taking stock*. European Food Safety Authority, Parma. Pp. 1-22.

Eischeid, A.C. & Linden, K.G. (2007). Efficiency of pyrimidine dimer formation in *Escherichia coli* across UV wavelengths. *Journal of Applied Microbiology*, **103**(5), 1650-1656.

Fane, A.G.T., Wang, R. & Jia, Y. (2008). Membrane technology: past, present and future. In: *Handbook of Environmental Engineering: Membrane and Desalination Technologies*, Vol. 13. (edited by L.K. Wang, J.P. Chen, Y-T, Hung & N.K. Shammas). Pp. 1-45. Totowa: Humana Press.

FAO (Food and Agricultural Organization of the United Nations) (2005). *Irrigation in Africa in figures – AQUASTAT Survey 2005*. Food and Agricultural Organization of the United Nations, Rome. Pp. 1-12.

Stellenbosch University https://scholar.sun.ac.za

FDA (Food and Drug Administration) (1998). *Guidance for industry: guide to minimize microbial food safety hazards for fresh fruit and vegetables*. U.S. Food and Drug Administration, Silver Spring. Pp. 1-43.

Figueras, M.J. & Borrego, J.J. (2010). New perspectives in monitoring drinking water microbial quality. *International Journal of Environmental Research and Public Health*, **7**(12), 4179-4202.

Forney, L. (2008). Advances in disinfection techniques for water reuse. In: *Handbook of Water and Energy Management in Food Processing* (edited by J. Klemeš, R. Smith & J-K. Kim). Pp. 700-719. Cambridge: Woodhead Publishing Limited.

Freese, S.D. & Nozaic, D.J. (2004). Chlorine: Is it really so bad and what are the alternatives? *Water SA*, **30**(5), 18-24.

Freese, S.D., Bailey, I. & Nozaic, D. (2003). *The evaluation of alternative disinfection processes for the removal of protozoan oocysts and cysts and other micro-organisms, in the treatment of final wastewater*. WRC Report no. 1030/1/03, Pretoria, South Africa.

Friedberg, E.C., Walker, G.C., Siede, W., Wood, R.D., Schultz, R.A. & Ellenberger, T. (2006). DNA damage. In: *DNA Repair and Mutagenesis*, 2nd ed. Pp. 9-57. USA: ASM Press.

Gayán, E., Condón, S. & Álvarez, I. (2014). Biological aspects in food preservation by ultraviolet light: a review. *Food and Bioprocess Technology*, **7**, 1-20.

Gayán, E., Monfort, S., Álvarez, I. & Condón, S. (2011). UV-C inactivation of *Escherichia coli* at different temperatures. *Innovative Food Science and Emerging Technologies*, **12**(4), 531-541.

Gemmell, M.E. & Schmidt, S. (2010). Potential links between irrigation water quality and microbiological quality of food in subsistence farming in KwaZulu-Natal, South Africa. *Applied Microbiology and Microbial Biotechnology*, **2**, 1190-1194.

Gerba, C.P., Gramos, D.M. & Nwachuku, N. (2002). Comparative inactivation of enteroviruses and adenovirus 2 by UV light. *Applied and Environmental Microbiology*, **68**(10), 5167-5169.

Gómez-López, V.M. (2012). Selected techniques to decontaminate minimally processed vegetables. In: Progress in Food Preservation (edited by R. Bhat., A.K. Alias & G. Paliyath). Pp. 3-14. West Sussex: Wiley & Blackwell Publishers.

Gordon, D.M., Clermont, O., Tolley, H. & Deamur, E. (2008). Assigning *Escherichia coli* strains to phylogenetic groups: Multi-locus sequence versus the PCR triplex method. *Environmental Microbiology*, **10**(10), 2484-2496.

Greig, J.D. & Ravel, A. (2009). Analysis of foodborne outbreak data reported internationally for source attribution. *International Journal of Food Microbiology*, **130**(2), 77-87.

Gulati, M., Jacobs, I., Jooste, A., Naidoo, D. & Fakir, S. (2013). The water-energy-food security nexus: Challenges and opportunities for food security in South Africa. *Aquatic Procedia*, **1**, 150-164.

Stellenbosch University https://scholar.sun.ac.za

Guo, M., Hu, H., Bolton, J.R. & El-Din, M.G. (2009). Comparison of low- and medium-pressure ultraviolet lamps: photoreactivation of *Escherichia coli* and total coliforms in secondary effluents of municipal wastewater treatment plants. *Water Research*, **43**(3), 815-821.

Guo, M., Huang, J., Hu, H. & Liu, W. (2011). Growth and repair potential of three species of bacteria in reclaimed wastewater after UV disinfection. *Biomedical and Environmental Sciences*, **24**(4), 400-407.

Guo, M., Huang, J., Hu, H., Liu, W. & Yang, J. (2012). UV inactivation and characteristics after photoreactivation of *Escherichia coli* with plasmid: health safety concern about UV disinfection. *Water Research*, **46**(13), 4031-4036.

Halliwell, B. & Gutteridge, J.M.C. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal*, **219**, 1-14.

Hallmich, C. & Gehr, R. (2010). Effect of pre- and post-UV disinfection conditions on photoreactivation of fecal coliforms in wastewater effluents. *Water Research*, **44**, 2885-2893.

Hanjra, M.A. & Qureshi, M.E. (2010). Global water crisis and future food security in an era of climate change. *Food Policy*, **35**, 365-377.

Harris, L.J., Farber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H. & Busta, F.F. (2003). Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety*, **2**, 78-86.

Harwood, V.J., Levine, A.D., Scott, T.M., Chivukula, V., Lukasik, J., Farrah, S.R. & Rose, J.B. (2005). Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. *Applied and Environmental Microbiology,* **71**(6), 3163-3170.

Hendricks, D.W. (2006). Slow Sand Filtration. In: *Water Treatment Unit Processes: Physical and Chemical* (edited by Taylor & Francis Group). Pp. 661-662. CRC Press: New York.

Henry, V., Helbronner, A. & Recklinghausen, M. (1910). Nouvelles recherches sur la sterilization de grandes quantited d'eau par les rayons ultraviolets. *Comptes rendus de l'Académie des sciences*, **151**, 677-680.

Hijnen, W.A.M., Beerendonk, E.F. & Medema, G.J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Research*, **40**(1), 3-22.

Hijnen, W.A.M., Dullemont, Y.J., Schijven, J.F., Hanzens-Brouwer, A.J., Rosielle, M. & Medema, G. (2007). Removal and fate of *Cryptosporidium parvum*, *Clostridium perfringens* and small-sized centric diatoms (*Stephanodiscus hantzschii*) in slow sand filters. *Water Research*, **41**, 2151-2162.

Hjertqvist, M., Johansson, A., Svensson, N., Abom, P.E., Magnusson, C., Olsson, M., Hedlund, K.O. & Andersson, Y. (2006). Four outbreaks of norovirus gastroenteritis after consuming

Stellenbosch University https://scholar.sun.ac.za

raspberries, Sweden, June-August 2006. [Internet document]. URL http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3038. Accessed 09/06/2014.

Holley, R.A., Arrus, K.M., Ominski, K.H., Tenuta, M. & Blank, G. (2006). *Salmonella* survival in manure-treated soils during simulated seasonal temperature exposure. *Journal of Environmental Quality*, **35**, 1170-1180.

Howe, K.J., Hand, D.W., Crittenden, J.C., Trussell, R.R. & Tchobanoglous, G. (2012). Disinfection. In: *Principles of Water Treatment.* Pp. 525-581.

Hoyer, O. (1998). Testing performance and monitoring of UV systems for drinking water disinfection. *Water Supply*, **16**(1/2), 424-429.

Hoyer, O. (2004). Water disinfection with UV radiation – requirements and realization. In: *Proceedings of the European Conference UV Karlsruhe, UV radiation. Effects and Technologies.* September 2003. Karlsruhe, Germany.

Hu, J., Chu, X.N., Quek, P.H., Feng, Y.Y. & Tan, X.L. (2005). Repair and regrowth of *Escherichia coli* after low- and medium-pressure ultraviolet disinfection. *Water Science and Technology: Water Supply*, **5**(5), 101-108.

Huang, J., Meador, D.P., Fisher, P.R., Decio, D.B. & Horner, W.E. (2011). Disinfectant chemicals to control waterborne pathogens are deactivated by peat particles in irrigation water. *Proceedings of the Florida State Horticultural Society*, **124**, 289-293.

Hugo, H.J. & Malan, A.P. (2006). *Literature Study on Control of Nematodes in Irrigation Water.* ARC Project no. 230063, Stellenbosch, South Africa.

Huisamen, N. (2012). *Assessment of microbial levels in the Plankenburg and Eerste Rivers and subsequent carry-over to fresh produce using source tracking as indicator*. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Huisman, L. & Wood, W.E. (1974). *Slow Sand Filtration*. Geneva: World Health Organization (WHO).

Ijabadeniyi, O.A. & Buys, E.M. (2012). Irrigation water and microbiological safety of fresh produce; South Africa as a case study: a review. *African Journal of Agricultural Research*, **7**(35), 4848-4857.

Ijabadeniyi, O.A., Debusho, L.K., Van der Linde, M. & Buys, E.M. (2011). Irrigation water as a potential preharvest source of bacterial contamination of vegetables. *Journal of Food Safety*, **31**, 452-461.

Ijpelaar, G.F., Harmsen, D.J.H., Beerendonk, E.F., Van Leerdam, R.C., Metz, D.H., Knol, A.H., Fulmer, A. & Krijnen, S. (2010). Comparison of low pressure and medium pressure UV lamps for UV/H_2O_2 treatment of natural waters containing micro pollutants. *Ozone: Science & Engineering,* **32**(5), 329-337.

Ingerson-Mahar, M. & Reid, A. (2011). *FAQ: E. coli: good, bad & deadly*. American Academy of Microbiology, Washington, DC. Pp. 1-13.

Stellenbosch University https://scholar.sun.ac.za

Jacangelo, J.G. & Noack, R.K. (2005). System concepts. In: *Microfiltration and Ultrafiltration Membranes for Drinking Water*, 1st ed. (edited by M. Christensen). Pp. 101-146. USA: American Water Works Association (AWWA).

Jagals, P., Traore, H.N. & Barnard, T.G. (2006). *Inflammatory potential measurement as a supplement to health-related microbial water-quality assessment*. WRC Report no. 1444/1/06, Pretoria, South Africa.

James, J. (2006). Overview of microbiological hazards in fresh fruit and vegetable operations. In: *Microbial Hazard Identification in Fresh Fruits and Vegetables* (edited by J. James). Pp. 1-36. New Jersey: John Wiley & Sons.

Jamieson, R., Gordon, R., Joy, D. & Lee, H. (2004). Assessing microbial pollution of rural surface waters: a review of current watershed scale modelling approaches. *Agricultural Water Management*, **70**, 1-17.

Johnston, L.M., Moe, C.L., Moll, D. & Jaykus, L. (2006). The epidemiology of produce-associated outbreaks of food borne disease. In: *Microbial Hazard Identification in Fresh Fruits and Vegetables* (edited by J. James). Pp. 38-52. New Jersey: John Wiley and Sons.

Jones, L.A., Worobo, R.W. & Smart, C.D. (2014). UV light inactivation of human and plant pathogens in unfiltered surface irrigation water. *Applied and Environmental Microbiology*, **80**(3), 849-854.

Joshi, K., Mahendran, R., Alagusundaram, T., Norton, T. & Tiwari, B.K. (2013). Novel disinfectants for fresh produce. *Trends in Food Science & Technology*, **34**(1), 54-61.

Kajitvichyanukul, P., Shammas, N.K., Hung, Y., Wang, L.K. & Ananpattarachai, J. (2011). Potable water biotechnology, membrane filtration and biofiltration. In: *Handbook of Environmental Engineering: Membrane and Desalination Technologies*, Vol. 13. (edited by L.K. Wang, J.P. Chen, Y-T. Hung & N.K. Shammas). Pp. 1-45. Totowa: Humana Press.

Kalisvaart, B.F. (2004). Re-use of wastewater: preventing the recovery of pathogens by using medium-pressure UV lamp technology. *Water Science and Technology*, **50**(6), 337-344.

Kalisvaart, B.F. (2011). Comparison of dark- and lightrepair tendencies between low and medium pressure UV lamps. [Internet document]. URL http://www.bestuv.com/wp-content/uploads/2012/05/Comparison-of-microbiological-effects-between-LP-and-MP-lamps-2012.pdf. Accessed 18/05/2014.

Kämpfer, P., Nienhüser, A., Packroff, G., Wernicke, F., Mehling, A., Nixdorf, K., Fiedler, S., Kolauch, C. & Esser, M. (2008). Molecular identification of coliform bacteria isolated from drinking water reservoirs with traditional methods and the Colilert-18 system. *International Journal of Hygiene and Environmental Health*, **211**(3-4), 374-384.

Kaper, J.B., Nataro, J.P. & Mobley, H.L.T. (2004). Pathogenic *Escherichia coli*. *Nature Reviews*, **2**, 123-140.

Stellenbosch University https://scholar.sun.ac.za

Kirk, M.D., Fullerton, K. & Gregory, J. (2008). Fresh produce outbreaks in Australia 2001-2006. Board 21. In: *2008 International Conference on Emerging Infectious Diseases Program and Abstracts*. Pp. 49-50. March 2008. Atlanta, Georgia.

Kitis, M. (2004). Disinfection of wastewater with peracetic acid: a review. *Environment International*, **30**(1), 47-55.

Knudson, G.B. (1985). Photoreactivation of UV irradiated *Legionella pneumophila* and other *Legionella* species. *Applied and Environmental Microbiology*, **49**(4), 975-980.

Koivunen, J. & Heinonen-Tanski, H. (2005a). Peracetic acid (PAA) disinfection of primary, secondary and tertiary treated municipal wastewaters. *Water Research*, **39**(18), 4445-4453.

Koivunen, J. & Heinonen-Tanski, H. (2005b). Inactivation of enteric microorganisms with chemical disinfectants, UV irradiation and combined chemical/UV treatments. *Water Research*, **39**(8), 1519-1526.

Konieczny, K., Sakol, D., Plonka, J., Rajca, M. & Bodzek, M. (2009). Coagulation-ultrafiltration system for river water treatment. *Desalination*, **240**, 151-159.

Kotzé, M. (2015). *Investigating the efficacy of medium pressure UV and hydrogen peroxide as on-farm treatment methods to reduce the microbial load of irrigation water.* MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Koutchma, T., Forney, L.G. & Moraru, C.L. (2009a). Microbial inactivation by UV light. In: *Ultraviolet Light in Food Technology: Principles and Applications* (edited by D-W. Sun). Pp. 69-99. Boca Raton: CRC Press.

Koutchma, T., Forney, L.G. & Moraru, C.L. (2009b). Sources of UV light. In: *Ultraviolet Light in Food Technology: Principles and Applications* (edited by D-W. Sun). Pp. 33-51. Boca Raton: CRC Press.

Kowalski, W. (2009). Introduction. In: *Ultraviolet Germicidal Irradiation Handbook*. Pp. 1-13. New York: Springer.

Krtinic, G., Duric, P. & Illic, S. (2010). *Salmonellae* in food stuffs of plant origin and their implications on human health. *European Journal of Clinical Microbiology and Infectious Diseases*, **29**, 1321-1325.

Ksibi, M. (2006). Chemical oxidation with hydrogen peroxide for domestic wastewater treatment. *Chemical Engineering Journal,* **119**(2-3), 161-165.

Labas, M.D., Zalazar, C.S., Brandi, R.J. & Cassano, A.E. (2008). Reaction kinetics of bacteria disinfection employing hydrogen peroxide. *Biochemical Engineering Journal,* **38**(1), 78-87.

Langenbach, K., Kuschk, P., Horn, H. & Kästner, M. (2009). Slow sand filtration of secondary clarifier effluent for wastewater reuse. *Environmental Science Technology*, **43**, 5896-5901.

Lazarova, V. & Bahri, A. (2005). Code of practices for health protection. In: *Water Reuse for Irrigation: Agriculture, Landscapes and Turf Grass* (edited by V. Lazarova & A. Bahri). Pp. 83-102. USA: CRC Press.

Stellenbosch University https://scholar.sun.ac.za

LeChevallier, M.W. & Au, K.K. (2004). Inactivation (disinfection) Processes. In: *Water Treatment and Pathogen Control: Process Efficiency in Achieving Safe Drinking Water*. Pp. 41-65. London: IWA Publishing.

Lenntech (2014). Water treatment solutions. [Internet document]. URL http://www.lenntech.com/index.htm. Accessed 25/03/2014.

León, H.B., Gómez-Aldapa, C.A., Rangel-Vargas, E., Vázquez-Barrios, E. & Castro-Rosas, J. (2013). Frequency of indicator bacteria, *Salmonella* and diarrhoeagenic *Escherichia coli* pathotypes on ready-to-eat cooked vegetable salads from Mexican restaurants. *Letters in Applied Microbiology*, **56**, 414-420.

Linden, K.G., Shin, G., Faubert, G., Cairns, W. & Sobsey, M.D. (2002). UV Disinfection of *Giardia lamblia* cysts in water. *Environmental Science and Technology*, **36**(11), 2519-2522.

Linden, K.G., Thurston, J., Schaefer, R. & Malley, J.P. (2007). Enhanced UV inactivation of adenoviruses under polychromatic UV lamps. *Applied and Environmental Microbiology*, **73**(23), 7571-7574.

Lotierzo, M., Feliers, C., Faure, N., Le Grand, L., Saby, S. & Cervantes, P. (2003). Synergistic effects of sequential treatment by UV irradiation and chemical disinfectant for drinking water disinfection. In: *Proceedings of the 2nd International Congress on Ultraviolet Technologies 2003*. Pp. 217-224. July 2003. Vienna, Austria.

Lötter, M. (2010). *Assessment of microbial loads present in two Western Cape rivers used for irrigation of vegetables*. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Ludwig, A. (2014). Slow Sand Filtration. [Internet document]. URL http://oasisdesign.net/water/treatment/slowsandfilter.htm. Accessed 08/05/2014.

Luukkonen, T., Teeriniemi, J., Prokkola, H., Rämö, J. & Lassi, U. (2014). Chemical aspects of peracetic acid based wastewater disinfection. *Water SA*, **40**(1), 73-80.

Lynch, M.F., Tauxe, R.V. & Hedberg, C.W. (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology and Infection*, **137**, 307-315.

Malley, J.P., Ballester, N.A., Margolin, A.B., Linden, K.G., Mofidi, A., Bolton, J.R., Crozes, G., Laine, G.M. & Janex, M.L. (2004). Overview of pathogen inactivation with UV. In: *Inactivation of pathogens with innovative UV technologies*. Pp. 1-7. USA: AWWA Research Foundation and American Water Works Association.

Mamane, H., Shemer, H. & Linden, K.G. (2007). Inactivation of *E. coli*, *B. subtilis* spores, and MS2, T4 and T7 phage using UV/H_2O_2 advanced oxidation. *Journal of Hazardous Materials*, **146**(3), 479-486.

Martínes, S.B., Pérez-Parra, J. & Suay, R. (2011). Use of ozone in wastewater treatment to produce water suitable for irrigation. *Water Resource Management*, **25**, 2109-2124.

Stellenbosch University https://scholar.sun.ac.za

Matthews, K.R. (2006). Microorganisms associated with fruits and vegetables. In: *Microbiology of Fresh Produce* (edited by K.R. Matthews). Pp. 1-21. Washington DC: ASM Press.

McDonnell, G. & Russel, A.D. (1999). Antiseptics and disinfectants: activity, action, and resistance. *Clinical Microbiology Reviews*, **12**(1), 147-179.

Melidis, P., Athanasoulia, E., Parisis, A. & Koppmann, M. (2009). Microbiological effluent control by UV-disinfection of a small-scale SBR system. *Desalination*, **248**(1-3), 915-922.

Mezzanotte, V., Antonelli, M., Citterio, S. & Nurizzo, C. (2007). Wastewater disinfection alternatives: Chlorine, ozone, peracetic acid and UV light. *Water Environment Research*, **79**(12), 2373-2379.

Michino, H., Araki, K., Minami, S., Tayaka, S., Sakai, N., Ono, A. & Yanagawa, H. (1999). Massive outbreak of *Escherichia coli* O157:H7 infection in school children in Sakai City, Japan, associated with consumption of white radish sprouts. *American Journal of Epidemiology*, **150**, 787-796.

Mody, R.K., Greene, S.A., Gaul, L., Sever, A., Pichette, S., Zambrana, I., Dang, T., Gass, A., Wood, R., Herman, K., Cantwell, L.B., Falkenhorst, G., Wannemuehler, K., Hoekstra, R.M., McCullum, I., Cone, A., Franklin, L., Austin, J., Delea, K., Behravesh, C.B., Sodha, S.V., Yee, J.C., Emanuel, B., Al-Khaldi, S.F., Jefferson, V., Williams, I.T., Griffin, P.M. & Swerdlow, D.L. (2011). National outbreak of *Salmonella* serotype Saintpaul infections: Importance of Texas restaurant investigations in implicating jalapeño peppers. *PLoS ONE*, **6**(2), 1-7.

Moeller, R.T., Douki, T., Cadet, J., Stackebrandt, E., Nicholson, W.L., Rettberg, P., Reitz, G. & Horneck, G. (2007). UV-radiation-induced formation of DNA bipyrimidine photoproducts in *Bacillus subtilis* endospores and their repair during germination. *International Microbiology*, **10**(1), 39-46.

Molden, D. (2007). Water responses to urbanisation. *Paddy and Water Environment*, **5**(4), 207-209.

Molden, D., Oweis, T., Steduto, P., Bindraban, P., Hanjra, M.A. & Kijne, J. (2010). Improving agricultural water productivity: between optimism and caution. *Agricultural Water Management*, **97**(4), 528-535.

Molden, D., Oweis, T.Y., Steduto, P., Kijne, J.W., Hanjra, M.A., Bindraban, P.S., Bouman, B.A.M., Cook, S., Erenstein, O., Farahani, H., Hachum, A., Hoogeveen, J., Mahoo, H., Nangia, V., Peden, D., Sikka, A., Silva, P., Turral, H., Upadhyaya, A. & Zwart, S. (2007). Pathways for increasing agricultural water productivity. In: *Water for Food, Water for Life: A Comprehensive Assessment of Water Management in Agriculture* (edited by D. Molden). Pp. 279-306. Colombo: International Water Management Institute.

Momba, M.N.B., Obi, C.L. & Thompson, P. (2008). *Improving disinfection efficiency in small drinking water treatment plants*. WRC Report no. 1531/1/08, Pretoria, South Africa.

Stellenbosch University https://scholar.sun.ac.za

Müller, A., Stahl, M.R., Graef, V., Franz, C.M.A.P & Huch, M. (2011). UV-C treatment of juices to inactivate microorganisms using Dean vortex technology. *Journal of Food Engineering,* **107**(2), 268-275.

Nasser, A.M. (2005). Persistence of Pathogens in Water. In: *Water Encyclopedia: Domestic, Municipal, and Industrial Water Supply and Waste Disposal* (edited by J. Lehr, J. Keeley & J. Lehr). Vol. 1-5. Pp. 521-523. USA: John Wiley & Sons, Inc.

Nath, K. (2006). Ultrafiltration. In: *Membrane Separation Processes*. Pp. 101-129. New Delhi: Prentice-hall of India Private Limited.

Ndiame, D. & Jaffee, S.M. (2005). Fruits and Vegetables: Global trade and competition in fresh and processed product markets. In: *Global Agricultural Trade and Developing Countries* (edited by M.A. Askoy & J.C. Beghin). Pp. 237-257. Washington: World Bank.

Newman, S.E. (2004). Disinfecting irrigation water for disease management. In: *20th Annual Conference on Pest Management on Ornamentals*. Pp. 1-10. February 2004. San Jose, California.

Niehaus, A.J., Apalata, T., Coovadia, Y.M., Smith, A.M. & Moodley, P. (2011). An outbreak of foodborne salmonellosis in rural KwaZula-Natal, South Africa. *Foodborne Pathogens and Disease*, **8**(6), 693-697.

Nuorti, J.P., Niskanen, T., Hallanvuo, S., Mikkola, J., Kela, E., Hatakka, M., Fredriksson-Ahomaa, M., Lyytikainen, O., Siitonen, A., Korkeala, H. & Ruutu, P. (2004). A widespread outbreak of *Yersinia pseudotuberculosis* O:3 infection from iceberg lettuce. *Journal of Infectious Diseases*, **189**, 766-774.

Nwachuku, N. & Gerba, C.P. (2008). Occurrence and persistence of *Escherichia coli* O157:H7 in water. *Reviews in Environmental Science and Biotechnology*, **7**, 267-273.

Odonkor, S.T. & Ampofo, J.K. (2013). *Escherichia coli* as an indicator of bacteriological quality of water: an overview. *Microbiology Research*, **4**(2), 5-10.

Oguma, K., Katayama, H. & Ohgaki, S. (2002). Photoreactivation of *Escherichia coli* after low- or medium-pressure UV disinfection determined by an endonuclease sensitive site assay. *Applied and Environmental Microbiology*, **68**(12), 6029-6035.

Oguma, K., Katayama, H. & Ohgaki, S. (2004). Photoreactivation of *Legionella pneumophila* after inactivation by low- or medium-pressure ultraviolet lamp. *Water Research*, **38**(11), 2757-2763.

Okeke, I.N. & Nataro, J.P. (2001). Enteroaggregative *Escherichia coli*. *Infectious Diseases*, **1**(5), 304-313.

Olaimat, A.N. & Holley, R.A. (2012). Factors influencing the microbial safety of fresh produce: a review. *Food Microbiology*, **32**, 1-19.

Oteiza, J.M., Giannuzzi, L. & Zaritzky, N. (2010). Ultraviolet treatment of orange juice to inactivate *E. coli* O157:H7 as affected by native microflora. *Food and Bioprocess Technology,* **3**(4), 603-614.

Stellenbosch University https://scholar.sun.ac.za

Ozaki, H. (2004). Rejection of micropollutants by membrane filtration. In: *Regional Symposium on Membrane Science and Technology 2004*. April 2004. Johor, Malaysia.

Pachepsky, Y., Shelton, D.R., McLain, J.E.T., Patel, J. & Mandrell, D.E. (2011). Irrigation waters as a source of pathogenic microorganisms in produce: a review. *Advances in Agronomy*, **113**, 73-138.

Parajuli, P.B., Mankin, K.R. & Barnes, P.L. (2009). Source specific fecal bacteria modelling using soil and water assessment tool model. *Bioresource Technology*, **100**, 953-963.

Paulse, A.N., Jackson, V.A. & Khan, W. (2007). Comparison of enumeration techniques for the investigation of bacterial pollution in the Berg River, Western Cape, South Africa. *Water SA*, **33**(2), 165-173.

Paulse, A.N., Jackson, V.A. & Khan, W. (2009). Comparison of microbial contamination at various sites along the Plankenburg- and Diep Rivers, Western Cape, South Africa. *Water SA*, **35**(4), 469-478.

Percival, S., Chalmers, R., Embrey, M., Hunter, P., Sellwood, J. & Wyn-Jones, P. (2004). *Escherichia coli*. In: *Microbiology of Waterborne Diseases: Microbiological Aspects & Risk*. Pp. 71-90. New York: Academic Press.

Pereira, V.J., Weinberg, H.S., Linden, K.G. & Singer, P.C. (2007). UV degradation kinetics and modelling of pharmaceutical compounds in laboratory grade and surface water via direct and indirect photolysis at 254 nm. *Environmental Science and Technology,* **41**(5), 1682-1688.

Perret, S.R. (2002). Water policies and small holding irrigation schemes in South Africa: a history and new institutional challenges. *Water Policy*, **4**, 283-300.

Peter-Varbanets, M., Zurbrügg, C., Swartz, C. & Pronk, W. (2009). Decentralized systems for potable water and the potential of membrane technology. *Water Research*, **43**(2), 245-265.

Poepping, C., Beck, S., Wright, H. & Linden K.G. (2014). Evaluation of DNA damage reversal during medium-pressure UV disinfection. *Water Research*, **56**, 181-189.

Popkin, B.M., Adair, L.S. & Ng, S.W. (2011). Global nutrition transition and the pandemic of obesity in developing countries. *Nutrition Reviews*, **70**(1), 3-21.

Pu, S. (2009). *Effects of plant maturity and bacterial inoculum level on the surface contamination and internalization of Escherichia coli O157:H7 in growing spinach*. MSc in Food Science Thesis, Louisiana State University and Agricultural and Mechanical College, USA.

Punyani, S., Narayana, P., Singh, H. & Vasudevan, P. (2006). Iodine based water disinfection: a review. *Journal of Scientific and Industrial Research*, **65**, 116-120.

Quek, P.H. & Hu, J. (2008a). Indicators for photoreactivation and dark repair studies following ultraviolet disinfection. *Journal of Industrial Microbiology and Biotechnology*, **35**(6), 533-541.

Quek, P.H. & Hu, J. (2008b). Influence of photoreactivating light intensity and incubation temperature on photoreactivation of *Escherichia coli* following LP and MP UV disinfection. *Journal of Applied Microbiology*, **105**(1), 124-133.

Raffellini, S., Schenk, M., Guerrero, S. & Alzamora, S.M. (2011). Kinetics of *Escherichia coli* inactivation employing hydrogen peroxide at varying temperatures, pH and concentrations. *Food Control*, **22**, 920-932.

Rastogi, R.P., Richa, K.A., Tyagi, M.B. & Sinha, R.P. (2010). Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *Journal of Nucleic Acids*, **2010**, 1-32.

Raudales, R.E., Parke, J.L., Guy, C.L. & Fisher, P.R. (2014). Control of waterborne microbes in irrigation: a review. *Agricultural Water Management*, **143**, 9-28.

Renner, J. (2012). Global irrigated area at record levels, but expansion slowing. [Internet document]. URL http://www.worldwatch.org/global-irrigated-area-record-levels-expansion-slowing-0. Accessed 26/08/2014.

Richardson, S.D., DeMarini, D.M., Kogevinas, M., Fernandez, P., Marco, E., Lourencetti, C., Ballesté, C., Heederik, D., Meliefste, K., McKague, A.B., Marcos, R., Font-Ribera, L., Grimalt, J.O. & Villanueva, C.M. (2010). What's in the pool? A comprehensive identification of disinfection by-products and assessment of mutagenicity of chlorinated and brominated swimming pool water. *Environmental Health Perspectives*, **118**(11), 1523-1530.

Rizvi, H., Ahmad, N., Yasar, A., Bukhari, K. & Khan, H. (2013). Disinfection of UASB-treated municipal wastewater by H_2O_2, UV, ozone, PAA, H_2O_2/sunlight, and advanced oxidation processes: regrowth potential of pathogens. *Polish Journal of Environmental Studies,* **22**(4), 1153-1161.

Rodríguez, R.A., Bounty, S. & Linden, K.G. (2013). Long-range quantitative PCR for determining inactivation of adenovirus 2 by ultraviolet light. *Journal of Applied Microbiology,* **114**(6), 1854-1865.

Rossi, S., Antonelli, M., Mezzanotte, V. & Nurizzo, C. (2007). Peracetic acid disinfection: a feasible alternative to wastewater chlorination. *Water Environment Research*, **79**(4), 341-350.

Russo, T.A. & Johnson, J.R. (2003). Medical and economic impact of extraintestinal infections due to *Escherichia coli*: Focus on an increasingly important endemic problem. *Microbes and Infection*, **5**(5), 449-456.

Russo, T.A. & Johnson, J.R. (2009). Extraintestinal pathogenic *Escherichia coli*. In: *Vaccines for Biodefense and Emerging and Neglected Diseases* (edited by A.D.T. Barret & L.R. Stanberry). Pp. 939-961. London: Elsevier, Ltd.

SADC (South African Development Community) (2008). *Southern Africa Environment Outlook*. South African Development Community, Gaborone. Pp. 57-58.

Stellenbosch University https://scholar.sun.ac.za

Salgot, M., Folch, M., Huertas, E., Tapias, J., Avellaneda, D., Gíros, G., Brissaud, F., Vergés, C., Molina, J. & Pigem, J. (2002). Comparison of different advanced disinfection systems for wastewater reclamation. *Water Science and Technology: Water Supply,* **2**(3), 213-218.

Savichtcheva, O. & Okabe, S. (2006). Alternative indicators of fecal pollution: relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. *Water Research*, **40**, 2463-2476.

Scaletsky, I.C.A., Fabbricotti, S.H., Carvalho, R.L.B., Nunes, C.R., Maranhão, H.S., Morais, M.B. & Fagundes-Neto, U. (2002). Diffusely adherent *Escherichia coli* as a cause of acute diarrhea in young children in Northeast Brazil: A case-control study. *Journal of Clinical Microbiology*, **40**(2), 645-648.

Scharff, R.L. (2010). Health-related costs from foodborne illness in the United States. [Internet document]. URL http://publichealth.lacounty.gov/eh/docs/ReportPublication/HlthRelatedCostsFromFoodborneIllinessUS.pdf. Accessed 10/06/2014.

Schmalwieser, A.W., Wright, H., Cabaj, A., Heath, M., Mackay, E. & Schauberger, G. (2014). Aging of low-pressure amalgam lamps and UV dose delivery. *Journal of Environmental Engineering and Science*, **9**(2), 113-124.

Scott, T., Rose, J., Jenkins, T., Farrah, S. & Lukasik, J. (2002). Microbial source tracking: current methodology and future directions. *Applied and Environmental Microbiology*, **68**, 5796-5803.

Selma, M.V., Allende, A., López-Gálvez, F., Conesa, M.A. & Gil, M.I. (2008). Disinfection potential of ozone, ultraviolet-C and their combination in wash water for the fresh-cut vegetable industry. *Food Microbiology*, **25**, 809-814.

Setlow, P. (2006). Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat and chemicals. *Journal of Applied Microbiology*, **101**(3), 514-525.

Sherchan, S.P., Snyder, S.A., Gerba, C.P. & Pepper, I.L. (2014). Online monitoring of *Escherichia coli* and *Bacillus thuringiensis* spore inactivation after advanced oxidation treatment. *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering,* **49**(8), 933-939.

Siddiqui, M. (2005). Ultraviolet Irradiation. In: *Water Encyclopedia: Domestic, Municipal, and Industrial Water Supply and Waste Disposal* (edited by J. Lehr, J. Keeley & J. Lehr). Vol. 1-5. Pp. 469-470. USA: John Wiley & Sons, Inc.

Sinha, R.P. & Hader, D.P. (2002). UV-induced DNA damage and repair: a review. *Photochemical and Photobiological Sciences*, **1**(4), 225-236.

Sivapalasingam, S., Friedman, C.R., Cohen, L. & Tauxe, R.V. (2004). Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *Journal of Food Protection*, **67**, 2342-2353.

Slavin, J.L. & Lloyd, B. (2012). Health benefits of fruits and vegetables. *Advances in Nutrition*, **3**, 506-516.

Stellenbosch University https://scholar.sun.ac.za

Solomon, E.B., Yaron, S. & Mathews, K.R. (2002). Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalisation. *Applied and Environmental Microbiology*, **68**, 397-400.

Stampi, S., De Luca, G. & Zanetti, F. (2001). Evaluation of the efficiency of peracetic acid in the disinfection of sewage effluents. *Journal of Applied Microbiology*, **91**(5), 833-838.

Steward-Wade, S.M. (2011). Plant pathogens in recycled irrigation water in commercial plant nurseries and greenhouses: their detection and management. *Irrigation Science*, **29**, 267-297.

Stewart, J.R., Gast, R.J., Fujioka, R.S., Solo-Gabriele, H.M., Meschke, J.S., Amaral-Zettler, L.A., Castillo, E.D., Polz, M.F., Collier, T.K., Strom, M.S., Sinigalliano, C.D., Moeller, P.D.R. & Holland, A.F. (2008). The coastal environment and human health: microbial indicators, pathogens, sentinels and reservoirs. *Environmental Health*, **7**, 1-14.

Stine, S.W., Song, I., Choi, C.Y. & Gerba C.P. (2005). Application of microbial risk assessment to the development of standards for enteric pathogens in water used to irrigate fresh produce. *Journal of Food Protection*, **68**, 913-918.

Suss, J., Volz, S., Obst, U. & Schwartz, T. (2009). Application of a molecular biology concept for the detection of DNA damage and repair during UV disinfection. *Water Research*, **43**(15), 3705-3716.

Tauxe, R., Kruse, H., Hedberg, C., Potter, M., Madden, J. & Wachsmuth, K. (1997). Microbial hazards and emerging issues associated with produce: a preliminary report to the national Advisory Committee on Microbiologic Criteria for Foods. *Journal of Food Protection*, **60**(11), 1394-1471.

Tawabini, B., Khalil, A. & Abussaud, B. (2013). Reduction of *Escherichia coli* bacteria from contaminated water by combining hydrogen peroxide, ozone and ultraviolet light. *Water Science and Technology: Water Supply*, **13**(3), 782-789.

Teksoy, A., Alkan, U., Eleren, S.Ç., Topaç, B.Ş., Şağban, F.O.T. & Başkaya, H.S. (2011). Comparison of indicator bacteria inactivation by the ultraviolet and the ultraviolet/hydrogen peroxide disinfection processes in humic waters. *Journal of Water and Health*, **9**(4), 659-669.

Thurston-Enriquez, J.A., Haas, C., Jacangelo, J., Riley, K. & Gerba, C.P. (2003). Inactivation of feline calicivirus and adenovirus type 40 by UV radiation. *Applied and Environmental Microbiology*, **69**(1), 577-582.

Timchak, E. & Gitis, V. (2012). A combined degradation of dyes and inactivation of viruses by UV and UV/H_2O_2. *Chemical Engineering Journal*, **192**, 164-170.

Todar, K. (2012). Pathogenic *E. coli*. In: *Todar's Online Textbook of Bacteriology*. [Internet document]. URL http://textbookofbacteriology.net/e.coli_4.html. Accessed 07/11/2014.

Turtoi, M. (2013). Ultraviolet light potential for wastewater disinfection. *Annals. Food Science and Technology*, **14**(1), 153-164.

Stellenbosch University https://scholar.sun.ac.za

UNDP (United Nations Development Programme) (2006). Water competition in agriculture. In: *Human Development Report 2006. Beyond Scarcity: Power, Poverty and the Global Water Crisis*. Pp. 171-199. New York: Palgrave Macmillan.

UNEP FI (United Nations Environment Programme Finance Initiative) (2009). Water sustainability of agribusiness activities in South Africa. In: *Chief Liquidity Series: Agribusiness*. Pp. 42-48. Genève: UNEP FI.

USEPA (Environmental Protection Agency) (2006). *Ultraviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule*. United States Environmental Protection Agency, Washington, DC. p. 1-4, 2-32.

USEPA (United States Environmental Protection Agency) (2011). *Water Treatment Manual: Disinfection*. United States Environmental Protection Agency, Washington, DC. p. 83-96.

Van Blommestein, A. (2012). *Impact of selected environmental factors on E. coli growth in river water and an investigation of carry-over to fresh produce during irrigation*. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Van der Bruggen, B., Vandecasteele, C., Van Gestel, T., Doyen, W. & Leysen, R. (2003). A review of pressure-driven membrane processes in wastewater treatment and drinking water production. *Environmental Progress*, **22**(1), 46-56.

Van der Merwe, M. & Britz, T.J. (1994a). Characterisation and numerical analysis of the microbial community in raw Baker's yeast factory effluent. *Water SA*, **20**(2), 161-168.

Van der Merwe, M. & Britz, T.J. (1994b). Selection and optimisation of naturally occurring bacterial strains from raw baker's yeast effluent for use in a pre-degradation step. *Mededelingen van de Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent* Belgium, **59**(4b), 2107-2120.

Van Vuuren, L. (2009). New water framework counts every drop. *The Water Wheel*, **8**(3), 28-30.

Vickers, J.C. (2005). Introduction. In: *Microfiltration and Ultrafiltration Membranes for Drinking Water*, 1st ed. (edited by M. Christensen). Pp. 1-6. USA: American Water Works Association (AWWA).

Voigt, E., Jaeger, H. & Knorr, D. (2013). Established basic methods for water decontamination. In: *Securing Safe Water Supplies: Comparison of Applicable Technologies*. Pp. 25-38. Oxford: Academic Press.

Wait, I.W. & Blatchley, E.R. (2010). Model of radiation transmittance by inorganic fouling on UV reactor lamp sleeves. *Water Environment Research,* **82**(11), 2272-2278.

Walters, E., Graml, M., Behle, C., Müller, E. & Horn, H. (2014). Influence of particle association and suspended solids on UV inactivation of fecal indicator bacteria in an urban river. *Water, Air, & Soil Pollution*, **225**(1), 1822-1828.

Wang X., Hu, X., Hu, C. & Wei, D. (2011). Sequential use of ultraviolet light and chlorine for reclaimed water disinfection. *Journal of Environmental Sciences*, **23**(10), 1605-1610.

Stellenbosch University https://scholar.sun.ac.za

Warriner, K. (2011). Shiga toxin producing: *Escherichia coli*: Germany 2011 *Escherichia coli* O104:H4 outbreak linked to sprouted seeds. *IUFoST Scientific Information Bulletin (SIB).* June 2011. International Union of Food Science and Technology. Ontario, Canada.

Warriner, K. & Namvar, A. (2010). The tricks learnt by human enteric pathogens from phytopathogens to persist within the plant environment. *Current Opinions in Biotechnology*, **21**, 131-136.

Warriner, K., Huber, A., Namvar, A., Fan, W. & Dunfield, K. (2009). Recent advances in the microbial safety of fresh fruits and vegetables. *Advances in Food and Nutrition Research*, **57**, 155-208.

Werschkun, B., Sommer, Y. & Banerji, S. (2012). Disinfection by-products in ballast water treatment: an evaluation of regulatory data. *Water Research*, **46**(16), 4884-4901.

WESGRO (Western Cape Trade and Investment Promotion Agency) (2003). *Fruit sector fact sheet*. Western Cape Trade and Investment Promotion Agency, Cape Town. Pp. 2-3.

WESGRO (Western Cape Trade and Investment Promotion Agency) (2006). *Fruit processing sector brief*. Western Cape Trade and Investment Promotion Agency, Cape Town. Pp. 36-40.

WESGRO (Western Cape Trade and Investment Promotion Agency) (2012). *Western Cape trade 2012*. Western Cape Trade and Investment Promotion Agency, Cape Town. Pp. 1-14.

Whipps, J.M., Hand, P., Pink, D.A. & Bending, G.D. (2008). Human pathogens and the phyllosphere. *Advances in Applied Microbiology*, **64**, 183-221.

WHO (World Health Organisation) (1989). *Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture.* WHO, Technical Report Series no. 778. Geneva, Switzerland.

WHO (World Health Organisation) (2001). *Water Quality: Guidelines, Standards, and Health: Assessment of risk and risk management for water-related infectious disease* (edited by L. Fewtrell and J. Bartram). London: IWA publishing.

Wolfe, R.L. (1990). Ultraviolet disinfection of potable water. *Environmental Sciences and Technology*, **24**(6), 768-773.

Wols, B.A. & Hofman-Caris, C.H.M. (2012). Review of photochemical reaction constants of organic micro pollutants required for UV advanced oxidation processes in water. *Water Research,* **46**(9), 2815-2827.

Wu, G-Q., Bi, W-H., Lü, J-M. & Fu, G-W. (2011). Determination of chemical oxygen demand in water using near-infrared transmission and UV absorbance method. *Spectroscopy and Spectral Analysis*, **31**(6), 1486-1489.

Xu, L., Tian, C., Lu, X., Ling, L., Lv, J., Wu, M. & Zhu, G. (2015). Photoreactivation of *Escherichia coli* is impaired at high growth temperatures. *Journal of Photochemistry and Photobiology B: Biology*, **147**, 37-46.

Stellenbosch University https://scholar.sun.ac.za

Yan, T. & Sadowsky, M.J. (2007). Determining sources of fecal bacteria in waterways. *Environmental Monitoring Assessment*, **129**, 97-106.

Yiasoumi, W., Evans, L. & Rogers, L. (2005). *Farm water quality and treatment*. New South Wales Department of Primary Industries, Orange. Pp. 1-35.

Yousef, A.E., Vurma, M. & Rodriguez-Roma, L.A. (2011). Basics of ozone sanitation and food application. In: *Non-Thermal Processing Technologies for Food* (edited by H.Q. Zhang., G.V. Barbosa-Canóvas., V.M. Balasubramaniam., C.P. Dunne., D.F. Farkas & T.C. Yuan). Pp. 289-294. West Sussex: Wiley-Blackwell.

Zheng, Y. & Dunets, S. (2014). Slow Sand Filtration. [Internet document]. URL http://www.ces.uoguelph.ca/water/PATHOGEN/SlowSand.pdf. Accessed 24/04/2014.

Zimmer, J.L. & Slawson, R.M. (2002). Potential repair of *Escherichia coli* following exposure to UV radiation from both medium- and low-pressure UV sources used in drinking water treatment. *Applied and Environmental Microbiology*, **68**(7), 3293-3299.

Zimmer, J.L., Slawson, R.M. & Huck, P.M. (2003). Inactivation and potential repair of *Cryptosporidium parvum* following low- and medium-pressure ultraviolet irradiation. *Water Research*, **37**(14), 3517-3523.

Zoutman, D., Shannon, M. & Brown, K. (2011). Effectiveness of a novel ozone and hydrogen peroxide gas-vapour system for the rapid high level disinfection of surfaces and healthcare spaces. *BMC Proceedings*, **5**(6), 038.

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Chapter 3

INVESTIGATING THE DISINFECTION EFFICACY OF LOW-PRESSURE ULTRAVIOLET AND ULTRAVIOLET/HYDROGEN PEROXIDE TREATMENTS CONSIDERING *ESCHERICHIA COLI* STRAIN VARIATION AND THE IMPACT OF WATER QUALITY

ABSTRACT

The disinfection ability of ultraviolet (UV) irradiation and combined UV/Hydrogen peroxide (H_2O_2) treatments were tested against six *Escherichia coli* (*E. coli)* strains. Two American Type Culture Collection (ATCC) reference *E. coli* strains (ATCC 25922 and ATCC 35218) and four environmental strains (MJ56, MJ58, M53 and F11.2) were exposed to a single UV dose (4 mJ.cm^{-2}) and a 4 mJ.cm^{-2}/20 mg.L^{-1} (10 min) combination treatment with H_2O_2 in Sterile Saline Solution (SSS). ATCC 25922, MJ56 and F11.2 were also exposed to solitary UV doses of 8, 10 and 13 mJ.cm^{-2} and 2.5, 100 and 200 mg.L^{-1} H_2O_2 under the same combination conditions (4 mJ.cm^{-2}/10 min contact). Reference strains were particularly sensitive to UV and UV/H_2O_2 treatments with reductions of 2.5 – 3.5 log recorded during initial experiments (4 mJ.cm^{-2} and 20 mg.L^{-1} H_2O_2). Environmental *E. coli* isolates were more resistant with strain F11.2 showing reductions of 1.58 and 1.34 log for the respective treatments. Compared to UV irradiation alone, UV/H_2O_2 combination treatments showed potential for better disinfection for some strains. For strains MJ56 and MJ58, respectively, reductions were 0.78 and 1.12 log greater for the latter. Increased UV doses resulted in enhanced disinfection with a > 3.5 log reduction reported at 10 mJ.cm^{-2} for the most resistant strain (F11.2). The same was observed when the H_2O_2 concentration was increased during UV/H_2O_2 treatments, yet a maximum reduction of 2.40 log was recorded for F11.2 at 200 mg.L^{-1} H_2O_2. The influence of water quality on treatment efficiency was also investigated. Significantly better reductions were achieved in SSS than in river water for both F11.2 and ATCC 25922. Flocculation resulted in improved river water quality but ATCC 25922, only, was sensitive to these changes. Reductions of 3.66 and 4.54 log were achieved in untreated and flocculated water, respectively, at a UV dose of 10 mJ.cm^{-2}. When low-pressure (LP) UV irradiation at doses of 5, 7 and 10 mJ.cm^{-2} was used to disinfect river water samples, a 3 log reduction of faecal coliforms (FC) could not be reached. Extreme faecal pollution (FC > 6 log cfu.100 mL^{-1}) and poor water quality largely influenced disinfection potential. The maximum dose tested (10 mJ.cm^{-2}) could not reduce FC to < 3 log cfu.100 mL^{-1}, indicating that the treated water did not meet guidelines for fresh produce irrigation. Informal settlements and industrial operations strongly contributed to poor water quality and the investigation of UV doses > 10 mJ.cm^{-2} for river water disinfection is advised.

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INTRODUCTION

Globally, and in South Africa, water is tremendously important for the sustainable production of agricultural food products. As a matter of fact, agricultural irrigation is key in ensuring universal food security (Molden *et al.*, 2007). Despite its significant role in food production, however, many factors are currently contributing to a decrement in the availability of water for irrigational purposes. It is further observed that the microbiological quality of the available water is rapidly deteriorating. Accordingly, various researchers have reported that pathogenic microorganisms are increasingly associated with irrigated minimally processed foods (MPFs) (Paulse *et al.*, 2009; Pachepsky *et al.*, 2011; Ijabadeniyi & Buys, 2012).

Considering the microbiological status of South African river water the levels of faecal indicator microorganisms often exceed the guidelines set by DWAF (1996) and WHO (1989) for water used for the irrigation of fresh produce (1 000 faecal coliforms per 100 mL). In this regard, the prevention of water contamination in South Africa is relegated by a range of political, social and financial factors. As a result, various methods of decontaminating irrigation water are now being investigated. Referring to the level of microbial contamination occurring in local rivers, a 3 – 4 log target reduction is suggested to obtain water that could be safely used for irrigation (Britz *et al.*, 2012; Britz *et al.*, 2013).

Amongst others, UV light has been described as an effective method of disinfection owing to its destructive effect on microbial DNA (Quek & Hu, 2008; Gayán *et al.*, 2014). Ultraviolet irradiation does not lead to the formation of disinfection by-products (DBPs) or other chemical residues, but several factors may compromise the effectiveness of the process (Quek & Hu, 2008). Water quality and microbiological properties, such as the phenomenon of DNA repair, are of primary concern (Koivunen & Heinonen-Tanski, 2005a & b). Nonetheless, various researchers have investigated the potential of UV light for the disinfection of contaminated water (Linden *et al.*, 2007; Mezanotte *et al.*, 2007; Wang *et al.*, 2011; Guo *et al.*, 2012; Jones *et al.*, 2014).

Doses ranging from 3 – 30 and 30 – 100 $mJ.cm^{-2}$, respectively, have been found to effectively reduce bacteria and viruses (Siddiqui, 2005). Additional research has indicated that UV doses of 6 – 18 and 22 – 38 $mJ.cm^{-2}$ are capable of reducing enteric bacteria and the coliphage MS2 virus by 1 – 3 log and 1 – 1.5 log, respectively (Koivunen & Heinonen-Tanski, 2005b). Numerous additional studies have been performed and doses required to achieve predetermined reductions of different microbial groups have been proposed. However, without the availability of standardised protocols these suggestions should be used with caution when applied for validation purposes since many factors may influence the potency of UV disinfection.

Recently, the use of UV light in combination with H_2O_2 has received much attention as method of water disinfection. Known as an advanced oxidation process (AOP) it is capable of destroying pollutants, spores and vegetative cells by means of generating reactive hydroxyl radicals ($OH^{\bullet}$) (Alkan *et al.*, 2007; Mamane *et al.*, 2007). In 2011, Teksoy *et al.* evaluated the

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disinfection efficacy of UV light alone and in combination with hydrogen peroxide in humic waters. Amongst the experimental strains, *E. coli* strain ATCC 25922 was the most sensitive. A 3 log reduction was achieved after 55 and 33 s following exposure to UV light alone and in combination with H_2O_2 at a concentration of 50 $mg.L^{-1}$, respectively. Ultraviolet light was emitted at an intensity of 40 $\mu W.cm^{-2}$ while fulvic acid (2 $mg.L^{-1}$) was used to signify humic substances. Insignificant inactivation was recorded when H_2O_2 was applied on its own as a single treatment under the same conditions. Tawabini *et al.* (2013) investigated the effect of UV/H_2O_2 combination treatments by utilising LP and medium-pressure (MP) UV light and H_2O_2 concentrations of 20, 50 and 100 $mg.L^{-1}$. An average decrease of 33% in the time required to achieve 8 log reductions of the *E. coli* K12 strain MG1655 was reported when LP UV light was used in combination with the different H_2O_2 concentrations in comparison to UV treatment alone. With reference to these results the combination of UV/H_2O_2 as disinfection technique shows potential for the decontamination of microbiologically polluted river water.

The aim of the research presented here was to investigate the efficiency of LP UV and UV/H_2O_2 combination treatments for the disinfection of microbiologically contaminated irrigation water. A series of studies were conducted to determine: firstly, the effect of UV dose and H_2O_2 concentration on the inactivation of reference and environmental *E. coli* strains in SSS; secondly, the influence of water quality, measured in terms of chemical oxygen demand (COD), UV transmission percentage (UVT%), turbidity, total suspended solids (TSS) content and electrical conductivity (EC), on the efficiency of such treatments performed in sterilised river water; and thirdly, the lethality of LP UV irradiation tested against the natural microbial population occurring in contaminated water sampled from the Plankenburg River, Stellenbosch.

MATERIALS AND METHODS

General materials and methods

UV and UV/H_2O_2 disinfection

A bench-scale collimator device (Berson, The Netherlands) (Fig. 1) was used to perform laboratory-scale UV and UV/H_2O_2 disinfection experiments in 0.85% SSS and river water. The instrument utilised an Amalgam LP mercury vapour lamp (UV-Technik, Germany) with power output of 40 W and arc length of 25 cm. Light was predominantly emitted at 253.7 nm.

Ultraviolet light intensity at the sample surface was determined before each treatment using an ILT1400 radiometer (International Light Technologies, USA) coupled with a XRL140T254 detector (International Light Technologies, USA). Subsequently, the required time of exposure to deliver a desired UV dose was calculated according to the following equations (Morowitz, 1950; Hallmich & Gehr, 2010):

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$$I_{\text{avg},\lambda\,(mW.cm^{-2})} = I_0\lambda \left[\frac{1 - e^{d\ln(\text{UVT}(\lambda))}}{-d\ln(\text{UVT}(\lambda))}\right] \quad [1]$$

$$Desired\ dose\ (mJ.cm^{-2}) = Average\ intensity\ (mW.cm^{-2}) \times Exposure\ time\ (s) \quad [2]$$

In the above, $I_{(avg,\lambda)}$ refers to the average intensity of UV light over the sample depth, *d*; UVT(λ) refers to the UV transmission at wavelength, λ, determined using an optical path length of 1 cm; $I_0(\lambda)$ is the intensity of UV light measured at the surface of the sample.

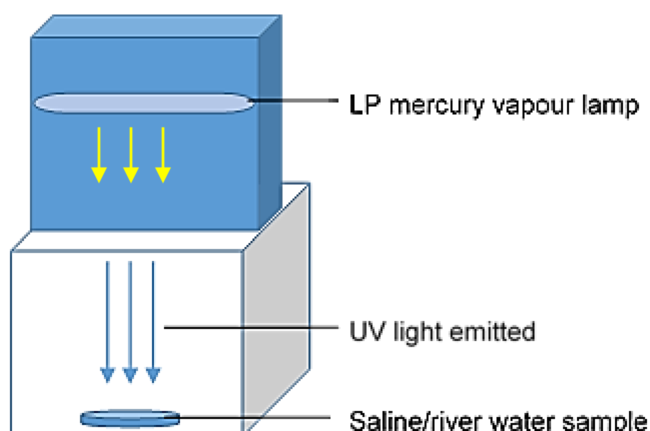

Figure 1 Schematic representation of the bench-top collimator beam device used for laboratory-scale UV and UV/H_2O_2 disinfection experiments.

Escherichia coli cultures used for disinfection experiments

Pure cultures of two ATCC reference *E. coli* strains and four environmental *E. coli* strains were used to investigate the efficacy of UV and UV/H_2O_2 disinfection treatments. Of these strains, three were known as antibiotic resistant (AR) isolates (Table 1). The environmental strains were isolated during previous studies performed by the Department of Food Science, Stellenbosch University. All *E. coli* strains were preserved at -80°C in 40% (v.v^{-1}) glycerol (Fluka Analytical, Germany).

Table 1 Characteristics of six *E. coli* strains used in laboratory-scale disinfection experiments

***E. coli* strain**	**Source of origin**	**Known antibiotic resistance**
ATCC 25922	Laboratory strain	None
ATCC 35218	Laboratory strain	AMP, C, STR
MJ58	Parsley	None
MJ56	Parsley	None
M53	Plankenburg River	T, TM, AMP, STR
F11.2	Plankenburg River	T

T – Tetracycline; TM – Trimethoprim; AMP – Ampicillin; STR – Streptomycin; C - Chloramphenicol

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Preparation of microbial cultures

The *E. coli* strains were removed from the -80°C freezer and 100 µL of the bacterial suspension was transferred to 5 mL sterile Nutrient Broth (NB) (Biolab, South Africa) which was then incubated at 37°C for 24 h. Following this, a loop full of the suspension was streaked out on Levine's Eosin Methylene-Blue Lactose Sucrose Agar (L-EMB) (Oxoid, South Africa) which was inversely incubated at 36°C for a further 24 h to obtain pure, single colonies of the respective strains. *Escherichia coli* appear in the form of metallic green colonies on L-EMB agar (Merck, 2005). Prior to disinfection experiments a single colony of the required strain was selected from a fresh L-EMB culture using a sterilised loop and transferred to 5 mL sterile NB (Biolab, South Africa). The suspension was incubated at 36°C for 24 h after which a volume of the prepared culture was transferred to SSS (0.85% $m.v^{-1}$ NaCl) or autoclaved river water to yield a cell density equivalent to a 0.5 McFarland standard (BioMèrieux, South Africa).

Preparation of H_2O_2 solution

A 300 $mg.L^{-1}$ H_2O_2 stock solution was prepared from 30% ($v.v^{-1}$) hydrogen peroxide (Merck, South Africa). A calculated volume of this was then transferred to the petri dishes representing the different treatments which contained a volume of the inoculum representing a 0.5 McFarland standard. A total volume of 16 mL, representative of the required H_2O_2 concentration, was obtained in this manner. A Spectroquant® Hydrogen Peroxide Cell Test (2.0 – 200 $mg.L^{-1}$) (Merck, South Africa) was used to confirm each of the respective concentrations.

River water sampling site and method

Unfiltered river water was sampled from the Plankenburg River (33°55'57.8''S,18°51'06.3''E) according to the procedure described by the South African National Standards (SANS) 5667-6 method (SANS, 2006). Samples were taken using two sterilised 5 L bottles and kept at refrigerated temperatures up until the point of analysis. The sampling bottles were submerged 30 cm below the surface of the water and only then were the caps removed. Once filled, the caps were replaced under water and the bottles were transferred to insulated cooler boxes and transported back to the laboratory for analysis.

Filtered river water was sampled from a pilot-scale water disinfection plant situated at an industrial site on the bank of the Plankenburg River in Stellenbosch, South Africa (33°56'15.4''S,18°50'53.0''E). Water was pumped from the river, through a sand filter, and samples were taken using sterile bottles. The samples were handled as described above until further analyses were performed.

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River water flocculation

A commercially available polymeric coagulant, Zetaflock Z553L (Zetachem, South Africa), was prepared as a 0.07% solution in a sterilised 1 L Schott bottle so that the addition of 50 mL of solution to 5 L of river water delivered a final concentration of 7 $mg.L^{-1}$. A rapid mixing speed of 100 rpm was applied for 2 min using an electronic Heidolph stirrer (Heidolph, Germany). This was followed by the application of a slow mixing speed, 40 rpm, for 15 min using the same instrument. The treated water sample was allowed to settle for 15 min prior to filtration through a Whatman No. 1 filter paper (Whatman, United Kingdom).

Microbiological analysis

Microbial analysis of river water samples were performed by using both standard plating methods as well as the standard membrane filtration (MF) method (USEPA, 2002). For the plating methods, serial dilutions were prepared according to the South African National Standards (SANS) method 6887-1 (SANS, 1999) in 9 mL SSS units. Serial dilutions were prepared in larger volumes (90 mL) of SSS for the MF analyses.

Total Coliform, Faecal coliform and E. coli enumeration

Serial dilutions (10^0 – 10^{-6}) were prepared before (control) and after all specific disinfection treatments. Following this, total coliforms (TC) were firstly determined according to the SANS method 4832 (SANS, 2007a). Violet Red Bile Agar (VRBA) (Merck, South Africa) was used for the preparation of duplicate pour plates which were incubated at 35°C for a period of 24 h. Faecal coliforms (FC) were enumerated at 44°C for 24 h using VRBA (Merck, South Africa) (Schraft & Watterworth, 2005). Coliforms were observed as red colonies surrounded by zones of red precipitate (Merck, 2005). Duplicate pour plates were also created using Chromocult® Coliform Agar Enhanced Selectivity (CES) for the enumeration of TC and *E. coli.* Again, the plates were incubated at 35°C for 24 h.

In addition, 100 mL of diluted water samples were filtered through sterile cellulose nitrate membrane filters with pore size of 0.45 μm and diameter of 47 mm (Whatman, England) according to the U.S. Environmental Protection Agency (USEPA) method 1604 (USEPA, 2002). The filters were transferred to CES plates which were inverted and incubated at 35°C for 24 h. Total coliforms and *E. coli* were observed as salmon to red and dark blue to violet colonies, respectively (Merck, 2005).

Heterotrophic plate count (HPC)

The heterotrophic plate count (HPC) was firstly determined according to the SANS method 4833 (SANS, 2007b). Water samples were serially diluted (10^0 – 10^{-6}) before (control) and after all disinfection treatments and duplicate pour plates were prepared using Plate Count Agar (PCA)

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(Merck, South Africa). For the MF analysis serial dilutions (100 mL) were filtered through sterile cellulose nitrate membrane filters with pore size of 0.45 µm and diameter of 47 mm (Whatman, England) and transferred to pre-poured PCA plates according to the USEPA method 1604 (USEPA, 2002). The plates were incubated at 30°C for 48 h where after those representing 30 – 300 colony forming units (cfu) were counted.

Escherichia coli identification and characterisation

Analytical Profile Index (API) for identification of E. coli strains

The phenotypic characteristics and identity of six *E. coli* strains were confirmed by means of using the Analytical Profile Index (API) 20E test (BioMérieux, South Africa) as well as additional tests described in the following section. The test was performed according to the manufacturer's instructions after which a seven-digit code, the API, was determined from the results. The codes were extended by performing the supplementary tests (described below) and submitted to the APIweb™ program (BioMérieux, South Africa) which identified the microorganisms with reference to their biochemical profiles.

Supplementary tests

Firstly, each of the *E. coli* strains were subjected to the gram-staining test. Also, motility was examined using the hanging drop method in which slides were microscopically studied under 100 x magnification (Gerhardt *et al*., 1981). Motility was recorded as a positive result. All of the strains were further subjected to the oxidase test in which the presence of a purple colour indicated a positive result. To detect catalase activity, a single colony of each *E. coli* strain was picked from Nutrient Agar (Biolab, South Africa) and transferred onto a glass slide as described by Gerhard *et al*. (1981). Three drops of 3% H_2O_2 was then used to cover each colony. A positive result was indicated by the immediate formation of bubbles. Lastly, the *E. coli* strains were streaked out on MacConkey agar. Following incubation at 37°C for 24 h, growth was recorded as a positive result.

River water analysis

Physico-chemical and microbiological properties of untreated, flocculated and filtered river water, respectively, were determined. The results of these analyses were subsequently compared to guidelines set for water intended to be used for fresh produce irrigation (Table 2).

Table 2 Limits suggested for quality parameters of fresh produce irrigation water (DWAF, 1996)

Water quality parameter	Limit
Faecal coliforms (FC)	1 000 cfu.100 mL^{-1}
Conductivity	40 $mS.m^{-1}$
pH	6.5 – 8.4
Total Suspended Solids (TSS)	50 $mg.L^{-1}$

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Chemical Oxygen Demand (COD)

The COD of river water samples was determined according to Standard Methods (APHA, 2005). Solutions A and B for the COD range of 10 – 150 $mg.L^{-1}$ (Merck Millipore, South Africa) were added to the sample to be analysed where after digestion for 2 h at 150°C followed. The DR 2000 spectrophotometer (Hach, USA) was used to determine COD at 585 nm. Analyses were performed in duplicate and an experimental blank was included.

Ultraviolet transmission percentage (UVT%)

The UVT% of river water samples was determined using a UVT-15 UV% Transmission Photometer (HF Scientific, USA) as well as a hand held Sense^{TM} T UV-Transmittance Monitor (Berson, The Netherlands) according to the instructions provided by the respective manufacturers. In each case, deionised water was used for the calibration of the instrument and represented UV transmission of 100%. Analyses were performed in duplicate using each of the two instruments.

Turbidity

In accordance with the manufacturer's instructions, a portable Orion AQ3010 Turbidity Meter (Thermo Scientific, USA) was used to determine the turbidity, measured as Nephelometric Turbidity Units (NTU), of river water samples. Prior to analyses calibration of the instrument was confirmed using solutions representing known turbidity values. Analysis of the river water was then performed in duplicate.

Alkalinity, pH, Total Suspended Solids (TSS), Volatile Suspended Solids (VSS) and temperature

Alkalinity, pH, TSS and VSS were determined with reference to instructions provided by Standard Methods (APHA, 2005). Twenty millilitres of water was titrated with 0.1 N H_2SO_4 to reach a pH of 4.3 for the calculation of alkalinity. A portable pH meter (WTW, Germany) was used, as instructed by the manufacturer, for the determination of the pH and temperature of river water samples. Each of the analyses were performed in duplicate on the sample to be tested.

Electrical Conductivity (EC)

A portable HI 8733 conductivity meter (Hanna Instruments, USA) was used to quantify the amount of dissolved salts in river water samples. Calibration of the instrument and the adjustment of measuring units were performed according to the instructions provided by the manufacturer. Duplicate measurements were performed on each of the water samples.

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Total Dissolved Solids (TDS)

As described by DWAF (1996) the total dissolved solids (TDS) content of river water samples was determined using a conversion factor and values recorded for conductivity. The following equation was used:

$$Electrical\ conductivity\ (mS.m^{-1}) \times 6.5 = TDS\ (mg.L^{-1})$$

Statistical analysis

Statistical analyses were performed using Statistica 12.5 software (StatSoft, USA). Data were analysed using a two-way analysis of variance (ANOVA) and the Fisher least significant difference (LSD) test was used to perform several post hoc analyses. Significant results were identified by means of using a 95% confidence interval i.e. a 5% significance level ($p<0.05$) as guideline.

Research study design

To evaluate the potential of LP UV and UV/H_2O_2 combination treatments for the reduction of microbial loads in river water prior to irrigation, the effectiveness of several disinfection approaches was studied in a laboratory-scale experiment. A preliminary study in SSS (utilising LP UV light at a single dose and H_2O_2 at a single concentration and contact time) was executed on two ATCC reference and four environmental *E. coli* strains (Table 1) to determine whether these respond differently in terms of log inactivation. Thereafter, a range of UV doses, as stand-alone treatment, and UV in combination with H_2O_2 at varying concentrations were tested against three of the *E. coli* strains. Furthermore, the influence of water quality on treatments showing potential for disinfection was investigated. Autoclaved river water was used to evaluate the influence of water quality, measured in terms of organic matter content (COD), ultraviolet transmission percentage (UVT%), turbidity, suspended solids content (TSS) and conductivity, on disinfection efficiency.

In a concluding study the effectiveness of UV irradiation for the disinfection of filtered river water was assessed, taking into account the influence of changing water quality and a heterogenic microbial population. Prior to performing disinfection experiments on the untreated river water techniques for enumerating TC, FC and the heterotrophic population were optimised. Where possible, results of the microbiological and water quality analyses were compared to guidelines for irrigation water quality set by DWAF (1996) and were used to interpret the effectiveness of the respective treatments.

Study A: Efficiency of UV and UV/H_2O_2 treatments tested against six E. coli strains in SSS.

Escherichia coli inoculums (0.5 McFarland standard equivalent) were prepared in sterile 0.85% SSS and aseptically transferred to a sterile petri dish before performing the respective treatments.

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In the case of UV irradiation as stand-alone treatment the petri dishes were immediately positioned in the centre of the sphere of UV light observed on the surface of the bottom section of the collimator device (Fig. 2). For the UV/H_2O_2 combination treatments, H_2O_2 was added to each petri dish and allowed to have contact with the inoculum before it was transferred to the collimator (Fig. 2). The contact time was initiated as soon as the H_2O_2 was mixed with the inoculum.

Six *E. coli* isolates (Table 1), including two ATCC reference (ATCC 25922 and ATCC 35218) and four environmental strains (MJ58, MJ56, M53 and F11.2), were exposed to a lower-limit UV dose (4 mJ.cm^{-2}) to determine whether they respond differently to UV light irradiation. Additionally, the same strains (ATCC 25922, ATCC35218, MJ58, MJ56, M53 and F11.2) were subjected to a single concentration (20 mg.L^{-1}) of H_2O_2 for a predetermined contact period (10 min) before being irradiated at the same UV dose (4 mJ.cm^{-2}). One of the reference strains (ATCC 25922) and two of the environmental strains (F11.2 and MJ56) were further irradiated with incremented UV doses (8, 10 & 13 mJ.cm^{-2}) and were also exposed to both lower and higher H_2O_2 concentrations (2.5, 100 & 200 mg.L^{-1}) using the contact time (10 min) and UV dose (4 mJ.cm^{-2}) mentioned earlier (Table 3). Enumeration of the respective *E. coli* strains was performed both before and after the different treatments in order to determine the reduction achieved for specific UV doses and combination treatments. This was done by serially diluting the untreated and treated inoculums whereafter duplicate pour plates were prepared using VRBA (Fig. 2). Following incubation at 35°C for 24 h the responses of the *E. coli* strains were expressed in terms of log inactivation. Experimental procedures were performed in triplicate.

Table 3 Ultraviolet and UV/H_2O_2 combination treatments executed on six *E. coli* strains in a laboratory-scale investigation

	***E. coli* Strain**					
Treatment	**ATCC 25922**	**ATCC 35218**	**MJ58**	**MJ56**	**M53**	**F11.2**
UV 4 mJ.cm^{-2}	✓	✓	✓	✓	✓	✓
UV 8 mJ.cm^{-2}	✓	-	-	✓	-	✓
UV 10 mJ.cm^{-2}	✓	-	-	✓	-	✓
UV 13 mJ.cm^{-2}	✓	-	-	✓	-	✓
UV 4 mJ.cm^{-2}/H_2O_2 2.5 mg.L^{-1}	✓	-	-	✓	-	✓
UV 4 mJ.cm^{-2}/H_2O_2 20 mg.L^{-1}	✓	✓	✓	✓	✓	✓
UV 4 mJ.cm^{-2}/H_2O_2 100 mg.L^{-1}	✓	-	-	✓	-	✓
UV 4 mJ.cm^{-2}/H_2O_2 200 mg.L^{-1}	✓	-	-	✓	-	✓

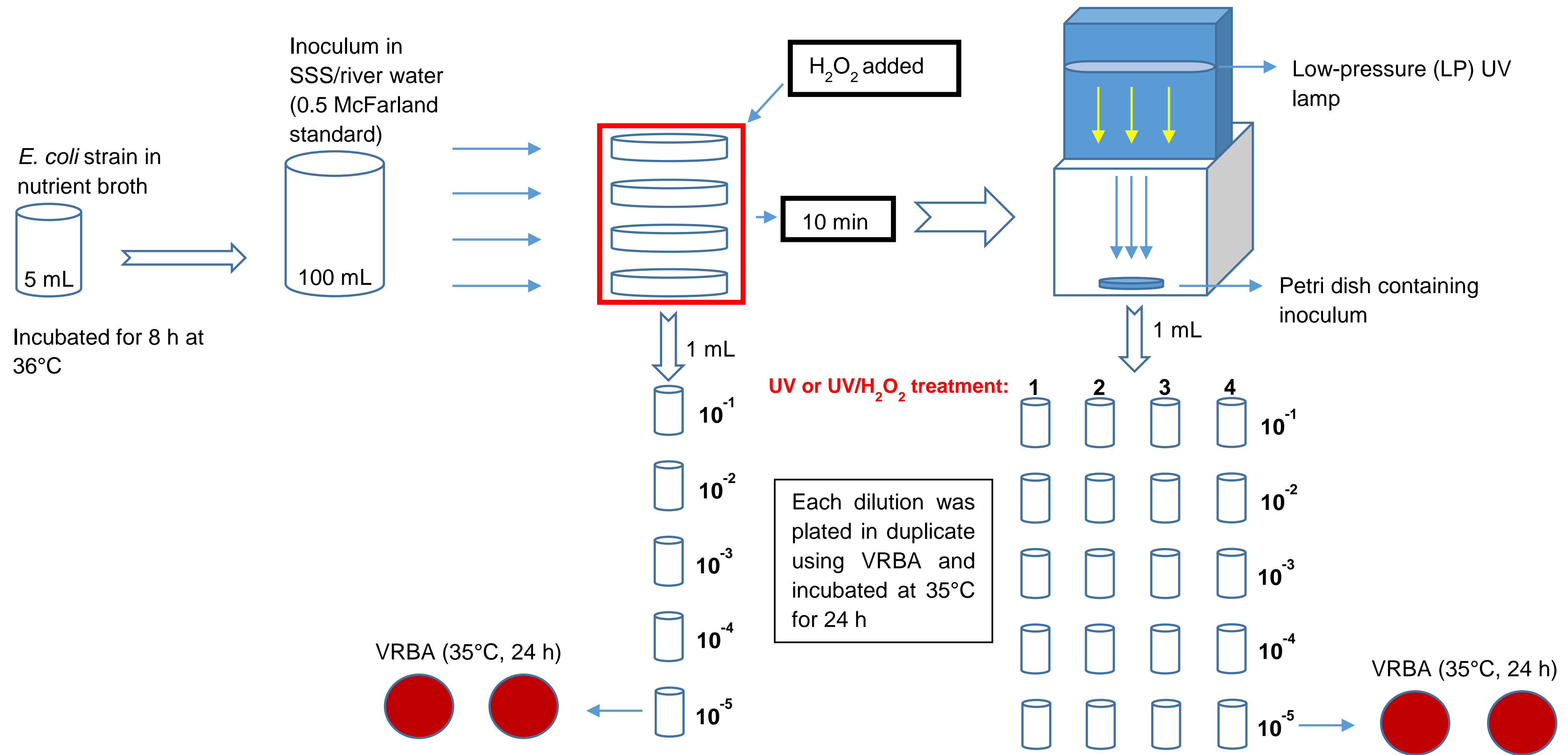

Figure 2 Experimental design used in Study A to determine the log inactivation achieved for each strain following the application of UV or UV/H_2O_2 disinfection treatments as summarised in Table 3.

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Study B: Influence of COD, UVT%, turbidity, TSS and conductivity on UV and UV/H_2O_2 disinfection

For the second part of the study, one reference strain (ATCC 25922) and one resistant environmental (F11.2) *E. coli* strain were selected and inoculated into both sterile untreated and flocculated river water. Both strains were exposed to an UV dose of 10 mJ.cm^{-2} while, F11.2 was further subjected to a UV/H_2O_2 combination treatment utilising a H_2O_2 concentration of 200 mg.L^{-1}, contact time of 10 min and UV dose of 10 mJ.cm^{-2}. This was done to determine if water quality (measured in terms of organic matter content (COD), UV transmission percentage (UVT%), turbidity, suspended solids content (TSS) and conductivity) had an impact on the efficiency of UV and UV/H_2O_2 treatments. Disinfection and subsequent enumeration procedures were performed as described for UV and UV/H_2O_2 disinfection in SSS.

Study C: Comparison of methods for the enumeration of coliforms and total heterotrophic bacteria

Filtered river water (sampled from the Plankenburg River on three respective days) was used to compare different techniques for the enumeration of TC, FC (*E. coli*) and the heterotrophic bacteria population (HPC). This was done to optimise enumeration procedures for subsequent disinfection studies performed on river water. Total coliforms and FC (*E. coli*) were enumerated using the pour plate and MF techniques in conjunction with VRBA and CES. The heterotrophic population (HPC) was enumerated using PCA, again to compare the pour plate technique with MF. For the plating methods, the experimental procedure was performed in triplicate i.e. three 1 mL river water samples were analysed. For MF, the procedure was performed once on a 100 mL river water sample.

Study D: UV disinfection of filtered river water

To investigate the efficiency of LP UV irradiation for the disinfection of river water, untreated samples (collected to excecute study C) were exposed to UV doses of 5, 7 & 10 mJ.cm^{-2} in three respective trials. Each of the trials represented triplicate UV irradiation of untreated river water for each of the respective UV doses.

The collimated-beam device was utilised to execute the treatments according to the procedure described in Study A. In this instance, however, water was aseptically transferred from the control sample container to sterilised 500 mL glass beakers, one for each dose, to reach a depth of 22 mm. The glass beaker was subsequently transferred to the lower section of the collimator device and irradiated with UV light at the respective UV doses (Fig. 2). Total and faecal coliforms and heterotrophic bacteria (HPC) were enumerated before and after each UV treatment using the pour plate technique and media (VRBA and PCA) selected based on the findings of Study C (comparison of different techniques for enumerating the same microbial groups). Deeper

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samples (22 mm) were irradiated in order to mimic the conditions presented in a pilot-scale system in which the water depth around the UV lamp would be approximately 22 mm. Where applicable, measured water quality parameters were compared to the South African guidelines for water intended to be used for fresh produce irrigation as described by DWAF (1996) (Table 2).

RESULTS AND DISCUSSION

Escherichia coli characterisation: Analytical Profile Index (API 20E)

Each of the six trains used in this study was confirmed as *E. coli* following the application of the API 20E test (Table 4). This system allows the use of 20 biochemical tests in conjunction with a large database (APIweb^{TM}) (BioMèrieux, South Africa) in order to accurately identify microorganisms.

Table 4 Recorded API profiles and % certainty for six *E. coli* strains investigated in this study

E. coli strain	API profile	% Certainty	Description
ATCC 25922	5144552	99.9	Excellent identification
ATCC 35218	5144570	99.5	Very good identification
M53	5144552	99.9	Excellent identification
MJ56	5144572	99.5	Very good identification
MJ58	5044552	99.9	Excellent identification
F11.2	5044552	99.9	Excellent identification

Importantly, all of the API profile codes recorded for environmental strains used in this study (M53, MJ56, MJ58 and F11.2) have been documented previously for *E. coli* strains isolated from surface water or soil (Brennan *et al*., 2010; Janezic *et al*., 2013). This implies that this group of strains shows potential of occurring in irrigation water and subsequently on irrigated fresh produce. Investigating their responses to different water treatment methods may thus yield valuable insights into water disinfection.

It is apparent that strains ATCC 25922 and M53 exhibit the same biochemical profile (5144552). The same was observed for strains MJ58 and F11.2 which both represented the API profile code 5044552 (Table 4). Interestingly, the two strains isolated from parsley (MJ56 and MJ58) differed from each other with regards to their ability to ferment saccharose. Strain MJ56 (5144572) was capable thereof while MJ58 (5044552) was not. It has been found that plant-associated bacteria are often those that use saccharose as carbon source (Méric *et al*., 2013). It might thus be that strains capable of fermenting saccharose (MJ56 and ATCC 35218) would show greater potential for surviving should they be transferred from irrigation water to plant material.

Study A: Effect of LP UV and UV/H_2O_2 combination treatments on ATCC reference and environmental *E. coli* strains

The effect of a single UV dose and UV/H_2O_2 combination treatment was evaluated on both reference (ATCC) and environmental *E. coli* strains. It was unmistakeably observed that substantial differences exist between the strains regarding their sensitivity towards the respective processes (Fig. 3). In this initial experiment it was observed that environmental *E. coli* strains showed slightly greater overall resistance towards both of the disinfection treatments in comparison to the reference strains tested.

Reductions ranging from 2.59 – 3.68 and 1.58 – 3.06 log, respectively, were achieved following exposure of reference and environmental strains to the UV treatment. This clearly indicates variation in strain-specific resistance to UV light. The two reference strains (ATCC 25922 and ATCC 35218) as well as M53 were greatly inactivated when UV light at a dose of 4 $mJ.cm^{-2}$ was applied. Reductions of 2.59, 3.68 and 3.06 log, respectively, were achieved. Importantly, for ATCC 35218 and M53 the desired reduction of 3 log was reached. A 3 log reduction implies that should *E. coli* populations in the order of 10^5 – 10^6 cfu.100 mL^{-1} occur in water, the treatment might be able to reduce the population size to within the guideline limit of 1 000 faecal coliforms per 100 mL. This is important as *E. coli* levels exceeding 500 000 cfu.100 mL^{-1} have been detected in irrigation water used in the Western Cape (Paulse *et al*., 2009). Also, Lamprecht *et al*. (2014) reported that *E. coli* concentrations as high as 250 000 – 1 000 000 MPN.100 mL^{-1} have been detected in the Plankenburg River. The environmental strains MJ56, MJ58 and F11.2 were more resistant and represented reductions of 1.60, 1.93 and 1.58 log, respectively (Fig. 3). At a confidence interval of 95.0% no significant difference was observed between the log reductions achieved for MJ56 and F11.2 ($p=0.90$) (Fig. 3).

Regarding disinfection with UV/H_2O_2 at a H_2O_2 concentration of 20.0 $mg.L^{-1}$ and contact time of 10 min F11.2 was again identified as the most resistant *E. coli* strain, showing a reduction of 1.34 log (Fig. 3). Overall, reductions in the range of 2.40 – 3.60 and 1.34 – 3.05 log were reached for reference and environmental strains, respectively. While no significant difference in the log reductions achieved for strains ATCC 25922, M53 and MJ58 were recorded ($p>0.05$) strain ATCC 35218 was again the most sensitive with a reduction of 3.60 log being achieved. The target 3 log reduction was also attained for MJ58 during this experiment while a reduction of 2.93 was observed for M53. Only for two of the strains (MJ56 and MJ58), a significant difference ($p<0.05$) in log reduction was observed between UV as stand-alone treatment and UV in combination with H_2O_2 (Fig. 3). Reductions increased with 0.78 and 1.12 log units for MJ56 and MJ58, respectively, for the combination treatment. Initially, however, these two strains were more resistant to the UV treatment. These results indicate that the UV/H_2O_2 combination might only present increased disinfection potential for certain strains in comparison to UV irradiation alone. Other strains may be capable of surviving both approaches. For some strains (ATCC 35218, M53 and F11.2) slightly

lower average log reductions were recorded following the combination treatment compared to the UV treatment (4 mJ.cm^{-2}) alone. These differences in log reductions were, however, not statistically significant ($p>0.05$).

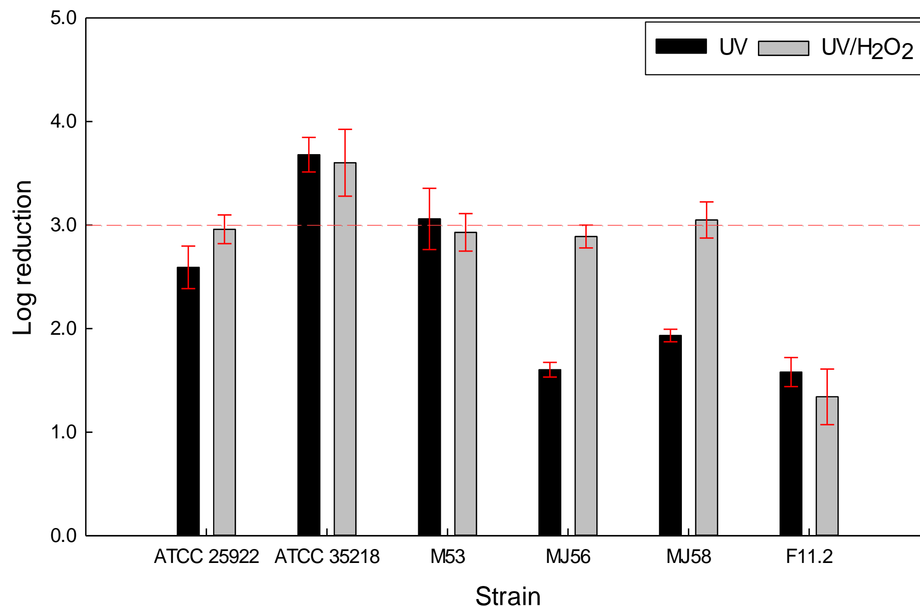

Figure 3 Disinfection efficiency of UV (4 mJ.cm^{-2}) and combined UV/H_2O_2 (4 mJ.cm^{-2}/20 mg.L^{-1}) treatments on six *E. coli* strains in SSS. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

The results presented here are in agreement with other work found in literature. Sommer *et al*. (1998, 2000) and Malley *et al.* (2004) reported on clear variation in UV sensitivity amongst different *E. coli* strains. The authors reported that the sensitivity of the strains (expressed in terms of the inactivation rate constant k (cm^2.mJ^{-1})) varied by factors of 5.8 and 3.7, respectively, in these studies. Furthermore, Mofidi *et al.* (2002) evaluated the effect of UV light on ATCC reference strains and a clinical environmental *E. coli* strain. In their work strain ATCC 23229 (standard, non-cytogenic *E. coli*) was most sensitive to UV while the cytogenic, clinical O157:H7 isolate was less susceptible (Mofidi *et al.*, 2002). Maya *et al.* (2003) also reported that environmental faecal coliform strains were more resistant to UV light in comparison to their seeded (laboratory stock-cultures) counterparts.

In this regard, Gayán *et al.* (2014) identifies process parameters, product/water parameters and microbial characteristics as major factors influencing microbial resistance to UV irradiation. For this study specifically, differences in microbial characteristics are key in understanding behavioural variances observed between the *E. coli* strains in response to the different treatments. Intrinsic microbial properties not only explain why different types of microorganisms and species react differently to UV light but may further clarify strain-to-strain variation (Gayán *et al.*, 2014). The size of the cells, the thickness of cell walls, cell pigmentation and properties related to the

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genetic material of different strains may all have an impact on the UV sensitivity of microorganisms. Extrinsic microbial factors such as the growth phase, growing conditions, stresses before and recovery conditions after UV treatment would be less influential in the present study as cultivation and experimental conditions were kept constant throughout the trial. Furthermore, literature states that mechanisms of DNA repair following UV irradiation have developed over time due to the exposure of microorganisms to UV light from the sun (Hijnen *et al.*, 2006). Environmental *E. coli* strains may thus show greater repair potential in comparison to those not previously exposed to the environment. Although the *E. coli* strains were not exposed to conditions favourable for DNA repair in this study, it is speculated that environmental strains might be distinguished from reference strains in terms of additional intrinsic characteristics modified by environmental stimuli over time. Such modifications could then possibly result in increased UV resistance. For instance, co-protective adaptation responses may be triggered if bacteria were previously exposed to heat, osmotic, starvation or acid shocks (Van der Veen & Abee, 2011).

The effect of combined chemical and UV disinfection have been investigated before. Possible synergistic benefits may be explained in terms of advanced oxidation but also simply in terms of a strategy of multiple damage (Koivunen & Heinonen-Tanski, 2005b). The latter implies that microorganisms are injured or destroyed as a result of the combined individual effects of UV light and H_2O_2 in addition to the effect of advanced oxidation (Timchak & Gitis, 2012). The synergistic benefits of the UV/H_2O_2 treatment on environmental strains MJ56 and MJ58, as seen in Figure 3, thus imply that they were sensitive to at least one of these mechanisms. None of the other strains, however, were seemingly more sensitive to the combination treatment implying that they were able to resist the effect of H_2O_2. Variation in the resistance amongst strains may thus be attributed to the presence (or absence) of multiple mechanisms of dealing with the effects of a UV/H_2O_2 combination treatment.

Literature states that resistance against the effects of antibiotics, biocides and oxidative stress could be readily developed by *E. coli* strains (Storz & Imlay, 1999; Davies & Davies, 2010). In addition, it has been proven that microorganisms occurring naturally in the environment show greater resistance to chemical disinfection compared to generally used reference strains (Mazzola *et al.*, 2006; Wojcicka *et al.*, 2007). In response to oxidative stress (such as that caused by advanced oxidation or the natural decomposition of H_2O_2 in water) microorganisms may express enzymes allowing them to protect themselves and repair damage caused by reactive oxygen species (Storz & Imlay, 1999; Iwase *et al.*, 2013). The toxicity of H_2O_2, for instance, can be supressed in the presence of hydroperoxidase 1 (HP1) and hydroperoxidase 2 (HP2) which are catalase enzymes capable of catalysing H_2O_2 into water and oxygen (Loewen *et al.*, 1985). Hydroperoxidase 1 and 2 are encoded by *Kat*G and *Kat*E genes which are induced by low concentrations of H_2O_2 and in the stationary phase, or the presence of other stress factors, respectively (Visick & Clarke, 1997; Storz & Imlay, 1999; Iwase *et al.*, 2013).

Keeping in mind that a low H_2O_2 concentration (20 mg.L^{-1}) was used in this experiment it was hypothesised that *E. coli* strains representing low HP1 activity may be sensitive to the combination treatment. Giddey *et al.* (2015) reported that both MJ56 and MJ58 represent low HP1 activity (catalase activity units (U) of < 50) in comparison to F11.2 and ATCC 25922 for which activity of approximately 120 and 230 U, respectively, were recorded. The increased disinfection observed for MJ56 and MJ58 in the presence of H_2O_2 is therefore in agreement with its inability to decompose H_2O_2 (low HP1 activity). Strains F11.2 and ATCC 25922 (showing higher levels of HP 1 activity) were less sensitive to the synergistic effects of UV light and H_2O_2 (Fig. 3).

In contrast, Giddey *et al.* (2015) further reported that strains ATCC 35218 and M53 exhibited HP1 activity of < 30 U while being insensitive to the presence of low concentrations of H_2O_2. This indicates that factors other than catalase activity are responsible for their resistance towards H_2O_2. Interestingly, these two strains are also resistant to multiple antibiotics (Table 1). It has been suggested that the mechanisms which protect bacteria against antibiotics may also provide them with resistance to H_2O_2 (Giddey *et al.*, 2015). For instance, non-specific efflux pumps might explain why strains ATCC 35218 and M53 (with low HP 1 activity) were resistant to the UV/H_2O_2 treatment. These pumps allow bacteria to protect themselves against antibiotics and non-specific biocides including hydrogen peroxide (Ortega Morente *et al.*, 2013).

Effect of increased UV dose and H_2O_2 concentration

It was decided to further investigate the effect of increased UV doses and both higher and lower H_2O_2 concentrations on disinfection efficacy using ATCC 25922, F11.2 and MJ56 as test strains. ATCC 25922 was chosen as popular reference strain, which is often encountered in literature, while F11.2 was selected due to its proven resistance against the respective treatments (Fig. 3). MJ56 showed less resistance toward the combination treatment in comparison to UV alone and was therefore chosen to further investigate this phenomenon at elevated UV doses and higher and lower H_2O_2 concentrations. Note that for the purpose of this discussion the absence of growth at the lowest dilution (10^{-1}) was recorded as 300 cfu.mL^{-1} (log value of 2.48).

The effect of increased UV doses on these test strains is presented in Figure 4. At a UV dose of 4 mJ.cm^{-2} the desired 3 log reduction was not achieved for any of the *E. coli* strains. It is, however, clear that an increase in UV dose resulted in increased inactivation of all tested strains. When the UV dose was amplified from 4 to 8 mJ.cm^{-2} complete inactivation was achieved for both ATCC 25922 and MJ56 (Fig. 4). The same was observed at UV doses of 10 and 13 mJ.cm^{-2} (Figs. 4 and 5). Slight log reduction variations observed between ATCC 25922 and MJ56 for UV doses of 8, 10 and 13 mJ.cm^{-2} in Figure 4 can be attributed to differences in the initial size of the microbial population prior to the application of the treatment. Strain F11.2 was confirmed to be the most resistant strain with the target 3 log reduction being reached only when the UV dose was increased from 8 to 10 mJ.cm^{-2}. A dose of 13 mJ.cm^{-2} was required to achieve complete inactivation. Additional reductions of 1.05 and 0.86 log were achieved for strain F11.2 when the

UV dose was increased from 8 to 10 $mJ.cm^{-2}$ and 10 to 13 $mJ.cm^{-2}$, respectively (Figs. 4 and 5). From these early results it was concluded that UV irradiation shows great potential for the disinfection of contaminated water since a reduction of 3 log was easily achieved for each of the tested strains.

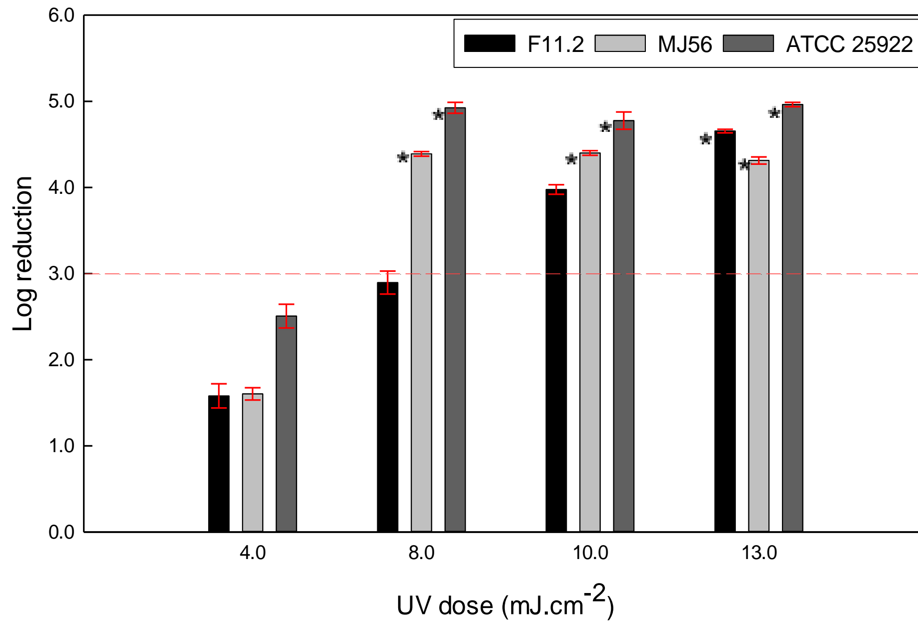

Figure 4 Effect of varying UV dose on the survival of three *E. coli* strains in SSS. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

* To the left of the bars indicate inactivation of the entire microbial population.

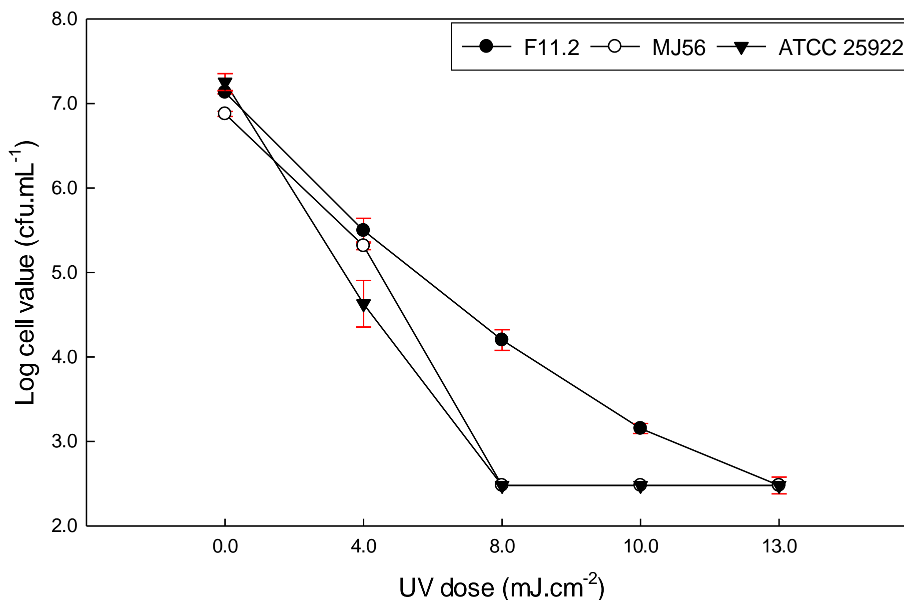

Figure 5 Effect of UV dose on the number of viable microorganisms following treatment in SSS. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

As was the case for increased UV dose, increases in the concentration of H_2O_2 in the UV/H_2O_2 combination treatments resulted in greater deactivation of all tested strains (Figs. 6 and 7). ATCC 25922 was the most sensitive and no growth was detected at a dose of 200 mg.L^{-1} while reductions of 2.40 and 4.10 was achieved for F11.2 and MJ56, respectively, under the same conditions (Fig. 6). Environmental strain F11.2 was again the most resistant strain and showed a significant difference ($p<0.05$) of only 1.08 log reductions between the highest (200 mg.L^{-1}) and lowest (2.5 mg.L^{-1}) concentrations of H_2O_2 (Fig. 6). The corresponding value for MJ56 is estimated at 1.32 (Fig. 6).

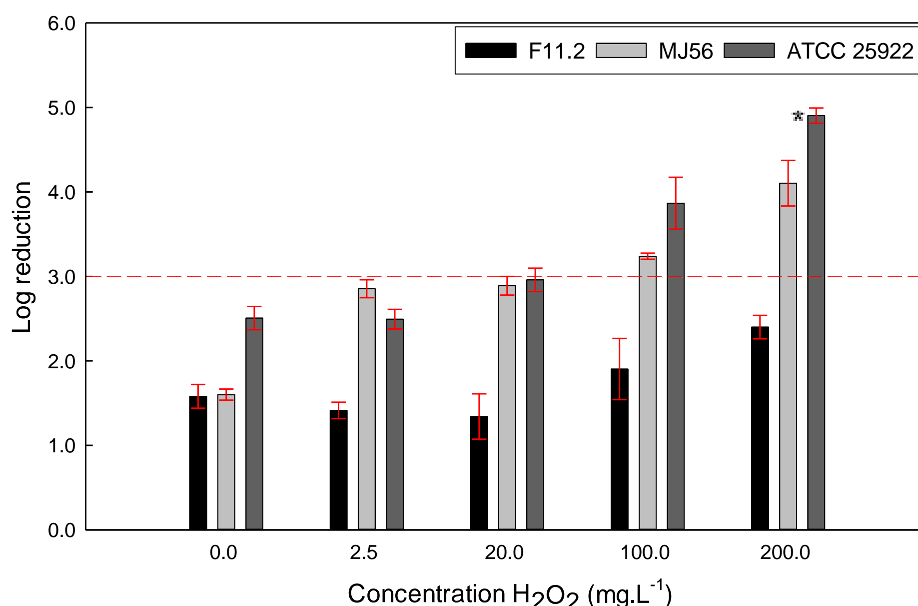

Figure 6 Effect of varying H_2O_2 concentrations used in combination with a UV dose of 4 mJ.cm^{-2} on the survival of three *E. coli* strains in SSS. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

* To the left of the bar indicate inactivation of the entire microbial population.

When considering the 3 log target, a 4 mJ.cm^{-2} UV treatment combined with H_2O_2 concentration of 20 mg.L^{-1} resulted in reductions of 2.89 and 2.96 for ATCC 25922 and MJ56, respectively (Fig. 6). When the H_2O_2 concentration was increased to 100 mg.L^{-1} the target reduction was easily reached. For F11.2, a 3 log reduction could not be achieved at a UV dose of 4 mJ.cm^{-2}, even in the presence of 200 mg.L^{-1} H_2O_2 (Fig. 6). Noteworthy is the fact that MJ56 is clearly more sensitive to UV treatment in the presence of low concentrations of H_2O_2. A significantly increased log reduction ($p<0.05$) of 1.20 was achieved by the addition of 2.5 mg.L^{-1} of the biocide before UV irradiation (Fig. 6). Again, this might be explained by referring to its low HP1 activity which restricts the strain to protect itself against low H_2O_2 concentrations. The log reduction achieved for MJ56 at 20 mg.L^{-1} H_2O_2 did, however, not differ significantly ($p=0.85$) from that observed at 2.5 mg.L^{-1} (Fig. 6) which indicates that the reaction conditions for effective AOP processes must be carefully optimised for maximum efficiency. As the concentration was

increased further, disinfection potency was also greater with a maximum reduction of 4.10 log achieved at 200 $mg.L^{-1}$ for strain MJ56.

For strains F11.2 and ATCC 25922, respectively, a 2.5 $mg.L^{-1}$ concentration of H_2O_2 had no statistically significant effect on log reduction in comparison to 0.0 $mg.L^{-1}$ ($p=0.32$ and $p=0.94$) (Figs. 6 and 7). It was only at higher concentrations (> 2.5 $mg.L^{-1}$) where greater log reductions were observed for these two strains. This is apparent for ATCC 25922 in particular. Significant increases ($p<0.05$) in the initial log reductions of 0.47, 0.91 and 1.03 log, respectively, were achieved when the H_2O_2 concentration was increased from 2.5 to 20 $mg.L^{-1}$, 20 to 100 $mg.L^{-1}$ and 100 to 200 $mg.L^{-1}$. Giddey *et al.* (2015) reported that ATCC 25922 shows high levels of HP1 activity (approximately 230 U) while HP2 was absent. It was, therefore, expected that the strain would be resistant to low concentrations of H_2O_2. Further, it is speculated that HP2 might also aid in protecting bacteria against high concentrations of the chemical in addition to its expression in the stationary phase and the presence of other stress factors. In this regard, the sensitivity of ATCC 25922 to higher H_2O_2 concentrations is explained. F11.2 showed fairly low and consistent log reductions as the concentration of H_2O_2 was increased from 2.5 to 200 $mg.L^{-1}$ (Fig. 6). This strain represents high activity of both HP1 and HP2 which enhances its general resistance to the range of tested H_2O_2 concentrations (Giddey *et al.*, 2015). These results indicate that the efficiency of the UV/H_2O_2 combination, be it via advanced oxidation or the multiple damage strategy (combining the individual biocidal effects of UV and H_2O_2), is certainly strain-specific.

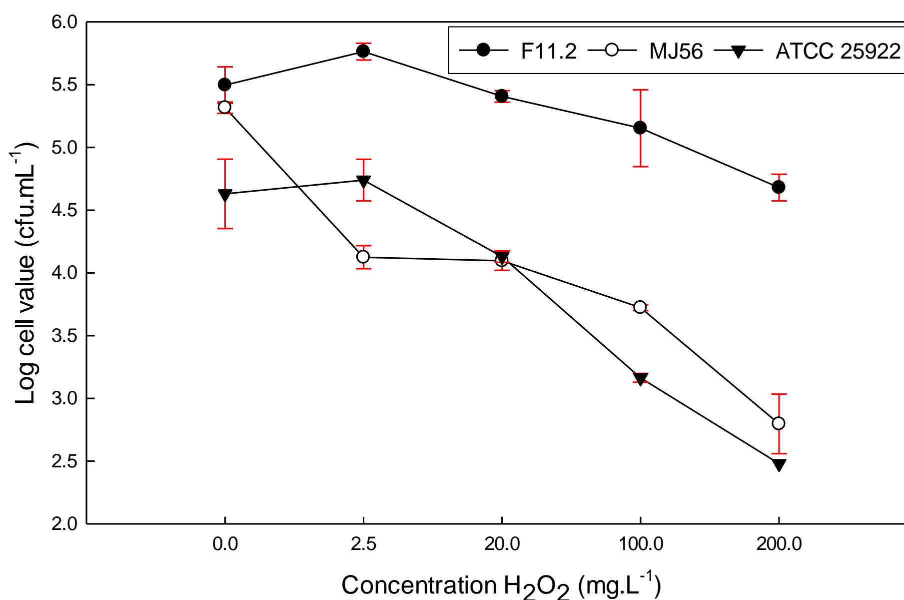

Figure 7 Effect of H_2O_2 concentration and a UV dose of 4 $mJ.cm^{-2}$ on the number of viable microorganisms following the application of UV/H_2O_2 combination treatments in SSS. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

Comparing results presented in Figures 4 and 6 it is evident that UV light as single treatment is capable of generating adequate (> 3 log) reductions for the most resistant strains. For

F11.2, reductions achieved at a UV dose of 8 $mJ.cm^{-2}$ and upwards were all better in comparison to the combination of 4 $mJ.cm^{-2}$ with H_2O_2 concentrations of 2.5, 20, 100 and 200 $mg.L^{-1}$. As a matter of fact, a reduction of 2.90 log was achieved using 8 $mJ.cm^{-2}$ while a 2.40 log reduction only was reached using a UV/H_2O_2 combination treatment of 4 $mJ.cm^{-2}$/200 $mg.L^{-1}$.

Study B: Influence of water quality on UV and UV/H_2O_2 disinfection potential

In order to evaluate the influence of water quality on the effectiveness of UV and UV/H_2O_2 treatments river water was flocculated using a commercial polymeric coagulant. The data presented in Table 5 indicates that the addition of coagulant at a concentration of 7 $mg.L^{-1}$ had a slight influence on the physico-chemical properties of the water. Results further show that both untreated and flocculated river water mostly met the guidelines regarding the physico-chemical qualities of water intended to be used for fresh produce irrigation (Table 2 and Table 5). Both samples, however, represented conductivity values exceeding the limit suggested by DWAF (1996) (Table 2 and Table 5) and was classified as being unfit for the use of fresh produce irrigation.

Table 5 Physico-chemical properties of untreated and flocculated river water

	Water sample	
Water quality parameter	**Untreated**	**Flocculated**
Chemical Oxygen Demand (COD) ($mg.L^{-1}$)	45.00	37.80
Ultraviolet Transmission Percentage (UVT%)[a]	54.70	62.70
Ultraviolet Transmission Percentage (UVT%)[b]	56.00	62.00
Turbidity (NTU)	14.68	07.22
Total Suspended Solids (TSS) ($mg.L^{-1}$)	21.00	19.50
Conductivity ($mS.m^{-1}$)	67.00	49.00
Total Dissolved Solids (TDS) ($mg.L^{-1}$)	435.50	318.50

[a]Measured using the hand held Sense™ T UV-Transmittance Monitor (Berson, The Netherlands)

[b]Measured using the UVT-15 UV% Transmission Photometer (HF Scientific, USA)

Following flocculation and filtration the COD of the river water decreased by 7.20 $mg.L^{-1}$, from 45.00 to 37.80 $mg.L^{-1}$ (Table 5). An increase of 8.00% and 6.00% in UV transmission was recorded for the same sample using the hand-held and UVT-15 UV% Transmission photometers, respectively. As was expected, the turbidity of the water was also lowered (by 7.46 units from 14.68 to 7.22 NTU). While the suspended solids content decreased only slightly, the conductivity was influenced more profoundly with a difference of 18.00 $mS.m^{-1}$ observed between the untreated and flocculated water. Electrical conductivity (EC) was measured in order to obtain an indication of the amount of total dissolved solids (TDS) present in each sample. By the incorporation of conversion factors TDS could be determined from conductivity values since EC is directly proportional to the dissolved solids concentration of a sample (DWAF, 1996). This method is

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routinely used in laboratories. The calculated TDS value for flocculated river water was 117 mg.L^{-1} lower than that of the untreated sample (Table 5).

It is well-known that the efficiency of UV disinfection is meaningfully influenced by the characteristics of the water to be treated (Salgot *et al.*, 2002; Selma *et al.*, 2008; Brahmi *et al.*, 2010; Howe *et al.*, 2012). In the literature, it is particularly stated that those factors related to the optical properties of liquids (UV absorbance and transmission percentage (UVT%), turbidity, total suspended solids (TSS), organic matter content (measured as COD), particle size and total dissolved solids (TDS) content) are greatly influential in terms of the lethality of UV irradiation (Koutchma, 2009a; Brahmi *et al.*, 2010; Gayán *et al.*, 2014). In this regard, both suspended and soluble compounds may scatter, absorb or reflect UV light (Koutchma, 2009b). In agreement with this, the absorption coefficient (α) of liquids are related to the penetration depth reached at specific UV doses and are strongly linked to the efficiency of UV disinfection (Koutchma, 2009a; Gayán *et al.*, 2011). Gayán *et al.* (2011) reported on a linear relationship between α and the rate of inactivation achieved for *E. coli*. The changes in water quality observed following flocculation (Table 5) therefore indicate that such treatment might present advantages in terms of UV and UV/H_2O_2 disinfection. This would fundamentally result from increased UV transmission in the water. Where UV is used as stand-alone treatment increased transmission could lead to increased photon absorption by microbial contaminants. For the UV/H_2O_2 combination the same applies, with the added benefit of more UV light being available for the generation of reactive oxygen species from the H_2O_2. The lower COD value represented by the flocculated water may further favour the efficacy of the UV/H_2O_2 treatment since more of the generated reactive oxygen species will be available for the oxidation of microbial material rather than other organic contaminants within the sample.

The results presented in Figure 8 firstly show that for both F11.2 and ATCC 25922, respectively, significantly better log reductions ($p<0.05$) were achieved in SSS compared to untreated river water at a UV dose of 10 mJ.cm^{-2}. Differences in reductions were calculated as 1.09 and 1.12 log, respectively. The restraining impact of organic matter and other suspended and dissolved substances on UV lethality was thus confirmed in this study. The significance of this observation is highlighted by the fact that the target 3 log reduction could not be achieved for strain F11.2 in untreated river water (Fig. 8).

When the quality of the water was changed by means of flocculation no significant difference ($p=0.29$) in the efficacy of the UV treatment against F11.2 (in terms of log inactivation) was observed in comparison to that observed in the untreated water (Fig. 8). Reductions of 2.89 and 3.06 log were achieved in untreated and flocculated water, respectively. In comparison to F11.2, it is clear that the reference strain (ATCC 25922) was sensitive to the changes in water quality. Reductions of 3.66 and 4.54 log were reached in untreated and flocculated water, respectively. A difference of 0.88 log was thus attained. For ATCC 25922 then, similar reductions (no growth detected) were recorded following UV treatment in SSS and flocculated river water

(Fig. 8). These results imply that the reference strain was sensitive to the influence of slight variation in water quality while F11.2 seems to be oblivious towards these changes. Thus, although flocculation was capable of enhancing water quality, the effect on the availability of UV photons for disinfection was too slight to have an impact on a resistant strain such as F11.2.

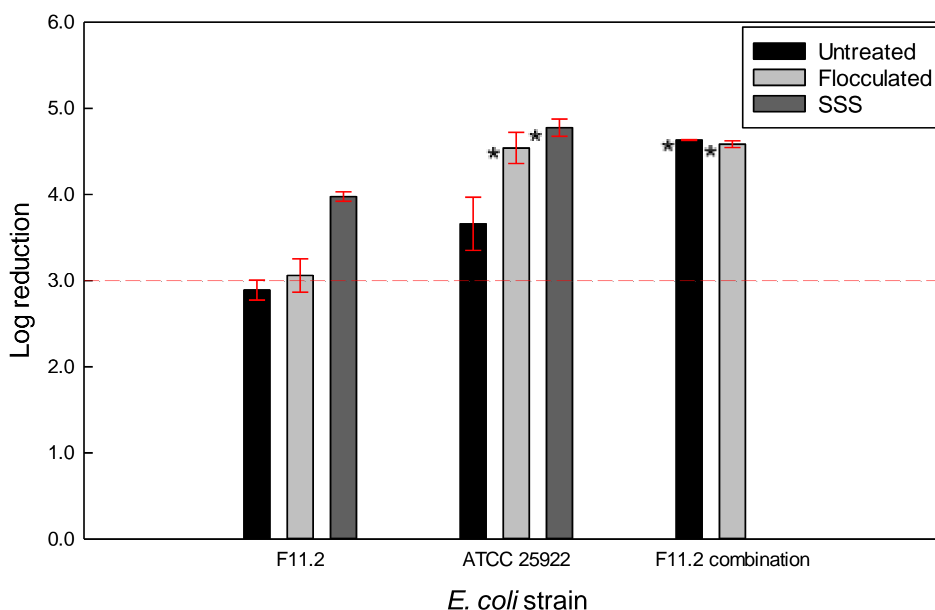

Figure 8 Log reduction of selected *E. coli* strains (F11.2 and ATCC 25922) exposed to a UV dose of 10 $mJ.cm^{-2}$ in SSS and untreated and flocculated river water and a UV/H_2O_2 combination treatment (F11.2 combination) in river water (untreated and flocculated). Error bars were calculated based on standard deviation at a confidence interval of 0.95.

* To the left of the bars indicate inactivation of the entire microbial population.

F11.2 combination – application of UV at 10 $mJ.cm^{-2}$ combined with H_2O_2 at a concentration of 200 $mg.L^{-1}$.

Recalling the results of Study A, it was concluded that a UV dose of 10 $mJ.cm^{-2}$ was required to achieve a 3 log reduction of F11.2 in SSS. ATCC 25922 was, however, much more sensitive with a 2.51 log reduction being achieved at a UV dose of 4 $mJ.cm^{-2}$ and complete inactivation (4.93 log reduction) occurring at 8 $mJ.cm^{-2}$. It was therefore expected that even a slight increase in the amount of available UV energy following flocculation would have a notable impact on the inactivation of strain ATCC 25922. This was clearly observed in the present study (Study B). In this regard, in both Studies A and B the time of exposure of water to UV light (to reach a specific UV dose) was calculated with reference to the UVT% of the water and essentially accounted for absorption effects that could restrict the delivered UV dose. The increased lethality of the treatment against strain ATCC 25922 (following flocculation in Study B), therefore, probably resulted from a lessened influence of scattering and/or blocking effects after the river water was flocculated and filtered. Flocculation and filtration could have resulted in the removal of larger particles from the water, for instance, thereby allowing a more direct disinfection effect. Referring to these results, water quality may have a tremendous impact on the efficacy of UV irradiation in

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practice. Slight changes in water quality could strongly influence the availability of UV photons on which the deactivation of resistant environmental strains are largely dependent.

When strain F11.2 was exposed to the UV/H_2O_2 combination treatment in untreated and flocculated river water complete inactivation was achieved in both samples (Fig. 8). In comparison to UV irradiation alone (10 $mJ.cm^{-2}$), the combination treatment (10 $mJ.cm^{-2}$/200 $mg.L^{-1}$) resulted in increased reductions of 1.74 and 1.51 log in the untreated and coagulated water, respectively.

Previously, in SSS, UV at a dose of 4 $mJ.cm^{-2}$ used in combination with 200 $mg.L^{-1}$ H_2O_2 resulted in a mere 2.40 log reduction of *E. coli* strain F11.2 (Study A). The meaningfully higher reduction (4.63 log in untreated water and 4.58 log in flocculated water) observed in this study (UV dose of 10 $mJ.cm^{-2}$ and 200 $mg.L^{-1}$ H_2O_2) must be related to the higher UV dose, particularly referring to the generation of reactive oxygen species through advanced oxidation. This result is interesting as one should keep in mind that the latter combination (using a higher UV dose) was performed in river water where physico-chemical properties may strongly influence treatment efficiency. Since complete inactivation of F11.2 occurred in both untreated and flocculated water samples, no conclusions regarding the effect of flocculation on reactive oxygen species (ROS) could be made. Nonetheless, the formation of ROS, when combination treatments are used, not only directly enhance the lethality of the treatment against *E. coli* but may also degrade additional organic contaminants. Subsequently, the potency of the available UV light will be enhanced (Tawabini *et al.*, 2013). In conclusion, the dynamics of UV/H_2O_2 combination treatments are extremely complex and further research is required to establish reaction conditions for optimal AOPs.

Study C: Comparison of methods for the enumeration of coliforms and total heterotrophic microorganisms

The use of different methods and media for the enumeration of the same microbial groups (TC, FC, HPC and *E. coli*) was evaluated and compared in order to standardise a procedure to be used in subsequent studies. This was done by determining microbial loads of the mentioned populations, in untreated river water, on three respective days (Days 1 – 3). Due to day-to-day variation in the microbial population present in the river water, methods could only be compared with reference to results that were obtained on the same day (from the same sample). Plating methods were performed in triplicate on each of the days using three 1 mL river water samples while membrane filtration was performed once a day using a 100 mL river water sample. Note that data recorded for the 1 mL samples (analysed using plating methods) were extrapolated to represent 100 mL samples.

Concerning the different techniques of enumeration it was expected that data recorded from VRBA plates incubated at 35°C (TC VRBA 35°C) would correlate well with that of TC enumerated on CES following membrane filtration (TC CES MF). On Day 1, TC levels of 7.02 and

7.40 log cfu.100 mL^{-1} were detected using the respective methods (TC VRBA 35°C and TC CES MF). The corresponding values for Days 2 and 3 were recorded as 6.76 and 7.01 log cfu.100 mL^{-1} and 6.94 and 7.24 log cfu.100 mL^{-1}, respectively (Fig. 9). The greatest difference observed between the methods, 0.38 log cfu.100 mL^{-1}, was thus recorded on Day 1. Differences of 0.25 and 0.30 log cfu.100 mL^{-1}, respectively, were observed on Days 2 and 3. For each of the three samples, the method using membrane filtration and CES (TC CES MF) resulted in the detection of greater cell concentrations (Fig. 9). Nonetheless, it was concluded that the two methods show good correlation as the difference between the detected cell concentrations were always lower than half (0.5) a log unit.

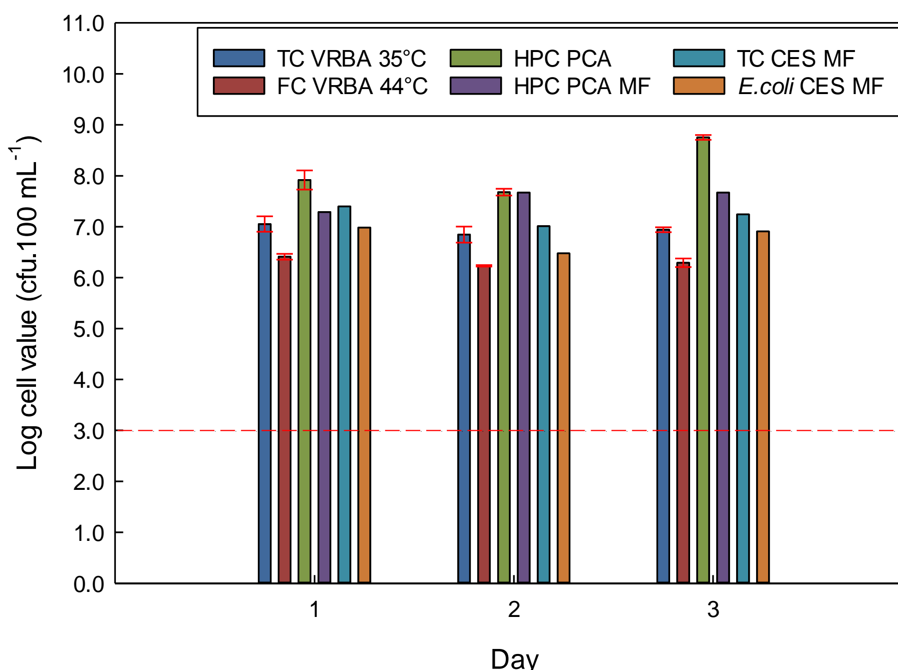

Figure 9 Microbial population size determined on three sampling days using different methods of enumeration**.** Error bars were calculated based on standard deviation at a confidence interval of 0.95.

TC – Total coliforms; FC – Faecal coliforms; HPC – Heterotrophic plate count.

MF – Membrane filtration.

VRBA – Violet Red Bile Agar; PCA – Plate Count Agar; CES - Chromocult® Coliform Agar Enhanced Selectivity.

According to literature, the majority of faecal coliforms, enumerated using VRBA incubated at 44°C (FC VRBA 44°C), are representative of *E. coli* (Schraft & Watterworth, 2005). It was therefore expected that the *E. coli* concentrations detected using the latter technique (FC VRBA 44°C) should correlate well with that determined using MF and CES (*E. coli* CES MF). On each of the three days, *E. coli* levels in the same log order were detected using the respective methods of enumeration (Fig. 9). On Day 1 values of 6.40 and 6.98 log cfu.100 mL^{-1} were recorded using the pour plate and MF techniques, respectively. For Days 2 and 3, the respective corresponding levels were determined as 6.24 and 6.48 log cfu.100 mL^{-1} and 6.34 and 6.91 log cfu.100 mL^{-1} (Fig. 9).

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Again, the greatest difference between the methods (0.58 log cfu.100 mL^{-1}) was observed on Day 1. For Days 2 and 3 differences of 0.24 and 0.57 log cfu.100 mL^{-1}, respectively, were recorded. In this case too, MF consistently resulted in the detection of higher levels of *E. coli* in comparison to the pour plate technique. It was also expected that TC levels would always exceed that of *E. coli* since *E. coli* is considered a subgroup of TC (Schraft & Watterworth, 2005). This implies that values for TC VRBA 35°C and TC CES MF should always be greater than those represented by FC VRBA 44°C and *E. coli* CES MF. For each of the three samples this was true, which confirms that the different methods are capable of distinguishing between TC and FC (*E. coli*).

When heterotrophic bacteria were enumerated using PCA, it was apparent that the pour plate technique (HPC PCA) resulted in higher levels being detected in comparison to MF (HPC PCA MF) (Fig. 9). Overall, differences between the two methods were also greater than those observed when TC and *E. coli* were estimated. The greatest difference (1.06 log cfu.100 mL^{-1}) was seen on Day 3 during which HPC levels were respectively recorded as 8.73 (HPC PCA) and 7.67 log cfu.100 mL^{-1} (HPC PCA MF). On Days 1 and 2, HPC levels of 7.86 and 7.29 log cfu.100 mL^{-1} (0.57 log difference) and 7.70 and 7.67 log cfu.100 mL^{-1} (0.03 log difference) were detected using the pour plate and MF methods, correspondingly.

The variability shown in the enumeration of the same microbial groups in the above mentioned results took into account the influence of different methods (pour plates and MF) as well as different media (VRBA, CES and PCA). In view of this, the reported differences between the techniques are regarded as being fairly small. However, it is important to take note thereof since the evaluation of UV disinfection efficiency may be slightly influenced depending on the technique used to enumerate specific microbial groups.

As suggested by Wohlsen *et al.* (2006), the absence of a standard reference inoculum (implicating enumeration by means of serial dilution) usually imparts some inconsistency in comparative studies such as the one discussed here. Variation in the calculated differences observed between the pour plate and MF methods (for TC, FC and HPC) could thus be as a result of such typical influences. Nevertheless, for the respective microbial groups, the same tendency was observed on each of the three days. With reference to the two techniques used (pour plate and MF) Wholsen *et al.* (2006) further found that MF detected mean *E. coli* counts that were significantly lower than the expected mean which was calculated with respect to a standardised inoculum. Pour plates, alternatively, produced means that were within the calculated specification. Membrane filtration also showed much higher than expected variability between replications (n=10). In the current study, however, higher concentrations of TC and FC were detected using MF in comparison to pour plates. This contrasting result could possibly be attributed to the use different media for enumeration. On the other hand, when heterotrophic microorganisms were enumerated on PCA, using the pour plate and MF techniques, the latter resulted in the detection of lower levels. This was in agreement with the findings of Wholsen *et al.* (2006).

In this study, two media were used for the enumeration of TC and *E. coli*. Violet Red Bile Agar (VRBA) is a solid medium containing lactose as fermentable carbohydrate together with neutral red as pH indicator. It utilises crystal violet and bile salts to inhibit the growth of gram-positive bacteria while the production of acid from lactose fermentation results in coliform bacteria being observed as red colonies surrounded by zones of red precipitate (Merck, 2005). Due to its inability to discriminate between *E. coli* and other coliforms, however, various media containing chromogenic substrates have now been developed to overcome this issue. Such products are becoming increasingly popular for the simultaneous quantification of coliforms and *E. coli* in surface water (Hallas *et al.*, 2008; Wohlsen *et al.*, 2008).

The Chromocult® Coliform Agar Enhanced Selectivity (CES) used in this study combines two substrates, Salmon™-β-D-GAL and X-β-D-glucuronide, to achieve the above mentioned task. The first is split by β-D-galactosidase (found in coliforms) resulting in coliform colonies being observed as having a salmon to red colour. The X-β-D-glucuronide substrate is capable of cleaving the β-D-glucuronidase (found in *E. coli*) resulting in a blue colour. Since *E. coli* will split both Salmon™-β-D-GAL and X-β-D-glucuronide colonies turn dark violet in colour and are easily distinguished from the salmon-red coliforms (Merck, 2005).

In view of the media used for coliform and *E. coli* enumeration in this study (as described above) researchers have reported on differences between standard and chromogenic products. Alonso *et al.* (1999), for instance, observed statistically significant differences ($p<0.05$) between such media when thermotolerant coliform levels in river and marine water samples were enumerated. Furthermore, false positive results have been reported following the use of chromogenic agars as a result of interference by *Aeromonas* spp. and *Vibrio* spp. (Geissler *et al.*, 2000). In such case, the levels of TC and *E. coli* would possibly be overestimated. In another study, the use of chromocult coliform agar produced violet colonies (possibly *E. coli*) which showed a slight pink colour that was very similar to that of TC colonies (Wang & Wanda, 2008). If this would occur, the detection of both TC and *E. coli* would be inaccurate. Adding to this, Maheux *et al.* (2008) evaluated the ability of Chromocult® Coliform Agar Enhanced Selectivity (CES) to detect an array of TC and *E. coli* strains. A fairly high level of false-negative detection of *E. coli* was observed, indicating that the method may be inappropriate for the identification of probable *E. coli* strains (Maheux *et al.*, 2008). Notwithstanding these results, a large number of studies reported on similar efficiencies observed for test methods such as Colilert, Readycult and those using MI and Chromocult agar in comparison to standard reference methods (Macy *et al.*, 2005; Bernasconi *et al.*, 2006; Hörman & Hänninen, 2006).

Having said all of this, VRBA and CES make use of different analytical principles for the enumeration of TC and FC (*E. coli*). While VRBA detects fermentation of lactose, the chromogenic agar simultaneously detects cleavage of β-D-glucuronidase and the activity of β-D-galactosidase. Both of these selective features are shared by coliforms, however, differences in methodology are expected to influence the obtained results (González *et al.*, 2003). In subsequent laboratory-scale

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experiments performed in this study, enumeration before and after disinfection was executed using the pour plate technique. This method requires the use of smaller sample volumes, consequently simplifying the analysis. Also, VRBA was selected as medium to be used for the enumeration of TC and FC. Despite the possible advantages presented by chromogenic media, discrepancies found in previous research, and the cost related to its use, were key in this decision. Furthermore, supporting research performed by Wohlsen *et al.* (2006) concluded that methods used to enumerate volumes of 1 mL delivered more accurate results in comparison to those such as MF which require volumes of 100 mL.

Study D: UV disinfection of filtered river water

River water analysis

Due to the fact that water quality may influence UV disinfection efficiency, and the need for fresh produce irrigation water to comply with guidelines set by the Department of Water Affairs (DWAF, 1996), a range of parameters was determined. As indicated by the results presented in Figure 9 and Table 6 it is clear that the Plankenburg River carries extremely high levels of microbial contaminants. The detected levels of TC and FC, as well as *E. coli,* always exceeded the recommended limit set for FC in water intended to be used for fresh produce irrigation (1 000 cfu.100 mL^{-1}/3 log cfu.100 mL^{-1}) (Fig. 9 and Table 6) (DWAF, 1996). In fact, contamination levels were so high that a reduction exceeding 3 log would be required to yield water that is fit for this purpose. The highest level of FC (6.41 log) was detected in Trial 1 and in this instance a reduction of 3.41 log would be required to produce water of acceptable microbiological quality. These findings are in agreement with that of previous research.

Work performed by Barnes & Taylor (2004) showed that the Plankenburg River represented FC levels of up to 1.74 x 10^7 cfu.100 mL^{-1}. Later on, Paulse *et al.* (2009) and Ackerman (2010) reported on FC levels of 3.5 x 10^6 and 4.9 x 10^2 – 1.6 x 10^5 cfu.100 mL^{-1}, respectively, indicating the persistence of the problem. Furthermore, Huisamen (2012) investigated microbial contamination of the Eerste and Plankenburg Rivers which are both located in the area of Stellenbosch. It was reported that FC counts reached 7 x 10^6 cfu.100 mL^{-1} in the latter. Counts ranging between 230 and 3 300 cfu.100 mL^{-1} were found in the Eerste River. Concerning these extreme levels of faecal pollution, the informal settlement of Kayamandi (which is situated close to the Plankenburg River) has been suggested as major source of contamination (Paulse *et al.*, 2009). Inadequate waterworks and poor service delivery in the area are key drivers of the problem. Referring to these results in the literature, and those of the current study, the urgent need for an effective method for river water disinfection is once again emphasised.

Considering the physico-chemical properties of the water the conductivity exceeded the suggested limit (Table 2) in each of the three trials (Table 6). The highest value (60 $mS.m^{-1}$) was recorded for Trial 1. For Trials 2 and 3, very similar levels were detected. According to DWAF

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(1996) these results indicate that a high level of inorganic salts may be dissolved in the water since EC is directly proportional to the total dissolved solids (TDS) content. Irrigation water containing salt, in particular, may lead to the formation of saline soil which, in turn, could result in poor crop yields. Although it would be economically unrealistic, the salt concentration of irrigation water could be reduced by means of technologies such as ion exchange and reverse osmosis (DWAF, 1996). Previous research has also indicated high levels of dissolved solids in the Plankenburg River. Van Blommestein (2012) reported on conductivity values ranging from 28 – 41 $mS.m^{-1}$ in samples taken from this river. Furthermore, higher levels (49 – 72 $mS.m^{-1}$) were recorded by Lötter (2010).

Table 6 Physico-chemical and microbiological properties of filtered river water prior to the execution of disinfection experiments

Quality Parameter	Trial 1	Trial 2	Trial 3
UVT%	36.00	35.05	37.60
COD ($mg.L^{-1}$)	96.30	46.80	63.00
Turbidity (NTU)	24.50	15.84	25.60
TSS ($mg.L^{-1}$)	29.00	18.00	25.00
VSS ($mg.L^{-1}$)	25.00	14.00	19.00
pH	7.23	7.42	7.29
Alkalinity (mg $CaCO_3.L^{-1}$)	118.00	118.00	131.00
Conductivity ($mS.m^{-1}$)	60.00	47.00	48.00
Faecal coliforms (FC) (log cfu.100 mL^{-1})[a]	6.41	6.23	6.29
Total coliforms (TC) (log cfu.100 mL^{-1})[b]	7.07	6.87	6.94
Heterotrophic plate count (log cfu.100 mL^{-1})[c]	7.94	7.68	8.75

[a] Determined using VRBA (pour plate technique) and incubation temperature of 44°C

[b] Determined using VRBA (pour plate technique) and incubation temperature of 35°C

[c] Determined using PCA (pour plate technique) and incubation temperature of 30°C

All other parameters met the guidelines regarding physico-chemical quality of water intended to be used for fresh produce irrigation (Table 2) (DWAF, 1996). In Trial 1, higher values for COD, Turbidity, TSS, VSS and conductivity were observed in comparison to Trials 2 and 3. Since these parameters include an array of absorbing substances, it was expected that UV transmission would be lower in comparison to that of the water analysed on Days 2 and 3. This was not the case, however, and UVT% was consistently low.

In Trial 2 the turbidity, TSS content and COD of the water were low in comparison to other days. This water was of the highest quality, so to speak. Again, the UVT% was not pointedly better as would be expected. As a matter of fact, it was for Trial 2 that the lowest UVT% (35.05%)

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was measured. This implies that the differences in water quality between the trials were too small to have a clear impact on the penetration/transmission of UV light. In spite of these results, a correlation was observed between the levels of COD, Turbidity, TSS and VSS in each of the respective trials. Referring to the influence of water quality, it was expected that comparable effectiveness for the respective treatments applied in each trial would be observed.

Efficacy of laboratory-scale LP UV irradiation for river water disinfection

Following UV treatment in Trial 1, enhanced disinfection as the UV dose was increased from 5 to 10 $mJ.cm^{-2}$ was clearly observed for TC, FC and heterotrophic microorganisms (bacteria) (Fig. 10). For TC and the heterotrophic population maximum reductions of 2.16 and 1.20 log, respectively, were recorded when a dose of 10 $mJ.cm^{-2}$ was applied. For the FC population, however, an UV dose of 7 $mJ.cm^{-2}$ resulted in slightly greater reductions (in comparison to 10 $mJ.cm^{-2}$) with values of 1.97 and 1.83 log being reached. Nevertheless, the difference of 0.14 log was statistically not significant ($p=0.32$). Furthermore, significant differences in log reductions ($p<0.05$) were observed between the lowest (5 $mJ.cm^{-2}$) and highest (10 $mJ.cm^{-2}$) UV dose for all enumerated groups (Fig. 10). These were calculated as 0.77, 0.69 and 0.82 log for TC, FC and heterotrophic organisms, respectively.

Considering the different microbial groups, it was observed that the greatest reduction was achieved for TC, followed by FC and heterotrophic microorganisms for most treatments. Nevertheless, with reference to Figure 10 it is seen that the difference in log reductions achieved for TC and FC at the respective UV doses were mostly insignificant ($p>0.05$). It was only at a UV dose of 10 $mJ.cm^{-2}$ where TC were significantly better inactivated ($p<0.05$) compared to FC in terms of log inactivation. At a dose of 5 $mJ.cm^{-2}$ TC were reduced by 1.39 log units. At the maximum dose the greatest reduction (2.16 log) was again recorded for the same group (Fig. 10). At 7 $mJ.cm^{-2}$, however, the largest reduction (1.97 log) was observed for FC but compared to TC, the log difference was insignificant ($p=0.08$). Regardless of the fact that similar inactivation of TC and FC was observed for most treatments, the levels of TC remaining following UV irradiation were generally greater compared to FC. At the maximum tested UV dose (10 $mJ.cm^{-2}$) TC and FC concentrations of 5.05 and 4.85 log $cfu.100\ mL^{-1}$, respectively, were detected in the irradiated river water. This was expected as higher levels of TC were detected initially in the untreated water (Table 6).

The total heterotrophic bacteria population, in particular, was very resistant to LP UV irradiation with a maximum reduction of 1.20 log being reached at a dose of 10 $mJ.cm^{-2}$. Corresponding values for irradiation at doses of 5 and 7 $mJ.cm^{-2}$ were recorded as 0.38 and 1.08 log, respectively. Thus, compared to TC and FC, each of the treatments resulted in significantly lower log reductions ($p<0.05$) of the heterotrophic population. These results indicate that within the heterotrophic population, microorganisms that are much more resistant to UV irradiation (in comparison to coliforms and *E. coli*) are present. Nonetheless, the similar inactivation recorded for

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total coliforms and faecal coliforms (*E. coli*) indicates similar susceptibility of the two groups to UV disinfection.

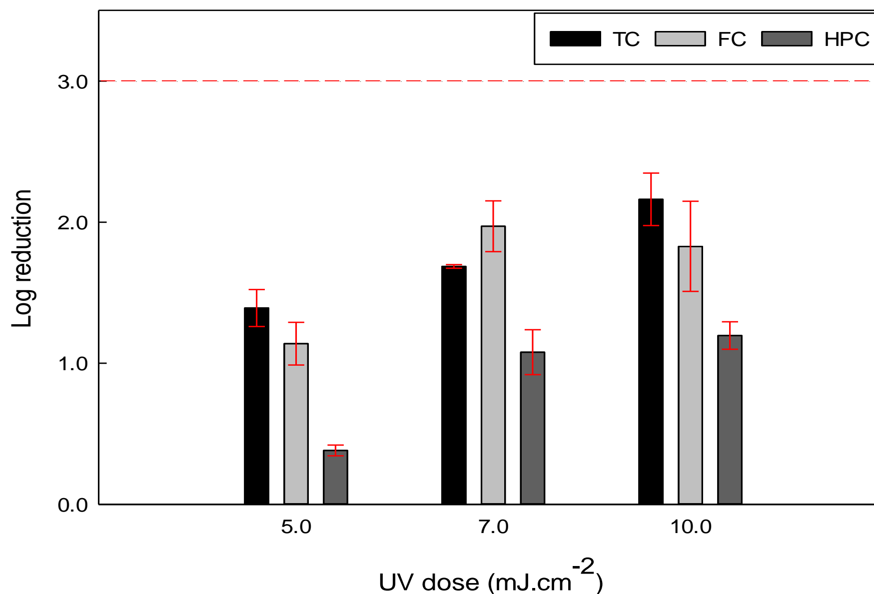

Figure 10 Microbial reductions achieved at three doses (5, 7 and 10 mJ.cm^{-2}) following laboratory-scale LP UV irradiation during experimental Trial 1. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

In this trial (Trial 1) it was observed that the suggested 3 log target reduction (indicated by the dotted red line) was never met. Besides, considering the initial FC population size (6.41 log cfu.100 mL^{-1}) (Table 6), reduction exceeding 3 log would be required to produce water that would be suitable for the irrigation of fresh produce. Looking at the results of this trial, treatment at the highest dose would thus be insufficient in reducing the level of FC to within the recommended 1 000 cfu.100 mL^{-1} (DWAF, 1996). This implies that doses greater than 10 mJ.cm^{-2} would be required to achieve adequate disinfection should the water to be treated be contaminated to the extent of that observed here. As is clear in Table 6, TC, FC and HPC concentrations detected in Trials 2 and 3 were comparable to that measured in Trial 1. In the discussion of coming results, it would, therefore, be important to emphasise the disinfection efficiency of UV at the highest dose (10 mJ.cm^{-2}), rather than elaborating on the effect of lower doses.

In the second trial, trends that were very similar to those observed in Trial 1 were seen. As was expected, increased UV doses again resulted in better disinfection (Fig. 11). Also, significant differences in log reductions ($p<0.05$) achieved between the lowest (5 mJ.cm^{-2}) and highest (10 mJ.cm^{-2}) UV doses were recorded for each of the enumerated microbial groups. These values were calculated as 1.19, 1.01 and 0.68 log for TC, FC and heterotrophic organisms, respectively. Maximum reductions for the same groups were calculated as 2.21, 2.08 and 1.36 log. In comparison to Trial 1, each of the microbial groups were thus reduced to a slightly greater extent. However, the 3 log target reduction was once again not reached (Fig. 11). As was observed in

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Trial 1, results show that the treatment will not be able to reduce the initial FC load of 6.23 log cfu.100 mL^{-1} (Table 6) to within the limit of 1 000 cfu.100 mL^{-1} (DWAF, 1996).

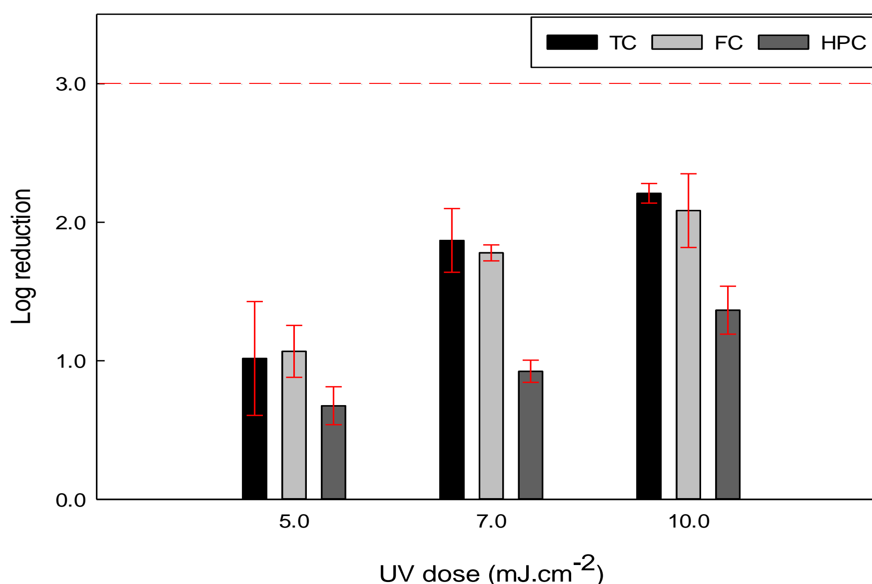

Figure 11 Microbial reductions achieved at three doses (5, 7 and 10 mJ.cm^{-2}) following laboratory-scale LP UV irradiation during experimental Trial 2. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

Furthermore, tendencies similar to those detected for the different microbial groups in Trial 1 were again seen. Total coliforms were most sensitive while the total heterotrophic bacteria population was the most resistant. While the differences in log reduction recorded for TC and FC were always insignificant ($p>0.05$), corresponding values for the heterotrophic population were mostly significantly lower ($p<0.05$). However, at a dose of 5 mJ.cm^{-2} the difference in log reduction achieved for the heterotrophic population was not significantly lower ($p>0.05$) compared to TC and FC, respectively. For the former group (HPC) reductions of 0.68, 0.92 and 1.36 log were recorded following irradiation at 5, 7 and 10 mJ.cm^{-2}, respectively. This was 33, 51 and 38% and 36, 49 and 35% lower than the corresponding values determined for TC and FC, respectively, at the same doses.

Results recorded for the third trial clearly deviated from those discussed for Trials 1 and 2. As the dose was increased from 5 to 7 mJ.cm^{-2} very similar reductions were achieved for TC and FC, separately. Values of 1.87 and 1.88 log and 1.23 and 1.22 log were recorded for the respective groups at 5 and 7 mJ.cm^{-2} (Fig. 12). Thus, for neither TC nor FC, 7 mJ.cm^{-2} resulted in significantly better log reductions compared to 5 mJ.cm^{-2} ($p=0.98$ and $p=0.94$) For the heterotrophic population it was observed that a lower degree of inactivation (0.83 log reduction) was achieved at 7 mJ.cm^{-2} in comparison to that achieved at 5 mJ.cm^{-2} (1.09 log reduction). The difference was, however, statistically insignificant ($p=0.23$). Increased UV doses resulted in increased inactivation of heterotrophic microorganisms in Trials 1 and 2 (Figs. 10 and 11).

Interestingly, the difference in reductions achieved for TC and FC in Trial 3 were greater in comparison to that observed at the same doses in previous trials, especially at 5 and 7 mJ.cm^{-2} (Fig. 12).

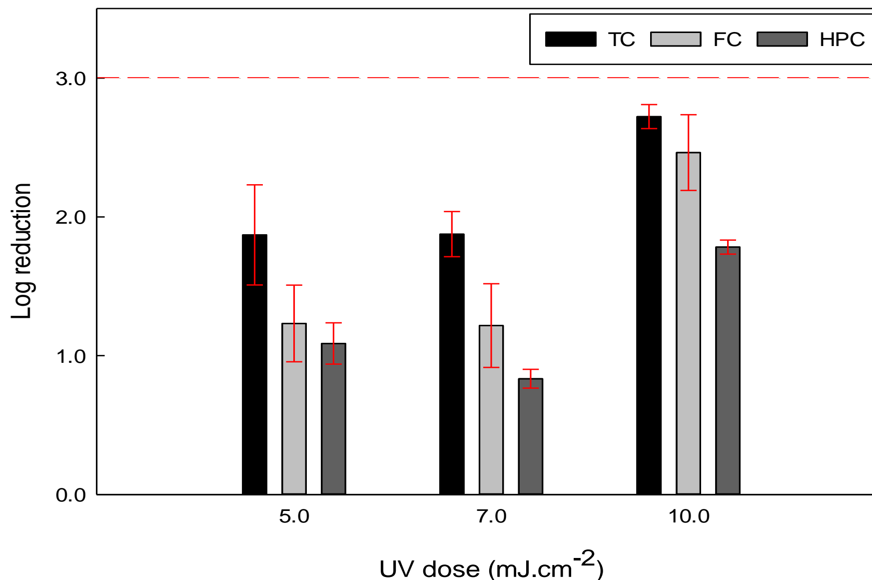

Figure 12 Microbial reductions achieved at three doses (5, 7 and 10 mJ.cm^{-2}) following laboratory-scale LP UV irradiation during experimental Trial 3. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

It was only at 10 mJ.cm^{-2} where significantly better log reductions ($p<0.05$) were reached for TC, FC and the heterotrophic population compared to UV doses of 5 and 7 mJ.cm^{-2}, respectively. The respective significant differences ($p<0.05$) in the reductions achieved at the lowest and highest UV doses (5 and 10 mJ.cm^{-2}) were calculated as 0.85, 1.23 and 0.69 log for the three groups. Maximum reductions were recorded as 2.72, 2.46 and 1.78 log for TC, FC and heterotrophic microorganisms, respectively (Fig. 12). In contrast to Trials 1 and 2 then, the first increment of the UV dose (from 5 to 7 mJ.cm^{-2}) did not result in increased disinfection. However, greater reductions at the highest dose (compared to 5 and 7 mJ.cm^{-2}) were observed for each of the enumerated groups. Also, in this trial (Trial 3), LP UV irradiation at 10 mJ.cm^{-2} was 21, 26 and 33% and 19, 15 and 24% more effective in comparison to similar treatment in Trials 1 and 2, respectively. Nonetheless, with reference to the limit set on FC, and the initial concentration of 6.29 log cfu.100 mL^{-1} (Table 6), the above mentioned maximum reduction (2.46 log) will not be sufficient in generating water that is acceptable for the irrigation of fresh produce. Regarding the different microbial groups, it was shown that TC were the most sensitive, followed by FC and the heterotrophic group. At 5 and 7 mJ.cm^{-2} log reductions achieved for TC were significantly greater ($p<0.05$) than that achieved for FC. However, at 10 mJ.cm^{-2} the difference in log reductions achieved for TC and FC was not statistically significant ($p=0.18$). Compared to TC and FC,

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significantly lower log reductions ($p<0.05$) were generally achieved for the heterotrophic bacteria population.

Based on the results achieved at the maximum dose evaluated in this study (10 $mJ.cm^{-2}$) LP UV irradiation was most effective in Trial 3. Poorer disinfection was observed in Trial 2 and the lowest lethality was recorded for Trial 1 (Table 7). While various factors may influence the efficiency of UV disinfection, the impact of water quality and microbiological properties are of primary concern.

Table 7 Log reductions achieved in river water following LP UV irradiation at a dose of 10 $mJ.cm^{-2}$ on three respective days

	Maximum reduction (Log)		
Microorganisms	**Trial 1**	**Trial 2**	**Trial 3**
TC	2.16±0.19	2.21±0.07	2.72±0.09
FC	1.82±0.30	2.08±0.27	2.46±0.27
HPC	1.20±0.10	1.36±0.17	1.78±0.05

TC – Total coliforms; FC – Faecal coliforms; HPC – Heterotrophic plate count

Considering the effect of changing water quality on UV disinfection (at the highest dose of 10 $mJ.cm^{-2}$) it was seen that the achieved microbial reductions often differed with respect to the different days of treatment (Table 7). Trial 3 clearly represented better disinfection efficiency, especially in comparison to that achieved in Trial 1. As a matter of fact, significantly greater log reductions ($p<0.05$) were achieved at a dose of 10 $mJ.cm^{-2}$ for TC, FC and HPC in Trial 3 compared to both Trials 1 and 2. This variability in disinfection efficiency (on different days) may possibly be attributed to the ever changing physico-chemical properties of the river water. Nevertheless, comparable reductions were reached on some days for TC, FC and the heterotrophic population, respectively. For instance, the difference in log reductions achieved for TC and FC, respectively, in Trials 1 and 2 was not statistically significant ($p=0.78$ and $p=0.11$) at a UV dose of 10 $mJ.cm^{-2}$.

In essence, UVT% indicates the amount of UV light absorbed by water and its different components and is defined as a function of the absorption coefficient (α) of the treated substance ($UVT = 100 \times 10^{-\alpha}$). Low UVT% indicates high absorption and results in the delivery of lower UV doses (Bolton & Cotton, 2008). Thus, decreased UVT% is expected to correlate with higher levels of water quality parameters such as COD, turbidity, TSS and total dissolved solids (TDS) which contribute absorbing substances (Koutchma *et al.*, 2009a; Brahmi *et al.*, 2010). In this laboratory-scale study, however, the applied UV dose was corrected with reference to the UVT% and the depth of the sample to be irradiated, as described by Morowitz (1950). This implies that, irrespective of water quality, the same UV dose was always delivered. It was thus expected that reductions recorded for TC, FC and the heterotrophic population (following the three trials),

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respectively, would be fairly similar regardless of differences in the levels of absorbing substances on the different days of treatment.

Looking at water quality, the UVT% recorded for Trial 3 (37.60%) was higher than that recorded for Trial 1 (36.00%) and correlated with lower COD, TSS, VSS and conductivity values (Table 6). The turbidity of the water, however, was slightly higher in the case of Trial 3. In view of this, it was explained earlier that the applied UV dose in each of the trials compensated for the influence of variability in UV transmission (UVT%). This implies that the influence of absorbing substances on the delivered UV dose was accounted for on the different days of UV treatment. As a result, the mentioned differences in the achieved reductions must be attributed to the influence of effects other than UV light absorption by the components present in the water. In this regard, some particulate substances in water may influence UV disinfection as a result of the light being scattered and/or blocked, as opposed to being absorbed. Particularly, interfering effects termed shading and encasement have been described by Howe *et al*. (2012). The latter implies that microorganisms may associate with particulate material and particles size may therefore also be influential (Walters *et al*., 2014). In the literature, it has been reported that higher levels of suspended solids, specifically, lower the efficiency of UV disinfection of water due to such impacts (Walters *et al*., 2014). In this regard also, the effect of turbidity on disinfection efficiency has also been investigated and proven to impede UV lethality (Christensen & Linden, 2003; Jones *et al*., 2014).

As mentioned, lower values for COD, TSS, VSS and conductivity were recorded for Trial 3 in comparison to Trial 1. Also, the measured values for turbidity were very similar (Table 6). Considering the influence of scattering and blocking effects on the number of photons available for inactivating microorganisms, it was thus expected that better inactivation would be observed in Trial 3. This was true and reductions that were 0.56, 0.64 and 0.58 log greater (compared to Trial 1) were recorded for TC, FC and the heterotrophic population, respectively, in Trial 3. If, however, variation in log inactivation resulted from these effects (scattering and/or blocking), one would expect to observe even larger differences between reductions achieved in Trials 1 and 2 as the latter represented the lowest levels of all influential parameters (Table 6). This was not the case and only slightly better inactivation was observed in Trial 2. The maximum efficiency observed in Trial 3 therefore indicates that factors other than water quality will influence the efficiency of LP UV irradiation. In the broader sense, the differences in water quality for the three trials were not that vast, as is confirmed by the comparable values of UVT%. Also, in the majority of instances, the physico-chemical properties adhered to the suggested limits for water to be used for irrigation. These results, therefore, complicate the discussion of the effect of water quality on the efficacy of laboratory-scale LP UV disinfection.

The disinfection efficiency of UV light (at a constant dose) may also be influenced by variability in the characteristics of the irradiated microorganisms. In this study, contaminated river water was sampled on different days, implying that the microbial population, unquestionably, were

heterogenic. In addition to the effect of water quality, the significant differences ($p<0.05$) observed in the inactivation of the respective microbial groups on different days may thus be related to variation in microbial properties. In this regard, both intrinsic and extrinsic factors will determine UV sensitivity (Gayán *et al.*, 2014). Therefore, UV resistance will vary amongst different types, species and strains. Intrinsic parameters, including cell and genome size, cell wall thickness and pigmentation may contribute to variation in the effectiveness of UV irradiation amongst microorganisms. The most influential extrinsic microbial properties, in terms of UV disinfection in this study, include conditions for growth and growth phase, as well as stress factors prior to irradiation.

Variation in the reductions achieved for TC and FC on the different days of treatment indicates that within the coliform group present in the Plankenburg River, species and strain variation were a given (Figs. 10 to 12). As expected, this was also true for the total heterotrophic population. Regardless of water quality, the microbial population irradiated in Trial 1 was the most resistant, followed by that of Trials 2 and 3. At 10 $mJ.cm^{-2}$, the achieved maximum reductions for TC, FC and the heterotrophic populations, respectively, were always lower in the first trial (Table 6). It is thus possible that the population irradiated on that day was inherently more resistant to UV irradiation in comparison to that in the other samples. From this point of view, the population investigated in Trial 3 was the most sensitive to UV light.

Additionally, exposure to stressors, including acid, alkali, oxidative conditions and starvation, before UV irradiation may have contributed to the greater resistance observed for the coliforms in Trial 1. These external influences may be significant considering that the river water characteristics on the different days of treatment will directly influence the growth phase of the microorganisms. In the literature, it has been reported that stationary-phase cells show increased UV resistance in comparison to their actively growing counterparts (Gayán *et al*, 2014). In this regard, it has been stated that the higher UV resistance observed for gram-negative bacteria, as growth rate decreases, is related to the stimulation of the general stress response sigma factor RpoS (s^{38}) (Child *et al.*, 2002; Berney *et al.*, 2006; Bucheli-Witschel *et al.*, 2010). Thus, should the river water population be exposed to environmental stress before UV irradiation, it may enhance its resistance by means of co-protective adaptation reactions (Van der Veen & Abee, 2011).

In this study, untreated water was sampled from the Plankenburg River which is exposed to several probable sources of pollution. The sampling site utilised for this investigation was situated downstream of agricultural and residential areas, the informal settlement of Kayamandi as well as several industrial operations. It is therefore quite possible that the composition of the river water varied extensively, depending on the level of pollution brought about on different sampling days. Informal settlements are reported to predominantly contribute untreated sewage and surface runoff to natural waters (Barnes, 2003). In addition to faecal pollution, it is speculated that this may introduce chemical residues and detergents into river water, possibly putting the microbial population under stress. In support of this statement, Jackson *et al.* (2009) reported that upstream

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of the sampling site, a storm-water drain flows directly from Kayamandi into the Plankenburg River. It was suggested that the effluent of waste and household products may enter the river via this route. In effect, increased UV resistance (as a result of increased stress) may possibly be acquired by the microbial population occurring in the river.

Industrial activities, on the other hand, may introduce substances that could either promote or negate the growth of microbial populations occurring in the polluted river water. Therefore, based on the nature of pollution, microorganisms in the receiving water may either become more resistant or sensitive to UV irradiation. Industries situated upstream of the sampling site in this study included cheese, yoghurt and clothing manufacturers, as well as spray painting and other workshops (Barnes, 2003; Jackson *et al.*, 2009). The range of possible contaminants is thus extremely diverse. While the extent of faecal contamination in the Plankenburg River has been emphasised, previous research has, for instance, also reported on high levels of metal contamination in the same water. In 2009, aluminium and iron concentrations reaching levels of 48 and 14 363 $mg.L^{-1}$, respectively, have been detected. Some microorganisms may utilise these metals to perform important (growth related) functions (Jackson *et al.*, 2009). In turn, this could again influence UV sensitivity. It is therefore concluded that the efficiency of UV disinfection in this study may have been influenced by the characteristics of the Plankenburg River on each of the sampling days.

Reductions achieved for FC were always slightly lower than those recorded for TC at the maximum UV dose (Table 7). However, these differences were observed to be statistically insignificant ($p>0.05$). On the other hand, in each of the trials it was seen that the maximum reduction achieved for the total heterotrophic population was significantly lower ($p<0.05$) compared to that reported for TC and FC, respectively (Table 7). Broadly, heterotrophs are described as microorganisms that require an external source of organic carbon in order to grow. Bacteria (gram-negative and gram-positive) are included in this group (WHO, 2003) which presents an array of types, strains and species of microorganisms. This diversity undoubtedly contributed to the observed resistance of the heterotrophic population against UV irradiation. In this regard, gram-positive bacteria, for instance, are known to be more resistant to UV light in comparison to the gram-negative types as a result of their thick peptidoglycan cell wall. The latter has been reported to hamper UV penetration within the bacterial cells (Beauchamp & Lacroix, 2012). Furthermore, cell size may also influence the efficiency of UV disinfection, with larger cells generally being more resistant. This is explained by the fact that photons are more likely to be absorbed by other cell components before reaching the microbial DNA (Oteiza *et al.*, 2010; Gabriel, 2012). Additional microbial characteristics (such as DNA condensation and cell pigmentation) may further provide specific microorganisms with increased UV resistance (Gayán *et al.*, 2014). In river water, a portion of the enumerated heterotrophic population will always be representative of the more resistant microorganisms, as described above. It was therefore expected that greater resistance

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(lower reductions) would be observed in comparison to that shown by TC and FC at each of the respective UV doses.

Having considered all of the above, it is evident that none of the tested LP UV treatments were able to achieve adequate disinfection in river water of the discussed physico-chemical and microbiological quality. The data presented in Table 8 shows the final concentrations of FC following each of the UV treatments. As indicated, the load was never reduced to within the guideline limit of 1 000 cfu.100 mL^{-1} (3 log cfu.100 mL^{-1}) for water intended to be used for fresh produce irrigation (DWAF, 1996).

Table 8 Faecal coliform concentrations in river water following LP UV disinfection at three doses (5, 7 and 10 $mJ.cm^{-2}$) in a laboratory-scale study

	Log cfu.100 mL^{-1}		
UV dose	**Trial 1**	**Trial 2**	**Trial 3**
5 $mJ.cm^{-2}$	5.84±0.30	5.03±0.14	4.84±0.14
7 $mJ.cm^{-2}$	5.06±0.38	4.41±0.05	4.73±0.19
10 $mJ.cm^{-2}$	4.85±0.12	4.04±0.13	3.83±0.21

CONCLUSIONS

In this study the disinfection potential of LP UV and UV/H_2O_2 treatments was investigated using six reference and environmental *E. coli* strains. Initially, clear strain-to-strain variation in the resistance against both treatments (UV dose of 4 $mJ.cm^{-2}$ and UV/H_2O_2 combination of 4 $mJ.cm^{-2}$/20 $mg.L^{-1}$) was seen. The general observation was that the reference strains were more sensitive. In each case, environmental isolate F11.2 was the most resistant and strain ATCC 35218 the most vulnerable. The use of popular reference strains might thus not be the most accurate method for the optimisation of disinfection treatments on laboratory-scale. In addition, the low doses utilised for both UV and the combination treatment were considered inadequate to generate a 3 log target reduction. In comparison to UV alone, the combination treatment showed potential for increased disinfection, yet the majority of strains reacted similarly towards the respective processes. Resistance mechanisms, including catalase activity and the use of efflux pumps, may allow *E. coli* strains to protect themselves against the combined effects of UV and H_2O_2.

In subsequent studies it was observed that increased UV doses and H_2O_2 concentrations resulted in overall greater reductions. Even for the most resistant strain a 3 log reduction was achieved at a UV dose of 10 $mJ.cm^{-2}$. Similarly, higher concentrations of H_2O_2 resulted in better disinfection but the effect was less profound. At a concentration of 200 $mg.L^{-1}$ (and UV dose of 4 $mJ.cm^{-2}$) the most resistant strain was reduced by 2.40 log units only. Variation in the responses of the different strains to incremented H_2O_2 concentrations was related to their catalase activity,

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although exceptions were observed. It became clear that the dynamics of combined treatments are complex and therefore further research is required to optimise the conditions for AOPs.

In the following study, it was observed that the physico-chemical properties of both untreated and flocculated river water failed to meet the guidelines for water intended to be used for fresh produce irrigation. Furthermore, significantly better reductions ($p<0.05$) were achieved in saline compared to sterile untreated river water for both test strains (ATCC 25922 and F11.2). The impact of suspended and dissolved compounds on the availability of UV photons (and consequent disinfection) was thus clear. It was also apparent that the use of a polymeric coagulant at 7 mg.L^{-1} resulted in a slightly increased water quality. The effect thereof on the delivered UV light, however, was only slight and could not result in greater reductions of a resistant environmental strain. When the same strain was exposed to a "shock" combination treatment, complete inactivation was achieved in both water samples. It is suggested that in the presence of high H_2O_2 concentrations the available UV light was influenced not as much by water quality, but rather it was absorbed by the chemical for the generation of reactive oxygen species.

Thus, if one should consider the most resistant strain as member of the river water population, UV at a dose of 10 mJ.cm^{-2} would possibly be able to generate a 3 log reduction. An UV dose of 4 mJ.cm^{-2} combined with 200 mg.L^{-1} H_2O_2 would not be able to do the same. However, the influence of water quality on the efficiency of UV disinfection was clearly observed and in this regard the use of combination treatments should be investigated further. Also, since resistant environmental *E. coli* was insensitive to water quality changes (in terms of available UV energy/photons) the cost and viability of pre-treatment should be thoroughly compared to that of simply increasing the applied UV dose to achieve better disinfection.

Different methods for the enumeration of TC, FC and total heterotrophic microorganisms were evaluated and compared in order to optimise methodologies for subsequent studies. For the respective microbial groups, the use of different media (VRBA, CES and PCA) and techniques (pour plates and membrane filtration) compared fairly well, irrespective of the absence of a standardised reference inoculum. Based on the results obtained, and those of similar studies reviewed in the literature, the pour plate technique was selected as suitable enumeration method.

The final study evaluated the potential of LP UV irradiation for the disinfection of three dissimilar water samples taken from the Plankenburg River. Firstly, it was observed that the water did not comply with guidelines set for physico-chemical and microbiological properties of water intended to be used for fresh produce irrigation (DWAF, 1996). Faecal coliforms were detected at levels of 6.41, 6.23 and 6.29 log cfu.100 mL^{-1} in the three respective samples. Sources such as informal settlements and industrial operations probably contributed to the high levels of pollution observed in the river water. Following treatment, similar inactivation was generally observed for TC and FC while the heterotrophic bacteria population showed much greater resistance. Increased UV doses resulted in improved disinfection but the maximum (10 mJ.cm^{-2}) could not deliver a 3 log reduction of TC, FC or the total heterotrophic population. In addition, it was seen

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that a reduction exceeding 3 log would be required to produce water that could be safely used for the irrigation of fresh produce. Considering the impact of water quality and microbiological aspects on UV disinfection it is recommended that doses > 10 mJ.cm^{-2} be evaluated in future studies.

REFERENCES

Ackerman, A. (2010). *Assessment of microbial loads of the Plankenburg and Berg Rivers and the survival of Escherichia coli on raw vegetables under laboratory conditions*. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Alkan, U., Teksoy, A., Atesli, A. & Başkaya, H.S. (2007). Efficiency of the UV/H_2O_2 process for the disinfection of humic surface waters. *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering*, **42**(4), 497-506.

Alonso, J.L., Soriano, A., Carbajo, O., Amoros, I. & Garelick, H. (1999). Comparison and recovery of *Escherichia coli* and thermotolerant coliforms in water with a chromogenic medium incubated at 41 and 44.5°C. *Applied and Environmental Microbiology*, **65**(8), 3746-3749.

APHA (American Public Health Association) (2005). *Standard Methods for the Examination of Water and Wastewater,* 21st ed. Washington, DC: American Public Health Association Press.

Barnes, J.M. (2003). *The Impact of Water Pollution from Formal and Informal Urban Developments along the Plankenburg River on Water Quality and Health Risk.* PhD Thesis in Epidemiology, University of Stellenbosch, South Africa.

Barnes, J.M. & Taylor, M.B. (2004). *Health risks assessment in connection with the use of microbiologically contaminated source waters for irrigation*. WRC Report no. 1226/1/04, Pretoria, South Africa.

Beauchamp, S. & Lacroix, M. (2012). Resistance of the genome of *Escherichia coli* and *Listeria monocytogenes* to irradiation evaluated by the induction of cyclobutane pyrimidine dimers and 6-4 photoproducts using gamma and UV-C radiations. *Radiation Physics and Chemistry*, **81**(8), 1193-1197.

Bernasconi, C., Volponi, G. & Bonadonna, L. (2006). Comparison of three different media for the detection of *E. coli* and coliforms in water. *Water Science and Technology*, **54**(3), 141-145.

Berney, M., Weilenmann, H.U., Ihssen, J., Bassin, C. & Egli, T. (2006). Specific growth rate determines the sensitivity of *Escherichia coli* to thermal, UVA, and solar disinfection. *Applied and Environmental Microbiology*, **72**(4), 2586-2593.

Bolton, J.R. & Cotton, C.A. (2008). Effects of water quality on UV disinfection. In: *The Ultraviolet Disinfection Handbook* (edited by M. Valentine). Pp. 77-86. USA: American Water Works Association (AWWA).

Brahmi, M., Belhadi, N.H., Hamdi, H. & Hassen, A. (2010). Modeling of secondary treated wastewater disinfection by UV irradiation: effects of suspended solids content. *Journal of Environmental Sciences,* **22**(8), 1218-1224.

Stellenbosch University https://scholar.sun.ac.za

Brennan, F.P., Abram, F., Chinalia, F.A., Richards, K.G. & O'Flaherty, V. (2010). Characterization of environmentally persistent *Escherichia coli* isolates leached from Irish soil. *Applied and Environmental Microbiology*, **76**(7), 2175-2180.

Britz, T.J., Sigge, G.O., Buys, E.M., Schmidt, S., Potgieter, N. & Taylor M.B. (2012). Baseline study on extent (types and quantities) of contamination found in irrigation water at selected sites (K5/1773). In: *Quantitative investigation into the link between irrigation water quality and food safety* (Volume 2). WRC Report no. K5/1773/4, Pretoria, South Africa.

Britz, T.J., Sigge, G.O., Huisamen, N., Kikine, T., Ackermann, A., Lötter, M., Lamprecht, C. and Kidd, M. (2013). Fluctuations of indicator and index microbes as indication of pollution over three years in the Plankenburg and Eerste Rivers, Western Cape, South Africa. *Water SA*, **39**(4), 457-465.

Bucheli-Witschel, M., Bassin, C. & Egli, T. (2010). UV-C inactivation in *Escherichia coli* is affected by growth conditions preceding irradiation, in particular by the specific growth rate. *Journal of Applied Microbiology*, **109**(5), 1733-1744.

Child, M., Strike, P., Pickup, R. & Edwards, C. (2002). *Salmonella typhimurium* displays cyclical patterns of sensitivity to UV-C killing during prolonged incubation in the stationery phase of growth. *FEMS Microbiology Letters,* **213**(1), 81-85.

Christensen, J. & Linden, K.G. (2003). How particles affect UV light in the UV disinfection of unfiltered drinking water. *Journal American Water Works Association*, **95**(4), 179-189.

Davies, J. & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews*, **74**(3), 417-433.

DWAF (Department of Water Affairs and Forestry) (1996). *South African Water Quality Guidelines*, 2nd ed. (edited by S. Holmes). *Volume 4: Agricultural Water Use: Irrigation*. Pp. 71-76. The Government Printer. Pretoria, South Africa.

Gabriel, A. (2012). Inactivation of *Escherichia coli* O157:H7 and spoilage yeasts in germicidal UV-C-irradiated and heat-treated clear apple juice. *Food Control*, **25**(2), 425-432.

Gayán, E., Condón, S. & Álvarez, I. (2014). Biological aspects in food preservation by ultraviolet light: a review. *Food and Bioprocess Technology*, **7**, 1-20.

Gayán, E., Monfort, S., Álvarez, I. & Condón, S. (2011). UV-C inactivation of *Escherichia coli* at different temperatures. *Innovative Food Science and Emerging Technologies*, **12**(4), 531-541.

Geissler, K., Manafi, M., Amoros, I. & Alonso, J.L. (2000). Quantitative determination of total coliforms and *Escherichia coli* in marine waters with chromogenic and fluorogenic media. *Journal of Applied Microbiology*, **88**(2), 280-285.

Gerhardt, P., Murray, R.G.E., Costilow, R.N., Nester, E.W., Wood, W.A., Krieg, N.R. & Phillips, G.B. (1981). *Manual of Methods for General Bacteriology*. Washington, DC: American Society for Microbiology.

Stellenbosch University https://scholar.sun.ac.za

Giddey, K., Kidd, M., Britz, T.J., Sigge, G.O. & Lamprecht, C. (2015). Impact of hydrogen peroxide treatment on environmental *Escherichia coli* strains. *Journal of Applied Environmental Microbiology*, **3**(2), 49-57.

Gonzáles, R.D., Tamagnini, L.M., Olmos, P.D. & De Sousa, G.B. (2003). Evaluation of a chromogenic medium for total coliforms and *Escherichia coli* determination in ready-to-eat foods. *Food Microbiology*, **20**(5), 601-604.

Guo, M., Huang, J., Hu, H., Liu, W. & Yang, J. (2012). UV inactivation and characteristics after photoreactivation of *Escherichia coli* with plasmid: health safety concern about UV disinfection. *Water Research*, **46**(13), 4031-4036.

Hallas, G., Giglio, S., Capurso, V., Monis, P.T. & Grooby, W.L. (2008). Evaluation of chromogenic technologies for use in Australian potable water. *Journal of Applied Microbiology*, **105**(4), 1138-1149.

Hallmich, C. & Gehr, R. (2010). Effect of pre- and post-UV disinfection conditions on photoreactivation of fecal coliforms in wastewater effluents. *Water Research*, **44**, 2885-2893.

Hijnen, W.A.M., Beerendonk, E.F. & Medema, G.J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Research*, **40**(1), 3-22.

Hörman, A. & Hänninen, M.L. (2006). Evaluation of the Tergitol-7, m-Endo LES, Colilert 18, Readycult Coliforms 100, Water-Check-100, 3M Petrifilm EC and DryCult Coliform test methods for the detection of total coliforms and *Escherichia coli* in water samples. *Water Research*, **40**(17), 3249-3256.

Howe, K.J., Hand, D.W., Crittenden, J.C., Trussell, R.R. & Tchobanoglous, G. (2012). Disinfection. In: *Principles of Water Treatment.* Pp. 525-581.

Huisamen, N. (2012). *Assessment of microbial levels in the Plankenburg and Eerste Rivers and subsequent carry-over to fresh produce using source tracking as indicator*. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Ijabadeniyi, O.A. & Buys, E.M. (2012). Irrigation water and microbiological safety of fresh produce; South Africa as a case study: a review. *African Journal of Agricultural Research*, **7**(35), 4848-4857.

Iwase, T., Tajima, A., Sugimoto, S., Okuda, K., Hironaka, I., Kamata, Y., Takada, K. & Mizunoe, Y. (2013). A simple assay for measuring catalase activity: a visual approach. *Scientific Report*, **3**, 3081; DOI:10.1038/rep03081.

Jackson, V.A., Paulse, A.N., Odendaal, J.P. & Khan, W. (2009). Investigation into the metal contamination of the Plankenburg and Diep Rivers, Western Cape, South Africa. *Water SA*, **35**(3), 289-299.

Janezic, K.G., Ferry, B., Hendricks, E.W., Janiga, B., Johnson, T., Murphy, S., Roberts, E.R., Scott, S.M., Theisen, A.N., Hung, K.F. & Daniel, S.L. (2013). Phenotypic and genotypic

Stellenbosch University https://scholar.sun.ac.za

characterization of *Escherichia coli* isolated from untreated surface waters. *The Open Microbiology Journal*, **7**, 9-19.

Jones, L.A., Worobo, R.W. & Smart, C.D. (2014). UV light inactivation of human and plant pathogens in unfiltered surface irrigation water. *Applied and Environmental Microbiology*, **80**(3), 849-854.

Koivunen, J. & Heinonen-Tanski, H. (2005a). Peracetic acid (PAA) disinfection of primary, secondary and tertiary treated municipal wastewaters. *Water Research*, **39**(18), 4445-4453.

Koivunen, J. & Heinonen-Tanski, H. (2005b). Inactivation of enteric microorganisms with chemical disinfectants, UV irradiation and combined chemical/UV treatments. *Water Research*, **39**(8), 1519-1526.

Koutchma, T., Forney, L.G. & Moraru, C.L. (2009a). Microbial inactivation by UV light. In: *Ultraviolet Light in Food Technology: Principles and Applications* (edited by D-W. Sun). Pp. 69-99. Boca Raton: CRC Press.

Koutchma, T., Forney, L.G. & Moraru, C.L. (2009b). Sources of UV light. In: *Ultraviolet Light in Food Technology: Principles and Applications* (edited by D-W. Sun). Pp. 33-51. Boca Raton: CRC Press.

Lamprecht, C., Romanis, M., Huisamen, N., Carinus, A., Schoeman, N., Sigge, G.O. & Britz, T.J. (2014). *Escherichia coli* with virulence factors and multidrug resistance in the Plankenburg River. *South African Journal of Science*, **110**, 1-6.

Linden, K.G., Thurston, J., Schaefer, R. & Malley, J.P. (2007). Enhanced UV inactivation of adenoviruses under polychromatic UV lamps. *Applied and Environmental Microbiology*, **73**(23), 7571-7574.

Loewen, P.C., Switala, J. & Triggs-Raine, B.L. (1985). Catalase HP1 and HPII in *Escherichia coli* are induced independently. *Archives of Biochemistry and Biophysics*, **243**(1), 144-149.

Lötter, M. (2010). *Assessment of microbial loads present in two Western Cape rivers used for irrigation of vegetables*. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Maheux, A.F., Huppé, V., Boissinot, M., Picard, F.J., Bissonnette, L., Bernier, J.T. & Bergeron, M.G. (2008). Analytical limits of four β-glucuronidase and β-galactosidase-based commercial culture methods used to detect *Escherichia coli* and total coliforms. *Journal of Microbiological Methods*, **75**(3), 506-514.

Macy, J.T., Dunne, E.F., Angoran-Benie, Y.H., Kamelan-Tano, Y., Kouadio, L., Djai, K.A. & Luby, S.P. (2005). Comparison of two methods for evaluating the quality of stored drinking water in Abidjan, Côte d'Ivoire, a review of other comparisons in the literature. *Journal of Water and Health*, **3**(3), 221-228.

Malley, J.P., Ballester, N.A., Margolin, A.B., Linden, K.G., Mofidi, A., Bolton, J.R., Crozes, G., Laine, G.M. & Janex, M.L. (2004). Overview of pathogen inactivation with UV. In:

Stellenbosch University https://scholar.sun.ac.za

Inactivation of pathogens with innovative UV technologies. Pp. 1-7. USA: Awwa Research Foundation and American Water Works Association.

Mamane, H., Shemer, H. & Linden, K.G. (2007). Inactivation of *E. coli*, *B. subtilis* spores, and MS2, T4 and T7 phage using UV/H_2O_2 advanced oxidation. *Journal of Hazardous Materials*, **146**(3), 479-486.

Maya, C., Beltrán, N., Jiménez, B. & Bonilla, P. (2003). Evaluation of the UV disinfection process in bacteria and amphizoic amoeba inactivation. *Water Science and Technology: Water Supply*, **3**(4), 285-291.

Mazzola, P.G., Martins, A.M.S. & Penna, T.C.V. (2006). Chemical resistance of gram-negative bacteria to different sanitizers in a water purification system. *BMC Infections Disease*, **6**, 131-142.

Merck (2005). *Merck Microbiology Manual*, 12th ed. Germany: Merck KGaA.

Méric, G., Kemsley, E.K., Falush, D., Saggers, E.J. & Lucchini, S. (2013). Phylogenetic distribution of traits associated with plant colonisation in *Escherichia coli*. *Environmental Microbiology*, **15**(2), 487-501.

Mezzanotte, V., Antonelli, M., Citterio, S. & Nurizzo, C. (2007). Wastewater disinfection alternatives: Chlorine, ozone, peracetic acid and UV light. *Water Environment Research*, **79**(12), 2373-2379.

Mofidi, A.A., Rochell, P.A., Chou, C.I., Mehta, H.M., Linden, K.G. & Malley, J.P. (2002). Bacterial survival after ultraviolet light disinfection: resistance, regrowth and repair. In: *Proceedings of the American Water Works Association Water Quality Technology Conference*. November 2002. Seattle, USA.

Molden, D., Oweis, T.Y., Steduto, P., Kijne, J.W., Hanjra, M.A., Bindraban, P.S., Bouman, B.A.M., Cook, S., Erenstein, O., Farahani, H., Hachum, A., Hoogeveen, J., Mahoo, H., Nangia, V., Peden, D., Sikka, A., Silva, P., Turral, H., Upadhyaya, A. & Zwart, S. (2007). Pathways for increasing agricultural water productivity. In: *Water for Food, Water for Life: A Comprehensive Assessment of Water Management in Agriculture* (edited by D. Molden). Pp. 279-306. Colombo: International Water Management Institute.

Morowitz, H.J. (1950). Absorption effects in volume irradiation of microorganisms. *Science*, **111**(2879), 229-230.

Ortega Morente, E., Fernández-Fuentes, M.A., Burgos, M.J.G., Abriouel, H., Publido, R.P. & Gálvez, A. (2013). Biocide resistance in bacteria. *International Journal of Food Microbiology*, **162**(1), 13-25.

Oteiza, J.M., Giannuzzi, L. & Zaritzky, N. (2010). Ultraviolet treatment of orange juice to inactivate *E. coli* O157:H7 as affected by native microflora. *Food and Bioprocess Technology,* **3**(4), 603-614.

Stellenbosch University https://scholar.sun.ac.za

Pachepsky, Y., Shelton, D.R., McLain, J.E.T., Patel, J. & Mandrell, D.E. (2011). Irrigation waters as a source of pathogenic microorganisms in produce: a review. *Advances in Agronomy*, **113**, 73-138.

Paulse, A.N., Jackson, V.A. & Khan. W. (2009). Comparison of microbial contamination at various sites along the Plankenburg- and Diep Rivers, Western Cape, South Africa. *Water SA*, **35**(4), 469-478.

Quek, P.H. & Hu, J. (2008). Indicators for photoreactivation and dark repair studies following ultraviolet disinfection. *Journal of Industrial Microbiology and Biotechnology*, **35**(6), 533-541.

Salgot, M., Folch, M., Huertas, E., Tapias, J., Avellaneda, D., Gíros, G., Brissaud, F., Vergés, C., Molina, J. & Pigem, J. (2002). Comparison of different advanced disinfection systems for wastewater reclamation. *Water Science and Technology: Water Supply,* **2**(3), 213-218.

Schraft, H. & Watterworth, L.A. (2005). Enumeration of heterotrophs, fecal coliforms and *Escherichia coli* in water: comparison of 3M^{TM} Petrifilm^{TM} plates with standard plating procedures. *Journal of Microbiological Methods*, **60**, 335-342.

SANS (South African National Standards) (1999). Method 6887-1. Microbiology of Food and Animal Feeding Stuffs: Preparation of test samples, initial suspension and decimal dilutions for microbiological examination Part 1: General rules for the preparation of the initial suspension and decimal dilutions. Pretoria: South Africa.

SANS (South African National Standards) (2006). Method 5667-6. Water quality: Sampling Part 6: Guidance on sampling of rivers and streams. Pretoria: South Africa.

SANS (South African National Standards) (2007a). Method 4832. Microbiology of Food and Animal Feeding Stuffs: Horizontal methods for the enumeration of coliforms: Colony-count technique. Pretoria: South Africa.

SANS (South African National Standards) (2007b). Method 4833. 2nd ed. Microbiology: General guidance for the enumeration of microorganisms: Colony count technique at 30°C. Pretoria: South Africa.

Selma, M.V., Allende, A., López-Gálvez, F., Conesa, M.A. & Gil, M.I. (2008). Disinfection potential of ozone, ultraviolet-C and their combination in wash water for the fresh-cut vegetable industry. *Food Microbiology*, **25**, 809-814.

Siddiqui, M. (2005). Ultraviolet Irradiation. In: *Water Encyclopedia: Domestic, Municipal, and Industrial Water Supply and Waste Disposal* (edited by J.H. Lehr, J. Keeley & J. Lehr). Vol. 1. Pp. 469-470. USA: John Wiley & Sons, Inc.

Sommer, R., Haider, T., Cabaj, A., Pribil, W. & Lhotsky, M. (1998). Time fluence reciprocity in UV disinfection of water. *Water Science and Technology*, **38**(12), 145-150.

Sommer, R., Lhotsky, M., Haider, T. & Cabaj, A. (2000). UV inactivation, liquid-holding recovery, and photoreactivation of *Escherichia coli* O157 and other pathogenic *Escherichia coli* strains in water. *Journal of Food Protection*, **63**(8), 1015-1020.

Stellenbosch University https://scholar.sun.ac.za

Storz, G. & Imlay, J. (1999). Oxidative stress. *Current Opinion in Microbiology*, **2**, 188-194.

Tawabini, B., Khalil, A. & Abussaud, B. (2013). Reduction of *Escherichia coli* bacteria from contaminated water by combining hydrogen peroxide, ozone and ultraviolet light. *Water Science and Technology: Water Supply*, **13**(3), 782-789.

Teksoy, A., Alkan, U., Eleren, S.Ç., Topaç, B.Ş., Şağban, F.O.T. & Başkaya, H.S. (2011). Comparison of indicator bacteria inactivation by the ultraviolet and the ultraviolet/hydrogen peroxide disinfection processes in humic waters. *Journal of Water and Health*, **9**(4), 659-669.

Timchak, E. & Gitis, V. (2012). A combined degradation of dyes and inactivation of viruses by UV and UV/H_2O_2. *Chemical Engineering Journal*, **192**, 164-170.

USEPA (United States Environmental Protection Agency) (2002). Method 1604. Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium), EPA-821-R-02-024. Washington D.C: United States of America.

Van Blommestein, A. (2012). *Impact of selected environmental factors on E. coli growth in river water and an investigation of carry-over to fresh produce during irrigation*. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Van der Veen, S. & Abee, T. (2011). Bacterial SOS response: a food safety perspective. *Current Opinion in Biotechnology*, **22**(2), 136-142.

Visick, J.E. & Clarke, S. (1997). RpoS- and OxyR-Independent induction of HPI catalase at stationary phase in *Escherichia coli* and identification of rpoS mutations in common laboratory strains. *Journal of Bacteriology*, **179**(13), 4158-4163.

Walters, E., Graml, M., Behle, C., Müller, E. & Horn, H. (2014). Influence of particle association and suspended solids on UV inactivation of fecal indicator bacteria in an urban river. *Water, Air, & Soil Pollution*, **225**(1), 1822-1828.

Wang, D. & Wanda, F. (2008). Evaluation of media for simultaneous enumeration of total coliform and *Escherichia coli* in drinking water supplies by membrane filtration techniques. *Journal of Environmental Sciences*, **20**(3), 273-277.

Wang X., Hu, X., Hu, C. & Wei, D. (2011). Sequential use of ultraviolet light and chlorine for reclaimed water disinfection. *Journal of Environmental Sciences*, **23**(10), 1605-1610.

WHO (World Health Organisation) (1989). *Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture.* WHO, Technical Report Series no. 778. Geneva, Switzerland.

WHO (World Health Organisation) (2003). Expert consensus. In: *Heterotrophic Plate Counts and Drinking-water Safety: The Significance of HPCs for Water Quality and Human Health* (edited by J. Bartram, J. Cotruvo, M. Exner, C. Fricker & A. Glasmacher). Pp. 1-11. London: IWA Publishing.

Stellenbosch University https://scholar.sun.ac.za

Wohlsen, T., Bates, J., Vesey, G., Robinson, W.A. & Katouli, M. (2006). Evaluation of the methods for enumerating coliform bacteria from water samples using precise reference standards. *Letters in Applied Microbiology*, **42**(4), 350-356.

Wohlsen, T., Bayliss, J., Bates, J., Gray, B., Johnson, S. and Schneider, P. (2008). An evaluation of membrane fecal coliform agar and Colilert-18^{TM} for the enumeration of *E. coli* bacteria in surface water samples. *Journal of Water Supply: Research and Technology – AQUA*, **57**(8), 569-576.

Wojcicka, L., Hofmann, R., Baxter, C., Andrews, R.C., Li ère, J., Miller, T., Chauret, C. & Baribeau, H. (2007). Inactivation of environmental and reference strains of heterotrophic bacteria and *Escherichia coli* O157:H7 by free chlorine and monochloramine. *Journal of Water Supply: Research and Technology-AQUA*, **56**(2), 137-149.

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Chapter 4

PILOT-SCALE INVESTIGATION OF MEDIUM-PRESSURE ULTRAVIOLET IRRADIATION FOR RIVER WATER DISINFECTION CONSIDERING THE IMPACT OF WATER QUALITY AND DNA DAMAGE-REPAIR

ABSTRACT

Medium-pressure (MP) ultraviolet (UV) irradiation at doses of 13, 17 and 24 mJ.cm^{-2} were firstly used to disinfect water from the Plankenburg River. Initial faecal coliform (FC) concentrations up to 6.41 log cfu.100 mL^{-1} were detected which, together with poor water quality, disallowed the treatment to reduce FC to below 3 log cfu.100 mL^{-1}. The maximum reduction of FC was estimated at 3.23 log. Limits proposed by the Department of Water Affairs (DWA) implied that the disinfected water could not be safely used for fresh produce irrigation. Later studies demonstrated that better UVT% (49.90% vs 36.00%) contributed to a 1.16 log increase for the inactivation of total coliforms (TC) at 13 mJ.cm^{-2}. Nevertheless, photoreactivation allowed for a significant increase ($p<0.05$) in TC concentrations following UV irradiation. Total recovery reached 49.18% and 35.37% in the presence of 3.5 kLux fluorescent light for UV doses of 13 and 24 mJ.cm^{-2}, respectively, within 5 h. Owing to greater microbial damage, the higher dose (24 mJ.cm^{-2}) resulted in slower repair and lower total recovery. In addition to DNA damage, MP UV light may also have affected the photolyase enzyme which has been shown in literature to absorb UV energy at 280 and 384 nm. Compared to 24 mJ.cm^{-2}, 40 mJ.cm^{-2} and a different irradiation procedure (2 x 20 mJ.cm^{-2}), respectively, reduced photoreactivation by 3.82% and 14.49% under 3.5 kLux light. Increased UV dosage resulted in lower repair while a sequential treatment (2 x 20 mJ.cm^{-2}) inhibited photoreactivation even better. Nonetheless, TC concentrations always exceeded 3 log cfu.100 mL^{-1} following 5 h exposure to 3.5 kLux light. Light of lower intensity (1.0 to 2.0 kLux) did not result in significantly different total reactivation compared to that seen previously for the 40 and 2 x 20 mJ.cm^{-2} treatments, respectively ($p=0.55$ and $p=0.40$). Slight regrowth in the absence of light was observed following the 40 mJ.cm^{-2} treatment but could not be accurately attributed to dark-repair. From this work, it is concluded that MP UV irradiation shows potential as effective method of water disinfection. However, surface water quality and post-inactivation conditions may significantly affect its efficacy and must be considered when suggesting parameters for water disinfection. The use of pre-treatments (such as flocculation) to improve water quality, combined disinfection methods (such as UV/Chlorine) and UV doses greater than 24 mJ.cm^{-2} should be investigated further to optimise UV efficacy.

INTRODUCTION

As reported previously, South African rivers are extensively polluted. Referring to those in the Western Cape, in particular, microbiological as well as chemical contamination have been

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identified as reasons for great concern (Paulse *et al.*, 2009; Huisamen, 2012; Britz *et al.*, 2013). Extremely high levels of faecal indicator bacteria are regularly detected, mainly resulting from the influence of informal settlements established close to the rivers (Britz *et al.*, 2013). Nevertheless, such water is often utilised for the irrigation of agricultural products and current decontamination methods predominantly rely on the conservative, costly application of chemical disinfectants. In this regard, water intended to be used for the purpose of fresh produce irrigation should be representative of a FC load not exceeding 1 000 colony forming units (cfu) per 100 mL of water (DWAF, 1996).

In addition to the use of chemical disinfectants, such as chlorine, UV irradiation is a well-established alternative method of disinfection which is frequently used for the treatment of water and wastewater (Hu *et al.*, 2005; Bohrerova *et al.*, 2015; Kollu & Örmeci, 2015). This photochemical process has increased in popularity in recent times due to its non-chemical, environmentally friendly nature (Hu *et al.*, 2005). Disinfection by means of UV irradiation predominantly results from the absorption of UV photons by microbial genetic materials and principally the formation of pyrimidine dimers within the DNA strand (Harm, 1980; Rubin *et al.*, 1981). As a consequence, the affected cells will not be able to replicate and eventually die off (Moné *et al.*, 2001; Guo *et al.*, 2013). Two types of UV lamps, low and medium-pressure mercury vapour lamps (LP and MP), are most frequently used and differ with regards to the wavelengths at which they emit light. Low-pressure lamps were traditionally used while MP UV lamps have only been developed in the last two decades. These emit light at 254 and a range of 200 to 400 nm, respectively (Poepping *et al.*, 2014). In comparison to LP lamps, MP lamps also emit light at a higher intensity, consequently being more effective for the purpose of disinfection. As a result, the use of UV disinfection systems utilising MP lamps have increased during recent times (Quek & Hu, 2008a).

As method of water disinfection, UV irradiation is not entirely flawless. A major limitation of the process is the fact that no residual is offered, consequently providing bacteria with an opportunity to increase post-disinfection (Guo *et al.*, 2011). In this regard, the ability of microorganisms to reverse UV-induced structural damage has been reported and extensively studied in the literature (Guo *et al.*, 2009; Vélez-Colmenares *et al.*, 2011). Two methods of repair have been identified and include light-mediated photoreactivation/photo-repair as well as dark-repair (which could occur in the absence of light). The latter has been stated to be significantly less influential, particularly over the short term (Guo *et al.*, 2011). Nevertheless, photoreactivation is described as a mechanism of reverse damage-repair which is carried out by cyclobutane pyrimidine dimer (CPD) lyases (or photolyases). These enzymes act against CPDs, specifically, and require the presence of visible light to perform their task. Photolyase is approximately 50 kDa in size and occurs as a monomeric protein. Two cofactors, flavin adenine dinucleotide ($FADH^-$) and 5, 10-methenyltetrahydrofolate (MTHF), are non-covalently bound to the enzyme. The former ($FADH^-$) is known to be essential for catalysing the process of photo-repair (Sancar, 2003; Xu *et*

al., 2015). Dark-repair, on the other hand, is described as a form of nucleotide excision repair (NER) performed by the UvrABC exinuclease (Truglio *et al*., 2006). Even though it is regarded as a less significant problem, compared to photoreactivation, dark-repair may occur in distribution systems following disinfection and the study thereof remains important.

Regarding the problem of damage-repair, the use of MP UV systems for water disinfection is gaining admiration. Several researchers have found MP UV light to be more effective in limiting photoreactivation following disinfection, compared to LP UV irradiation (Oguma *et al*., 2002; Zimmer & Slawson, 2002; Kalisvaart, 2004; Hu *et al*., 2005; Quek & Hu, 2008a). As mentioned by Quek & Hu (2013) the former authors suggest that a possible explanation is the fact that MP UV lamps emit light of a broader spectrum. This implies that the irradiation may result in greater formation of dimers as well as additional damage to amino acids, important enzymes and possibly also photolyase. Low-pressure UV light, on the other hand, will primarily result in the formation of dimers due to its monochromatic emission at a wavelength of 254 nm (Quek & Hu, 2013).

Nevertheless, following UV disinfection of irrigation water, pathogens may still be present in fairly high numbers as the limit set by DWAF (1996) requires FC to not exceed 1 000 cfu.100 mL^{-1}. In this regard, growth of the representative microorganisms, as well as the phenomenon of photoreactivation, may significantly threaten the microbiological quality of the treated water. These are likely events as irradiated river water may still provide nutrients for growth, while exposure to light following disinfection is often difficult to evade. The investigation of MP UV irradiation (and the phenomenon of damage-repair) is therefore important so that adequate control measures and suggestions regarding river water disinfection can be made.

The aim of the current study was to investigate the potential of MP UV irradiation for the decontamination of microbiologically polluted water from the Plankenburg River. Pilot-scale disinfection was followed by laboratory-scale experiments in which the influence of damage-repair (post-inactivation) was investigated. The series of studies focussed on: the effect of MP UV dose on the inactivation of the microbial population occurring in the Plankenburg River; the influence of water quality and microbiological properties on disinfection efficiency; the impact of photo and dark-repair on the efficacy of different MP UV doses and varied irradiation protocols.

MATERIALS AND METHODS

General materials and methods

Pilot-plant site description

Larger-scale disinfection experiments were performed at a customised water treatment facility installed on the bank of the Plankenburg River at an industrial site (33°56'15.4''S,18°50'53.0''E) in Stellenbosch, South Africa. Water was pumped from the river, through a sand filter, and directed to three holding tanks representing volumes of 2 500 L each. Once filled, water was drawn from

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these tanks to perform continuous flow tests. The pilot-scale system was designed to allow flow rates in the range of 30 to 200 litres per minute (LPM) with sampling points situated before the sand filter (1), after the sand filter before exposure to UV light (2) and following exposure to UV light (3) (Fig. 1). Experiments were performed in both summer and late autumn (January/February and April/May) which, in terms of rainfall, may differ meaningfully in the Western Cape region.

Pilot-scale MP UV treatment

Experiments were performed using a Berson InLine 40+ UV disinfection system (Berson, The Netherlands). This utilises a B810H medium-pressure (MP) UV lamp installed perpendicular to the flow of water in the piping network. Light was emitted in the range of 220 to 580 nm.

On the day of each trial flow rates required to deliver the desired UV doses were calculated with reference to the UV transmission percentage (UVT%) of the river water at the time. The computerised UV system, having an advanced in-line sensor, allowed the operator to adjust the flow rate, in units of $m^3.h^{-1}$, and quantified the delivered UV dose in the desired units of $mJ.cm^{-2}$. The flow rate was adjusted on the digital interface of the UV system in order to establish the value that corresponded to the respective UV doses. The system, however, was not capable of automatically regulating the set flow rate and this was performed manually by manipulating a valve installed in the piping system before the UV lamp. The flow rate was measured by means of an in-line rotameter. Thus, the set flow rate shown on the display of the computer system was converted from $m^3.h^{-1}$ to LPM and adjusted sequentially to expose the water to the predetermined doses.

Water sampling

Water was sampled in sterilised 2 L bottles and these were drawn from the sampling taps shown in Figure 1. One control sample was taken at point 2 (after the sand filter) where after another sample was taken at point 3 following treatment at the respective UV doses. The filled bottles were labelled and kept in insulated cooler boxes that contained frozen ice-bricks to ensure that the water remained at low temperatures. Further analyses were performed within one hour.

During pilot-scale experiments protective clothing was always worn since the level of contamination presented by the water could have posed risks to the health of the operators. Standard safety precautions were undertaken during laboratory analyses and all additional experimental procedures.

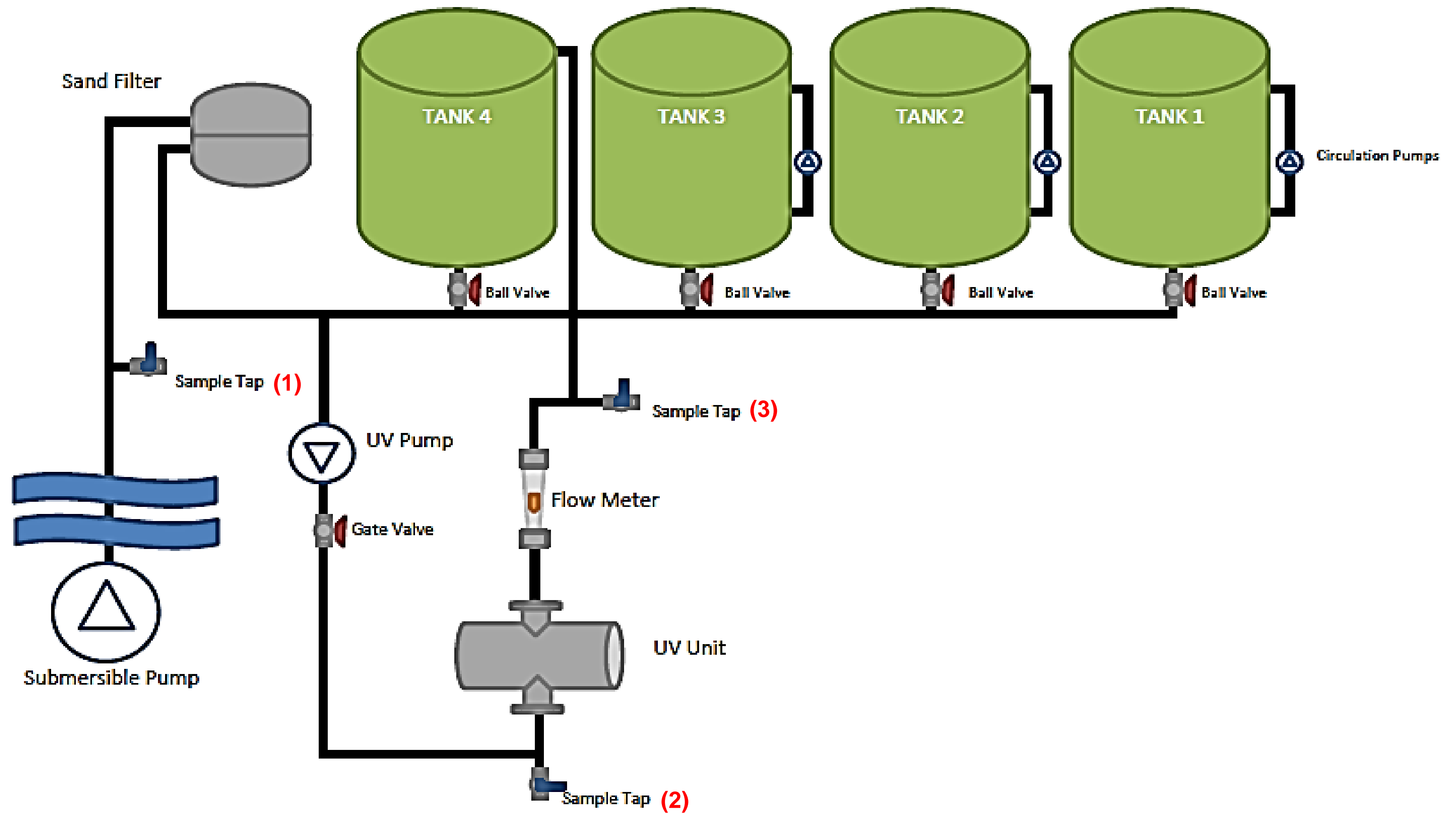

Figure 1 Diagrammatic representation of the pilot-scale MP UV system used in this study.

Photo-repair following MP UV irradiation

The potential for photo-repair (photoreactivation) following specific UV treatments was investigated using a closed system and light emitted at an intensity of 3.5 kilolux (kLux) as measured using a portable Jaz spectrometer (Ocean Optics, USA). For this purpose, two 10 W fluorescent lamps (STR-GX3006A, C10W, Eurolux, South Africa) were mounted on the top section of a closed container (Fig. 2). The UV irradiated water samples were exposed to the fluorescent light in 500 mL glass beakers while being agitated using a magnetic stirrer and bar. Water temperature was maintained at 23 ± 1°C throughout the period of irradiation.

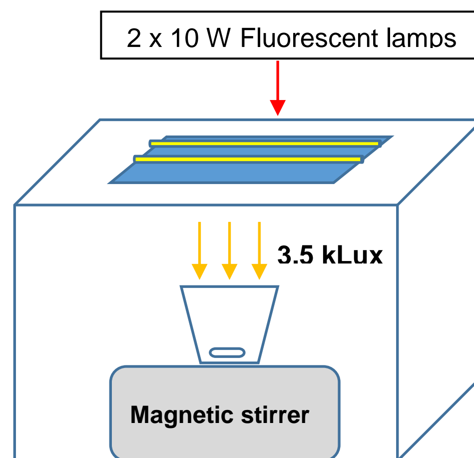

Figure 2 UV irradiated water samples exposed to fluorescent light in a closed container.

Regrowth was expressed in terms of log-reactivation and the percentage recovery was calculated using the following equation as defined by Lindenauer & Darby (1994):

$$Percentage\ photoreactivation\ (\%) = \frac{(N_p - N)}{(N_0 - N)} \times 100\%$$

In the above equation, N_p = the number of cells in reactivated sample (cfu.mL^{-1}), N = cell number immediately after UV irradiation (cfu.mL^{-1}) and N_0 = the number of cells before UV irradiation (cfu.mL^{-1}).

Microbiological analysis

Before (control) and after all specific disinfection and photo/dark-repair experiments dilution series ($10^0 - 10^{-6}$) were prepared in triplicate. Enumeration procedures were performed subsequently.

Total and Faecal coliform enumeration

Total coliforms (TC) and FC were enumerated according to the South African National Standards (SANS) method 4832 (SANS, 2007a). Violet Red Bile Agar (VRBA) (Merck, South Africa) was

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used to prepare duplicate pour plates which were inverted and incubated at 35°C and 44°C for 24 h to determine TC and FC, respectively.

Heterotrophic plate count (HPC)

The total heterotrophic bacteria population was enumerated according to the SANS method 4833 (SANS, 2007b). Duplicate pour plates were prepared using Plate Count Agar (PCA) (Merck, South Africa), inverted and incubated at 30°C for 48 h.

Water quality analysis

Physico-chemical parameters of sand-filtered, non-irradiated river water were determined according to Standard Methods (APHA, 2005) and included chemical oxygen demand (COD), total suspended solids (TSS), volatile suspended solids (VSS), alkalinity, conductivity and water temperature and pH. A DR2000 spectrophotometer (Hach, USA) was used to measure COD and results were expressed as mg $O_2.L^{-1}$. A portable HI 8733 conductivity meter (Hanna Instruments, USA) was used to measure conductivity in units of $mS.m^{-1}$. The UV transmission percentage (UVT%), turbidity and total dissolved solids (TDS) content of the water were also determined. Furthermore, total and faecal coliforms and the total heterotrophic population were enumerated in order to quantify the level of microbiological contamination occurring in the untreated river water. Following these analyses, the estimated values were compared to guidelines set by DWAF (1996) for water intended to be used for the irrigation of fresh produce (Table 1).

Ultraviolet transmission percentage (UVT%)

A Sense^{TM} T UV-Transmittance Monitor (Berson, The Netherlands) was used to measure the ultraviolet transmission percentage (UVT%) of filtered river water. The instrument was used as described by the manufacturer and deionised water was used for calibration. The latter represented UV transmission of 100%.

Turbidity

The turbidity of untreated river water was determined using an Orion AQ3010 Turbidity Meter (Thermo Scientific, USA), as described by the manufacturer. Solutions of known turbidity were used to verify that the instrument was calibrated.

Total Dissolved Solids (TDS)

The TDS content of water samples were calculated from the measured values for conductivity using the following equation (DWAF, 1996):

$$Electrical\ conductivity\ (mS.m^{-1}) \times 6.5 = TDS\ (mg.L^{-1})$$

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Table 1 Limits for physico-chemical and microbiological qualities of water used for the irrigation of fresh produce as suggested by DWAF (1996)

Water quality parameter	Limit
Faecal coliforms	1 000 cfu.100 mL^{-1}
Conductivity	40 $mS.m^{-1}$
pH	6.5 – 8.4
Total Suspended Solids (TSS)	50 $mg.L^{-1}$

Statistical analysis

Statistica 12.5 software (StatSoft, USA) was used to perform statistical analyses. Data were analysed using a two-way analysis of variance (ANOVA) and a mixed model repeated measures ANOVA. Post hoc analyses were performed using the Fisher least significance difference (LSD) test and significant results were identified using a 5% significance level ($p<0.05$) as guideline.

Research study design

Pilot-scale experiments were performed to investigate the efficiency of MP UV light for the disinfection of river water. In this regard, filtered water from the Plankenburg River was exposed to several UV doses on three respective days. A range of water quality parameters were determined on each of the three days in order to examine the influence of water quality on the efficacy of the respective treatments. The response of the microbial population in the water towards UV irradiation was expressed in terms of log-inactivation.

The potential for photo and dark-repair following MP UV irradiation was also investigated. River water was irradiated using a range of UV doses and a varied irradiation regime. Following UV irradiation the samples were exposed to different conditions of light and dark to analyse possible recovery. For each of these damage-repair trials the same range of water quality parameters were again determined.

Study A: Pilot-scale MP UV disinfection

River water was exposed to UV doses of 13, 17 and 24 $mJ.cm^{-2}$ as described earlier. Samples were taken before (point 2) and after (point 3) UV treatment where after TC, FC, and the heterotrophic population were enumerated to determine the log-inactivation achieved at the respective doses. The results of microbiological and water quality analyses were compared to guidelines for fresh produce irrigation water quality set by DWAF (1996) (Table 1) and were used to interpret the effectiveness of the respective treatments. For each trial the experimental procedure was performed in triplicate.

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Study B: Photo-repair following pilot-scale MP UV irradiation

The potential for photo-repair (photoreactivation) was investigated following pilot-scale MP UV disinfection. River water was irradiated at UV doses of 13 and 24 $mJ.cm^{-2}$, transferred to sterile 500 mL glass beakers (one for each dose) and subsequently exposed to artificial light at an intensity of 3.5 kLux. The samples were stirred using a magnetic stirrer and bar (Fig. 2). Total coliforms (TC) were enumerated before (time -1.0 h) and directly after (time 0 h) UV treatment and again after 1, 3 and 5 h of exposure to the fluorescent light. A control sample (untreated river water) was handled in the same manner. Regrowth was expressed in terms of log-reactivation and the percentage recovery was calculated.

Study C: Photo and dark-repair under varied conditions following pilot-scale MP UV disinfection

A higher UV dose and a modified irradiation technique (compared to Study B) was applied to river water and the extent of photo and dark-repair were investigated. The water was irradiated using UV doses of either 40 or 2 x 20 $mJ.cm^{-2}$ (sequential irradiation at 20 $mJ.cm^{-2}$). These treatments were chosen in order to evaluate the effect of using multiple UV lamps (simulated by the 2 x 20 $mJ.cm^{-2}$ treatment) in comparison to the use of one stronger lamp (simulated by the 40 $mJ.cm^{-2}$ treatment) to optimise disinfection and minimise the potential for recovery following larger-scale UV disinfection. The irradiated samples were exposed to the following conditions post UV treatment: Artificial light as in study B (3.5 kLux); ambient light (inside laboratory); complete darkness (closed container).

For the first condition (3.5 kLux light) samples were treated as described in Study B. The irradiated river water was also exposed to the lighting found in the laboratory which was a combination of artificial and natural lighting with intensity in the range of 1.0 to 2.0 kLux (measured using the Jaz spectrometer). River water was transferred to sterile 500 mL beakers (one for each dose), covered, and left on a work-bench inside the laboratory. To investigate dark-repair, water was again transferred to 500 mL beakers which were moved to a closed container, resulting in complete darkness. Total coliforms were again enumerated before (time -1.0 h) and after (time 0 h) the UV treatments and 1, 3 and 5 h following exposure to the different conditions described above. In all instances, regrowth was expressed as log-reactivation and the percentage recovery (for light and dark-repair) was calculated using the equation defined by Lindenauer & Darby (1994).

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RESULTS AND DISCUSSION

Study A: Medium-pressure (MP) UV disinfection of contaminated river water

River water quality

The quality characteristics of water that was sampled from the Plankenburg River were evaluated before disinfection in each of the pilot-scale trials. The microbiological and physico-chemical properties determined are presented in Table 2.

The results of the analyses show that extremely high levels of TC, FC and heterotrophic bacteria were present in the water on each of the respective days (Table 2). Even for FC, the size of the population always exceeded 1 000 0000 cfu.100 mL^{-1} (6 log) with the maximum (6.41 log cfu.100 mL^{-1}) observed in Trial 1. With regard to faecal contamination, it was thus demonstrated that the water did not comply with limits set for water intended to be used for fresh produce irrigation (DWAF, 1996) (Table 1). These results are in agreement with those of other researchers reported in the literature which show that the Plankenburg River is extensively contaminated with faecal bacteria (Paulse *et al*., 2009; Ackerman, 2010; Huisamen, 2012). In this study it was found that reductions of up to 3.41 log would be required to yield microbiologically acceptable irrigation water. In support of this, Britz *et al*. (2013) recommended a target reduction of 3 to 4 log based on an investigation of microbiological contamination of Western Cape Rivers and the limits set by DWAF (1996) and WHO (1989). Total coliforms (TC) and the heterotrophic population (HPC) reached maximum levels of 7.07 (Trial 1) and 8.75 log (Trial 3) cfu.100 mL^{-1}, respectively, again indicating the severity of microbial contamination in the water (Table 2). The urgent need for an affordable, safe and effective method of water disinfection is thus apparent.

Except for the conductivity, all of the physico-chemical parameters determined for the respective water samples adhered to the above mentioned guidelines set by DWAF (1996) (Table 1 and 2). However, the water tested in Trial 1 was generally of poorer quality in comparison to that tested in Trials 2 and 3. Poor water quality, in this sense, refers to greater concentrations of substances that could potentially interfere with the efficiency of UV disinfection. Measured values for COD, for instance, were 49.50 and 33.30 mg.L^{-1} higher in Trial 1 than in Trials 2 and 3, respectively. Water sampled during Trial 1 also represented the greatest magnitudes of TSS, VSS and conductivity (Table 2). Furthermore, the turbidity, expressed in terms of Nephelometric Turbidity Units (NTU), measured in Trial 1 was higher than that determined in Trial 2 and very similar to that represented by the water in Trial 3. Based on these results, it was expected that the water used in Trial 1 would be representative of lower UVT% as the mentioned parameters contribute a variety of substances that could absorb UV photons (Brahmi *et al*., 2010; Gayán *et al*., 2014). This was not the case, however, and UV transmission was poor throughout with a maximum of 37.60% detected in Trial 3. It was also clear from the results in Table 2 that better UV transmission did not always correlate positively with lower levels of parameters that impact water

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quality. The lowest values for COD, turbidity, TSS, VSS and conductivity were detected in Trial 2, yet UV transmission was the poorest (35.05%). This could suggest that the variation observed in some of the measured physico-chemical parameters were too slight to have a clear influence on the transmission of UV light within the samples. Nevertheless, in each trial the levels of the individual quality parameters could be correlated. For instance, compared to Trial 2, higher levels of COD were accompanied by higher levels of turbidity, TSS and VSS in Trials 1 and 3 (Table 2). In view of the UVT% differences determined for each trial it was expected that on each of the days of treatment, similar efficiency of MP UV irradiation would be observed.

Table 2 Quality characteristics of filtered water from the Plankenburg River before exposure to MP UV irradiation

Quality Parameter	Trial 1	Trial 2	Trial 3
UVT%	36.00	35.05	37.60
COD ($mg.L^{-1}$)	96.30	46.80	63.00
Turbidity (NTU)	24.50	15.84	25.60
TSS ($mg.L^{-1}$)	29.00	18.00	25.00
VSS ($mg.L^{-1}$)	25.00	14.00	19.00
pH	7.23	7.42	7.29
Alkalinity (mg $CaCO_3.L^{-1}$)	118.00	118.00	131.00
Conductivity ($mS.m^{-1}$)	60.00	47.00	48.00
Total coliforms (TC) (log cfu.100 mL^{-1})	7.07	6.87	6.94
Faecal coliforms (FC) (log cfu.100 mL^{-1})	6.41	6.23	6.29
Heterotrophic plate count (HPC) (log cfu.100 mL^{-1})	7.94	7.68	8.75

Efficiency of pilot-scale MP UV irradiation for river water disinfection

The results presented in Figure 3 clearly show how microbial inactivation was enhanced as the UV dose was increased from 13 to 24 $mJ.cm^{-2}$ during the first experimental trial. The initial increase, however, had a very slight effect on disinfection. Reductions that were 0.19, 0.18 and 0.43 log greater were achieved at 17 $mJ.cm^{-2}$ in comparison to those at 13 $mJ.cm^{-2}$ for TC, FC and the heterotrophic population, respectively (Fig. 3). For TC and FC, respectively, these differences were not statistically significant ($p=0.13$ and $p=0.08$). For the same three groups, an UV dose of 24 $mJ.cm^{-2}$ resulted in reductions that were 1.37, 0.89 and 1.09 log greater than those achieved at 13 $mJ.cm^{-2}$. The latter differences in log reduction were statistically significant ($p<0.05$) in each case. Maximum reductions were recorded as 3.51, 3.23 and 2.42 log, respectively, for TC, FC and HPC (Fig. 3).

As is evident, the target 3 log reduction (indicated by the dotted red line) was met for TC and FC at a dose of 24 $mJ.cm^{-2}$ (Fig. 3). However, considering the initial level of FC

(6.41 log cfu.100 mL^{-1}) (Table 2) treatment at the maximum dose (24 $mJ.cm^{-2}$) was not capable of reducing the load to below 1 000 cfu.100 mL^{-1}. The treated water was thus unfit for the application of fresh produce irrigation (DWAF, 1996). Doses exceeding 24 $mJ.cm^{-2}$ would, therefore, be required to reach the limit set on FC levels in irrigation water of similar poor quality. In this trial the lower UV doses (13 and 17 $mJ.cm^{-2}$) showed poor disinfection potential.

For each of the treatments the greatest reduction was achieved for TC, followed by FC and the heterotrophic population (Fig. 3). While FC is regarded as a subgroup of TC, the heterotrophic population comprises different microorganisms, such as gram-positive bacteria, of which some may be more resistant to MP UV irradiation. In this regard, log reductions achieved for the latter group (HPC) were always significantly lower ($p<0.05$) than for the others (TC and FC). At a dose of 24 $mJ.cm^{-2}$, a low 2.42 log reduction in HPC was reached. Corresponding reductions at doses of 13 and 17 $mJ.cm^{-2}$ were recoded as 1.33 and 1.76 log, respectively. The results also imply that some faecal coliform species are more resistant to UV irradiation in comparison to their non-faecal counterparts. However, reductions achieved for TC and FC were in most instances not statistically different ($p>0.05$). It was only at a UV dose of 24 $mJ.cm^{-2}$ where a significantly better log reduction ($p=0.019$) was achieved for TC compared to FC.

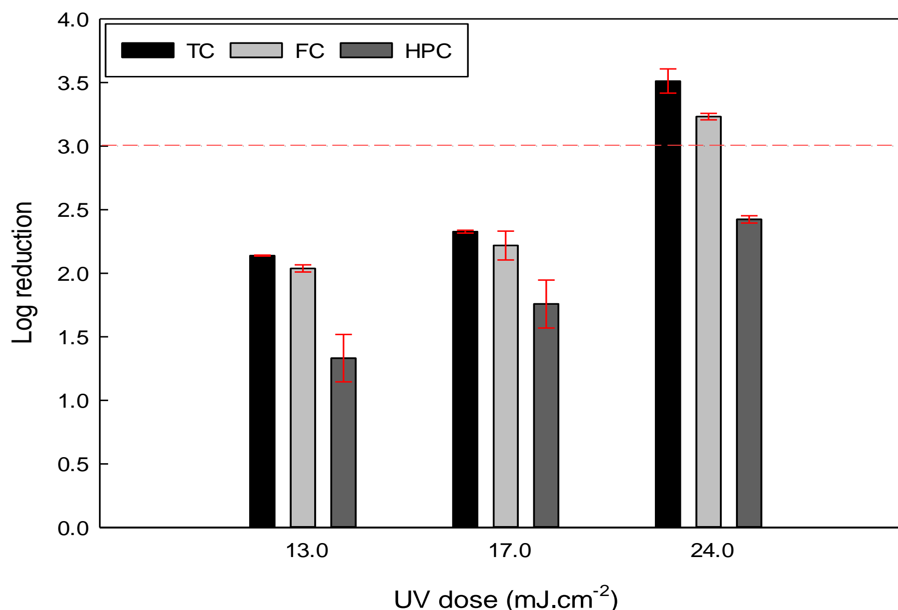

Figure 3 Reductions achieved for TC, FC and HPC following pilot-scale MP UV irradiation at three doses (13, 17 and 24 $mJ.cm^{-2}$) during experimental Trial 1. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

Results recorded for the second trial differed from that observed in Trial 1. The effect of elevating the applied UV dose was seemingly less profound (Fig. 4) but higher reductions were achieved at 13 and 17 $mJ.cm^{-2}$ compared to that seen in Trial 1. None of the enumerated groups (TC, FC and HPC) were inactivated significantly better (in terms of log reduction) ($p>0.05$) at 17

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mJ.cm^{-2} in comparison to 13 mJ.cm^{-2} in Trial 2 (Fig. 4). Furthermore, the difference in reductions achieved at the highest (24 mJ.cm^{-2}) and lowest (13 mJ.cm^{-2}) UV doses were calculated as 0.18, 0.31 and 0.64 log for TC, FC and the total heterotrophic population, respectively (Fig. 4). For TC the difference was again insignificant ($p=0.09$). Nonetheless, the heterotrophic population was the most resistant group (although more sensitive at 13 and 17 mJ.cm^{-2} compared to HPC in Trial 1), followed by FC and TC. For each treatment, significantly lower log reductions ($p<0.05$) were achieved for HPC in comparison to TC and FC, respectively. As observed in Trial 1, log reductions achieved for TC and FC were in some instances not statistically different ($p>0.05$).

The maximum reductions achieved (at 24 mJ.cm^{-2}) were calculated as 2.96, 2.74 and 2.45 log for TC, FC and the heterotrophic population, respectively. In comparison to Trial 1, reductions achieved for TC and FC (at 24 mJ.cm^{-2}) were thus 0.55 and 0.49 log lower in Trial 2. The latter differences were each statistically significant ($p<0.05$). The heterotrophic population showed very similar reductions in Trial 1 (2.42 log) and Trial 2 (2.45 log) at 24 mJ.cm^{-2} and the difference of 0.03 log was statistically insignificant ($p=0.66$). These results also show that the suggested 3 log target reduction was not achieved in Trial 2 (Fig. 4). As was observed in Trial 1, the maximum UV dose (24 mJ.cm^{-2}) was again incapable of reducing the initial FC population (6.23 log cfu.100 mL^{-1}) to below 3 log cfu.100 mL^{-1}.

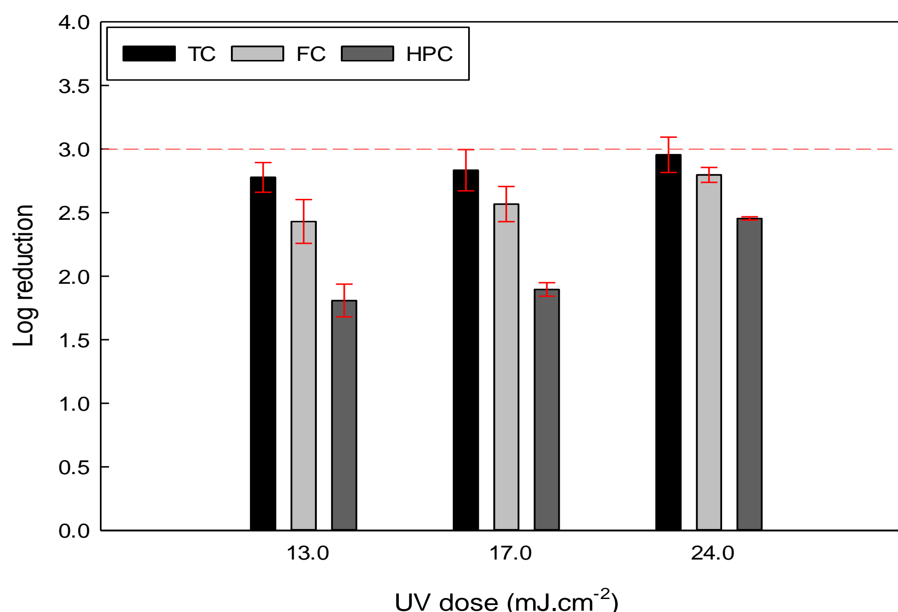

Figure 4 Reductions achieved for TC, FC and HPC following pilot-scale MP UV irradiation at three doses (13, 17 and 24 mJ.cm^{-2}) during experimental Trial 2. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

The results presented in Figure 5 show that tendencies similar to that observed in Trial 2 (Fig. 4) were seen during Trial 3. Again, slight differences in reductions were recorded for TC, FC and HPC, respectively, following irradiation at 13, 17 and 24 mJ.cm^{-2}. At a dose of 24 mJ.cm^{-2}, the

mentioned groups were reduced by 3.01, 2.69 and 2.94 log, correspondingly. These reductions were 0.32, 0.21 and 0.33 log, respectively, higher than those achieved at 13 $mJ.cm^{-2}$. The differences were significant ($p<0.05$) in each case. At the maximum dose (24 $mJ.cm^{-2}$) reductions achieved in Trial 3 were very similar to that achieved in Trial 2, with the exception of HPC. A significant difference ($p<0.05$) of 0.49 log in reduction of the latter group was observed, with the treatment in Trial 3 being more effective.

It was clearly observed that the total heterotrophic population could not be regarded as being the most resistant in this trial (Trial 3). At doses of 13 and 24 $mJ.cm^{-2}$ HPC were reduced to a greater extent than FC, while similar reductions were achieved at a dose of 17 $mJ.cm^{-2}$ for the respective groups (FC and HPC) (Fig. 5). The TC population was again the most sensitive. However, it was only at a dose of 17 $mJ.cm^{-2}$ that significantly better inactivation ($p<0.05$) (in terms of log reduction) was observed for TC in comparison to both FC and the total heterotrophic group. In this trial, it was clearly observed that the heterotrophic population was less resistant in comparison to that seen in Trials 1 and 2. Even at 13 $mJ.cm^{-2}$ a reduction of 2.63 log was achieved in comparison to the corresponding reductions of 1.33 and 1.81 log reached in Trials 1 and 2, respectively.

Considering the guideline limit set on FC, and the initial concentration of 6.29 log cfu.100 mL^{-1} (Table 2), the maximum reduction of FC in Trial 3 (2.69 log) will not be sufficient in rendering the water acceptable for fresh produce irrigation.

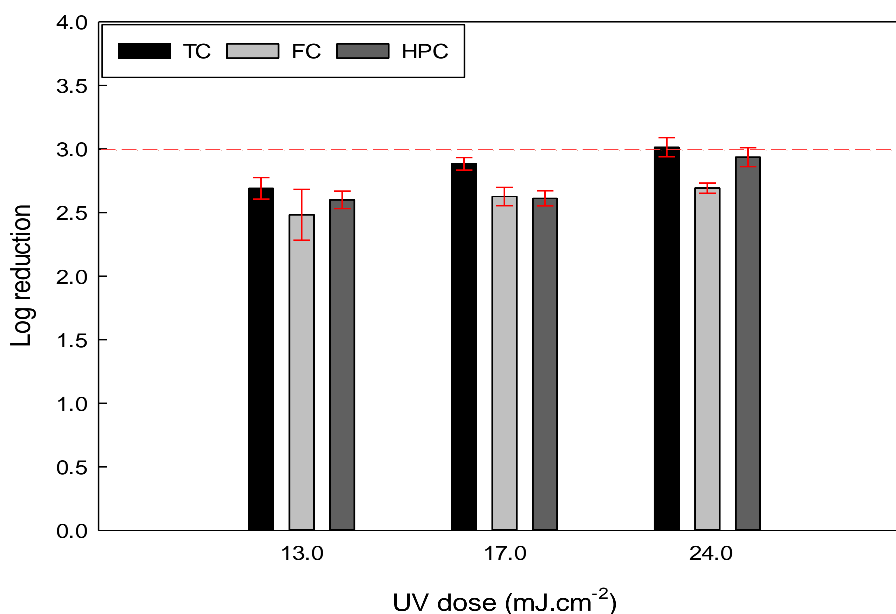

Figure 5 Reductions achieved for TC, FC and HPC following pilot-scale MP UV irradiation at three doses (13, 17 and 24 $mJ.cm^{-2}$) during experimental Trial 3. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

Results obtained in these individual trials were difficult to compare to the literature since investigations into the disinfection potential of UV light often describe efficiency in terms of

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inactivation kinetics (Hijnen *et al.*, 2006). Furthermore, it is also seen that previous research has often been conducted using LP UV lamps rather than the MP UV source utilised here (Mezanotte *et al.*, 2007; Selma *et al.*, 2008; Melidis *et al.*, 2009; Wang *et al.*, 2011; Jones *et al.*, 2014). Due to the difference in the emission spectra of LP and MP Mercury vapour lamps such results could not be compared to those recorded in the current study.

Nonetheless, Quek & Hu (2008a) reported that MP UV doses ranging from 4.5 to 9.0 mJ.cm^{-2} were required to produce a 4 log reduction of *E. coli*. The experiments were, however, conducted using seeded *E. coli* strains and sterile distilled water, which explains the greater lethality observed in comparison to that of the current research. Guo *et al.* (2009), however, reported a reduction of < 2.5 log when TC were enumerated following MP UV irradiation of a wastewater sample at 15 mJ.cm^{-2}. This result exemplifies the restraining influence of water quality (and the characteristics of a naturally occurring microbial population) on the efficiency of UV disinfection and was more in line with that found in this investigation.

From the results obtained in Trials 1 to 3 it was concluded that MP UV irradiation, even at a dose of 24 mJ.cm^{-2}, was incapable of delivering inactivation that will reduce the FC load of irrigation water to within the acceptable limit. Although the suggested 3 log target reduction was met in some instances (Table 3), the extreme levels of faecal contamination observed at the start of each trial were too high to allow for sufficient reductions. Future discussions will elaborate on effects observed at 24 mJ.cm^{-2}, in particular, as the lower doses could not be considered as being potentially effective for river water disinfection. Referring to this dose (24 mJ.cm^{-2}), and reductions achieved for TC and FC, the treatment was most effective in Trial 1. Very similar results were recorded for Trials 2 and 3, however, slightly better inactivation of FC was observed in Trial 2 (Table 3).

Table 3 Reductions achieved for TC, FC and HPC following MP UV treatment at a dose of 24 mJ.cm^{-2} during three respective trials

	Maximum reduction (Log)		
Microorganisms	**Trial 1**	**Trial 2**	**Trial 3**
TC	3.51±0.10	2.96±0.14	3.01±0.07
FC	3.23±0.03	2.74±0.04	2.69±0.04
HPC	2.42±0.03	2.45±0.01	2.94±0.07

TC – Total coliforms; FC – Faecal coliforms; HPC – Heterotrophic plate count

With reference to earlier discussions and the results presented in Table 3, reductions achieved for TC, FC and HPC, respectively, were often similar on the different days of treatment at a dose of 24 mJ.cm^{-2}. However, significant differences ($p<0.05$) were recorded in some instances, indicating that irrespective of the applied UV dose additional factors may impact the efficiency of

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UV disinfection. Specifically, the influence of water quality and microbiological properties, as well as technical limitations associated with the pilot-scale system, may be important.

Due to the fact that the Berson InLine 40+ UV system accounted for variability in UVT% (defined as a function of the absorption coefficient) it can be said with certainty that the same UV dose was delivered in each of the respective trials. Referring to the influence of water quality then, differences in the achieved reductions (Table 3) could not be frankly ascribed to differences in the levels of absorbing substances in the water. Nonetheless, in addition to the absorption of UV light, some particulate matter may influence UV disinfection efficiency as a result of blocking and/or scattering effects. The lethality of the treatments in this study would not necessarily correlate with the levels of these particles, however, since additional factors such as particle size and the extent of particle association become important.

More specifically, particulate substances have been suggested to influence UV disinfection efficiency as a result of light scattering, refraction or reflection (collectively referred to as shading) (Walters *et al*., 2014). A phenomenon known as encasement has also been shown to be influential. Incidentally, research has identified a positive correlation between the size and concentration of suspended particles and the efficiency of UV irradiation in contaminated water (Whitby & Palmateer, 1993; Örmeci & Linden, 2002). In this regard, microbial populations are well-known to be able to associate with particles occurring in their surrounding environment (Fries *et al*., 2008; Droppo *et al*., 2009). When associated with particles, microorganisms may be better protected compared to those freely suspended in the water. The former may obtain valuable nutrients from the substances to which they adhere while also being protected from a range of environmental stress factors (Sinton *et al*., 1999; Davies & Bavor, 2000).

Walters *et al*. (2014) evaluated the effect of particle association and suspended solids on the inactivation of faecal bacteria using UV light. Their work identified a reversed correlation between the total suspended solids (TSS) concentration and the rate of bacterial inactivation. Also, *E. coli* which were associated with smaller particles, particle diameter (d_p) ≤ 12 μm, were inactivated two times faster in comparison to those attached to particles with size 12 < d_p ≤ 63 μm. In the current study, the greater reductions achieved for TC and FC in Trial 1 (Table 3) could, however, not be related to lower levels of suspended solids (Table 2). Furthermore, the effect of TSS could also not be observed when comparing reductions achieved in Trials 2 and 3. Even though the TSS concentration was 7 mg.L^{-1} lower in Trial 2 than 3, very similar reductions were achieved for TC and FC, respectively (Table 2).

In the literature, UV efficiency has also been discussed with reference to the influence of turbidity. It is stated that high turbidity levels may contribute to absorption and, importantly, blocking of UV light. Pathogens may consequently be protected against the harmful UV rays (Jones *et al*., 2014). Nonetheless, the correlation between UV efficiency and the turbidity level in water is inconsistent. This results from the fact that substances which contribute to turbidity represent highly variable properties in terms of UV blocking and absorption (Jones *et al*., 2014).

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Generally, however, increased levels of turbidity result in lower efficiency of UV disinfection (Spellman, 2003; Qian, 2011). In this study, the influence of turbidity on UV lethality could also not be clearly observed. The water treated in Trial 2 was representative of the lowest turbidity (15.84 NTU), as well as TSS, yet reductions achieved for TC, FC and HPC, respectively, were never significantly higher ($p<0.05$) in comparison to the other days. Also, while the water treated in Trial 1 was very turbid (24.50 NTU), and representative of the highest TSS concentration (29.00 $mg.L^{-1}$), significantly higher log reductions ($p<0.05$) were reached for TC and FC in comparison to that achieved in Trials 2 and 3.

From these results it is evident that the study of the influence of scattering or blocking effects (imparted by particulate material) is more complex than simply referring to differences in concentrations of the influential particles. In this regard, it is apparent that it was difficult to correlate the effectiveness of the respective treatments with variation in water quality in Trials 1 to 3. Owing to this, and the fact that UV transmission was fairly similar throughout the days of disinfection, it might be that the differences in water quality were too small to have a clearly visible influence on the efficiency of UV disinfection. The impact of the characteristics of the microbial community occurring in the river water (on each day) may, however, be of great importance. While the level of microbial contamination of the water may vary daily, the presence or absence of particularly resistant strains or species may greatly influence disinfection efficiency.

It is well-known that the effectiveness of UV disinfection is largely dependent on the extent of DNA damage induced by the treatment, as well as the degree of subsequent DNA repair (López-Malo & Palou, 2004). DNA damage and repair, in turn, are influenced by environmental, process and microbial factors which may be prominent prior to, during or following UV irradiation (Gayán *et al.*, 2014). Earlier in this section the impact of environmental factors (water quality) was discussed and it was difficult to correlate the physico-chemical properties of the water with UV lethality. However, owing to the fact that the water was sampled from the Plankenburg River (on different days) it was certain that the representative microbial populations were diverse. Both intrinsic and extrinsic microbial factors could, therefore, affect the efficiency of the treatment (Gayán *et al.*, 2014). Intrinsic factors refer to properties such as genetic material conformation and cell and genome size, while extrinsic factors refer to influences from outside, including growing conditions, growth phase, environmental stressors and conditions for recovery. Variation in UV resistance will, therefore, vary by the type of microorganism and also by the species and strains present in the treated substance (Gayán *et al.*, 2014). This is clearly observed when looking at the results presented in Figures 3 to 5 and Table 3.

For TC, FC and the heterotrophic population, respectively, variation in reductions could be observed on different days of treatment. For TC and FC, specifically, better inactivation at a dose of 24 $mJ.cm^{-2}$ were observed in Trial 1, compared to that recorded for Trials 2 and 3 (Table 3). The heterotrophic population, however, was maximally inactivated in Trial 3. Thus, while the coliform community in Trial 1 was the most sensitive, the total heterotrophic population in the same sample

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was the most resistant in comparison to other days of treatment (Table 3). It is, therefore, evident that within the same water sample great variation in the UV sensitivity of different types of microorganisms may be observed. Nonetheless, coliforms irradiated in Trials 2 and 3 showed greater resistance to UV light (in comparison to those in Trial 1), as was the case for the total heterotrophic population in Trials 1 and 2 (compared to that in Trial 3) (Table 3). These populations possibly represented greater levels of intrinsic resistance to the harmful effects of UV irradiation if the influence of water quality was not to be considered.

On the other hand, extrinsic factors may also contribute to observed differences in UV resistance of the different microbial groups. In this study, the sampling site was situated down-stream of local industries and an informal settlement which have been suggested as probable sources of pollution of the Plankenburg River (Britz *et al.*, 2013). In addition to microbial contamination, substances such as chemical and/or food waste may also enter the river via this route. These could influence the growth characteristics of the representative microbial population by either providing additional nutrients or by introducing substances that would put the microorganisms under stress. While actively growing microorganisms are more sensitive to UV light, stimulation of RpoS (s^{38}) (a stress response factor) may impart added resistance if co-protective reactions are instigated (Child *et al.*, 2002; Berney *et al.*, 2006; Bucheli-Witschel *et al.*, 2010; Van der Veen & Abee, 2011). In this regard, the extent of pollution contributed by waste from informal settlements and industrial operations may vary on a day-to-day basis. It is therefore possible that the differences in the observed reductions achieved for TC, FC and HPC, respectively, (on different days of treatment) may be attributed to variation in the degree of pollution.

Referring to the effect of MP UV irradiation at a dose of 24 $mJ.cm^{-2}$ it was observed that the heterotrophic bacteria population was more resistant in comparison to coliforms in each of the respective trials (Table 3). This was expected as HPC is inclusive of all bacteria (WHO, 2003). In a review of the literature, Gayán *et al.* (2014) states that vegetative bacteria are most sensitive to UV disinfection, followed by yeast cells, spores of bacteria, viruses and lastly protozoa. In this regard, factors such as cell size and pyrimidine levels within the microbial DNA become important (Oteiza *et al.*, 2010; Fredericks *et al.*, 2011; Gabriel, 2012). Also, it has been stated that gram-positive bacteria show increased resistance in comparison to the gram-negatives (Gayán *et al.*, 2014). It was therefore expected that the heterotrophic population in the river will be more resistant than the coliforms since the former will always include some of the more resistant microorganisms mentioned above. As confirmation, Britz *et al.* (2013) reported that samples taken from the Plankenburg River frequently tested positive for the presence of *Staphylococcus* spp. and *Listeria* spp., which are more resistant, gram-positive bacterial species. The results in Table 3 also demonstrate that FC were reduced to a lesser extent in comparison to TC. However, the difference in the log reductions achieved for these two groups at 24 $mJ.cm^{-2}$ were, in most instances, statistically insignificant ($p>0.05$).

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Considering the similar reductions achieved at UV doses of 13, 17 and 24 mJ.cm^{-2} in Trials 2 and 3, respectively, the influence of process factors may be important. During pilot-scale experiments the flow rate of water through the irradiation chamber was regulated by means of a gate valve to deliver the respective UV doses. If the flow meter was slightly inaccurate, or the flow rate adjusted inaccurately, the delivered UV dose would have been different from the predetermined value, consequently not showing the expected increase in reduction with increase in UV dose. It might be, however, that the difference in UV doses were just too small to contribute large differences in inactivation potential. This could be possible if it is accepted that the pilot-scale system was precisely operated, since the delivered UV dose was accurately computed with reference to the measured UVT%. Inaccurate operation of the pilot-scale system may also contribute to the variation in reductions achieved at 24 mJ.cm^{-2} on the respective days of UV treatment (Table 3). However, experiments were always performed in a comprehensive manner and the influence of inaccurate procedures will therefore not be considered in this discussion.

The data presented in Table 4 shows the final concentrations of FC following each of the MP UV treatments performed on pilot-scale. None were capable of reducing the FC load to within the limit of 3 log cfu.100 mL^{-1} suggested by DWAF (1996) for fresh produce irrigation water.

Table 4 Levels of FC detected in river water following MP UV irradiation at three doses

	Log cfu.100 mL^{-1}		
UV dose	**Trial 1**	**Trial 2**	**Trial 3**
13 mJ.cm^{-2}	4.37±0.05	3.80±0.17	3.81±0.22
17 mJ.cm^{-2}	4.19±0.09	3.67±0.14	3.67±0.06
24 mJ.cm^{-2}	3.21±0.01	3.44±0.04	3.60±0.04

Study B: Photo-repair following MP UV irradiation

The data presented in Figure 6 show TC cell concentrations before (-1.0 h) and after (0.0 h) MP UV disinfection at doses of 13 and 24 mJ.cm^{-2}, respectively. The period 0.0 to 5.0 h represents exposure to photoreactivating light at an intensity of 3.5 kLux. During this time, regrowth was investigated and recorded in terms of percentage log recovery. Investigating photo-repair of the TC population was credible since the majority of the group is representative of the faecal coliforms (*E. coli*) subgroup. This is seen in Table 2 where the difference in the initial concentrations of TC and FC detected in the river water (Trials 1 to 3) was in the range of 0.64 to 0.66 log cfu.100 mL^{-1}.

Disinfection efficiency compared to Study A

Reductions of 3.30 and 3.36 log, respectively, were achieved when river water was irradiated with MP UV light at 13 and 24 mJ.cm^{-2} (Fig. 6). The difference between the reductions (0.06 log) was statistically not significant ($p=0.62$). In comparison to the inactivation of TC at 13 mJ.cm^{-2} in Trials

1, 2 and 3 (Study A), respectively, the treatment in this study was significantly more effective ($p<0.05$) and resulted in reductions that were 1.16, 0.52 and 0.61 log greater. At a dose of 24 mJ.cm^{-2} the treatment in the current study produced reductions that were 0.40 and 0.35 log greater compared to those reached in Trials 2 and 3 (Study A) while the treatment in Trial 1 (Study A) showed better inactivation (0.15 log) compared to that achieved in this study. Nevertheless, the general observation was that, at the same dose, MP UV irradiation was more lethal in Study B than Study A. Furthermore, the difference in reduction between the maximum (24 mJ.cm^{-2}) and minimum (13 mJ.cm^{-2}) UV dose in Study A was always larger than the corresponding value recorded for Study B (0.06 log).

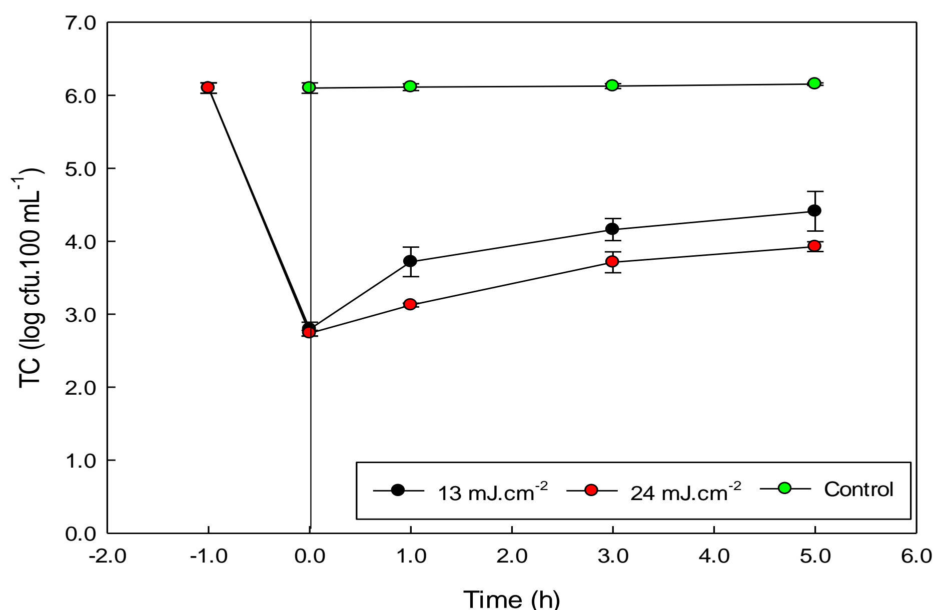

Figure 6 Total coliform (TC) population size before and 1, 3 and 5 h after exposure to photoreactivating light following MP UV irradiation. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

Looking at physico-chemical water characteristics it is evident that the untreated (sand-filtered) water could not be used for fresh produce irrigation as the conductivity was higher than the allowed limit suggested by DWAF (1996) (Tables 1 and 5). Nonetheless, the quality of water in this study was generally better than that observed in Study A. In comparison to each of the three trials in Study A, lower values for COD, turbidity, TSS and VSS were detected (Tables 2 and 5) in this trial. Also, UV transmission in the present investigation was 13.90, 14.85 and 12.30% greater than the values recorded in Trials 1, 2 and 3 (Study A), respectively (Table 5). This implies that UV absorption by water and its components would be lower in the current study and serves as an indication of better water quality, overall. Regarding microbiological contamination of the Plankenburg River, water used in Study B was less polluted as the TC concentration (6.10 log)

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was lower compared to that detected in Trial 1 (7.07 log), Trial 2 (6.87 log) and Trial 3 (6.94) of the previous investigation.

Owing to improved water quality, in terms of COD, turbidity, TSS, VSS and UVT%, the better disinfection observed in this experiment (compared to Study A) was expected. While the same UV dose was always delivered, lower levels of interfering substances probably resulted in a lesser degree of light scattering and blocking, consequently resulting in greater inactivation. Also, a larger proportion of the microbial population might have been suspended/free-floating, rather than being associated with particles. Referring to the discussion in Study A, such microorganisms would be more susceptible to the harmful effects of UV irradiation. Concerning microbiological aspects, it was possible that the TC population in Study B was inherently more sensitive to UV irradiation in comparison to that encountered previously. River water characteristics, in terms of the type and levels of contaminants being present, could also have influenced the UV sensitivity of the population due to their influence on microbial growth and expression of the RpoS (s^{38}) stress response factor.

Table 5 Water quality indicators as measured for sand-filtered water from the Plankenburg River prior to performing photo-repair experiments

Quality Parameter	Measured value
UVT%	49.90
COD ($mg.L^{-1}$)	27.70
Turbidity (NTU)	12.06
TSS ($mg.L^{-1}$)	11.00
VSS ($mg.L^{-1}$)	5.00
pH	7.32
Alkalinity (mg $CaCO_3.L^{-1}$)	118.75
Conductivity ($mS.m^{-1}$)	53.0
Total coliforms (TC) (log cfu.100 mL^{-1})	6.10

Photoreactivation following UV irradiation

The data presented in Figure 6 clearly indicate that upon exposure to visible light, UV inactivated coliforms were able to regenerate and repopulate the water in which they were initially active. Following 5 h of exposure to 3.5 kLux light, the TC population size reached 4.41 and 3.93 log cfu.100 mL^{-1} in water irradiated with UV doses of 13 and 24 $mJ.cm^{-2}$, respectively (Fig. 6). This equated to significant increases ($p<0.05$) of 1.62 and 1.19 log, respectively, with reference to the size of the initial TC population following UV irradiation. Clearly, the level of photo-repair was significantly greater ($p<0.05$) following irradiation at the lower UV dose (13 $mJ.cm^{-2}$) (Fig. 6). The control in this experiment was untreated river water which was exposed to identical conditions used to evaluate photo-repair i.e. 5 h exposure to 3.5 kLux fluorescent light. A statistically

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insignificant increase ($p=0.40$) in population size (0.05 log cfu.100 mL^{-1}) was observed over the 5 h period (0 to 5 h), signifying that most of the growth in the two irradiated samples resulted from light-induced DNA repair.

The data presented in Figure 7 show the percentage log recovery recorded for TC in the two test samples following 1, 3 and 5 h of exposure to photoreactivating light. In water exposed to a MP UV dose of 13 $mJ.cm^{-2}$ recovery reached a total of 49.18% after 5 h. The corresponding value for water irradiated at 24 $mJ.cm^{-2}$ was 35.37% (Fig. 7). For the first sample (13 $mJ.cm^{-2}$) it was seen that the greater part of the total reactivation occurred within the first hour of exposure to visible light. After 1 h, 28.08% of the inactivated coliforms were again active. In the 1 to 3 and 3 to 5 h intervals additional recovery of 13.39% and 7.72%, respectively, were observed. For the 24 $mJ.cm^{-2}$ treated sample, however, photoreactivation was slightly delayed with only 11.45% of the microorganisms being revived within the first hour. This is also clear in Figure 6 which shows a smaller gradient for the 24 $mJ.cm^{-2}$ sample in the time interval 0 to 1 h compared to that represented by the 13 $mJ.cm^{-2}$ sample. Additional regrowth of 17.62% and 6.30% was measured between hours 1 to 3 and 3 to 5, respectively. Note that the bars denoted 1, 3 and 5 h in Figure 7 show total reactivation at each point in time and not the individual contributions during the different time intervals mentioned above. The results discussed here show that the higher UV dose constantly (following each time interval) represented significantly lower ($p<0.05$) levels of total microbial regrowth (Fig. 7).

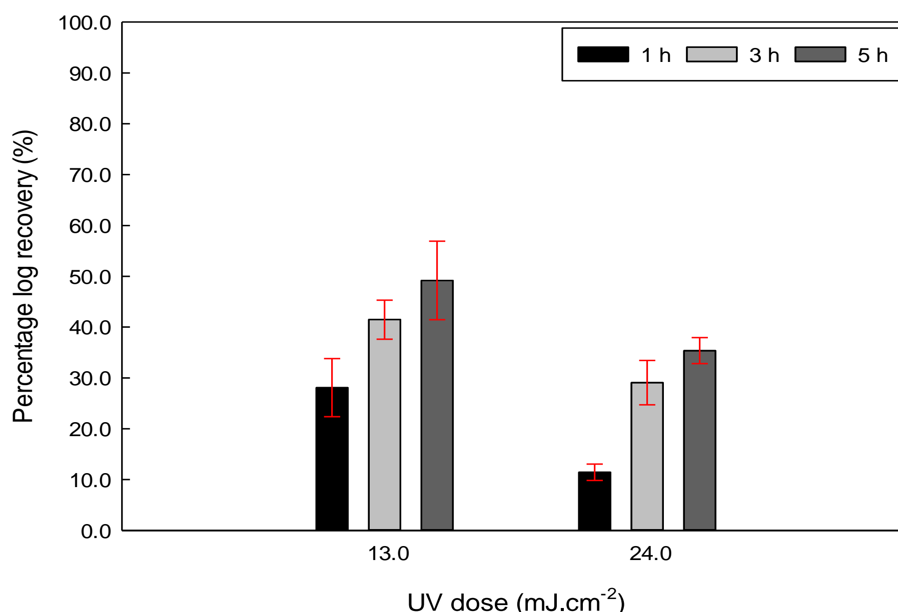

Figure 7 Percentage log recovery of TC after 1, 3 and 5 h of exposure to photoreactivating light following two MP UV treatments (13 & 24 $mJ.cm^{-2}$). Error bars were calculated based on standard deviation at a confidence interval of 0.95.

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Ultraviolet light is widely acknowledged and used as method of water disinfection, mainly due to its known effectiveness against a range of pathogenic microorganisms without leading to the formation of harmful disinfection by-products (Linden *et al.*, 2003; Locas *et al.*, 2008; Vélez-Colmenares *et al.*, 2011; Guo *et al.*, 2013). However, UV-induced DNA damage may be repaired by microorganisms through the action of photolyase in the process of photoreactivation, which was investigated in the current study. The phenomenon is well-known and has been thoroughly scrutinised by various researchers. The majority of studies, however, were conducted on laboratory-scale using low-pressure (LP) mercury vapour lamps (Guo *et al.*, 2011; Hu *et al.*, 2012; Guo *et al.*, 2012; Guo *et al.*, 2013). The current research is therefore significant, as the effect of photoreactivation was investigated on pilot-scale using MP equipment. This scenario is more representative of a UV disinfection system that would typically be used for surface water disinfection on larger scale. In this regard, some researchers have investigated photo-repair following MP UV irradiation of *E. coli* or coliform bacteria (Oguma *et al.*, 2002; Quek & Hu, 2008a & b; Guo *et al.*, 2009; Poepping *et al.*, 2014; Bohrerova *et al.*, 2015).

Trends observed in the present study are in agreement with those seen in previous research. As is evident in Figure 7, significantly lower repair (in terms of percentage log recovery) was observed at each of the time intervals (1, 3 and 5 h) for a UV dose of 13 $mJ.cm^{-2}$ compared to 24 $mJ.cm^{-2}$ ($p<0.05$). Guo *et al.* (2009) also reported that higher UV doses resulted in a lesser degree of photoreactivation. They suggested that the increased formation of pyrimidine dimers (at higher doses) may lower the extent of repair that could occur within a specific time frame. Furthermore, Quek *et al.* (2006) evaluated photo-repair of *E. coli* following MP UV irradiation at doses in the range of 1.6 to 19.7 $mJ.cm^{-2}$. Again, it was reported that lower magnitudes of photoreactivation were observed when the UV dose was higher.

In addition to DNA damage, i.e. the formation of pyrimidine dimers, the extent of photoreactivation in MP UV disinfection, specifically, may be related to the condition of the photolyase enzyme. It has been reported that the latter contains a cofactor (flavin adenine dinucleotide (FAD)) which shows meaningful absorbance at a wavelength of 280 nm (Harm, 1980). If FAD absorbed UV photons, potential damage to the photolyase enzyme could result in impaired photo-repair potential. In order to evaluate the effect of MP UV irradiation on the activity of endogenous *E. coli* photolyase Hu & Quek (2008) exposed the enzyme to doses ranging from 10 to 40 $mJ.cm^{-2}$. It was shown that with an increase in UV dose a consequent decrease in dimer repair rate could be observed. This implies that, within a given space of time, higher UV doses could allow for lower degrees of total reactivation.

As discussed earlier, it was observed for the 13 $mJ.cm^{-2}$ sample that the major part of photoreactivation (28.08%) occurred within the first hour of exposure to fluorescent light (Fig. 7). In the following hours, levelling off of the regrowth curve was observed (Fig. 6). This effect was less pronounced for the 24 $mJ.cm^{-2}$ treated sample with only 11.45% reactivation being observed in the first hour (Fig. 7). Also, the representative regrowth curve in Figure 6 only began to slightly level

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off in the 3 to 5 h time interval. Nonetheless, Quek & Hu (2008b) reported on results similar to those observed for the 13 $mJ.cm^{-2}$ treatment following photoreactivation of American Type Culture Collection (ATCC) *E. coli* strains ATCC 15597 and 11229. For strain ATCC 15597 the phenomenon was particularly evident with approximately 60% of a total of approximately 70% photoreactivation occurring in the first hour of a 4 h period. This result was also consistent with those of other researchers in the literature (Oguma *et al.*, 2001; Zimmer & Slawson, 2002; Quek *et al.*, 2006).

An increase in microbial population size following UV irradiation may, however, also be attributed to elements other than photo-repair. As suggested by Guo *et al.* (2011) the following three factors are of particular importance: normal growth of unharmed microorganisms; photo-repair of the damaged microorganisms; and normal growth of the rejuvenated microorganisms. Although the influence of the latter could not be quantified, normal growth of unharmed microorganisms served as control in this experiment. Since a very slight increase (0.05 log) was observed in this sample, the influence thereof on the total percentage recovery was very small. Thus, one can clearly observe the impact that photoreactivation may have on the final concentrations of indicator bacteria in UV disinfected waters. The results of the current study serve as a very applicable example. Each of the two UV doses (13 and 24 $mJ.cm^{-2}$) were capable of reducing the initial TC population (6.10 log $cfu.100\ mL^{-1}$) to within the limit set for FC in water used for fresh produce irrigation (3 log $cfu.100\ mL^{-1}$) (Fig. 6) (DWAF, 1996). However, within the first hour of exposure to visible light the TC population, in both samples, exceeded the mentioned limit. This is worrisome since FC represent the major portion of the TC population and are expected to react in similar fashion since the two groups were similarly affected by UV irradiation in Study A.

Furthermore, Quek & Hu (2008b) found that with an increase in light intensity, the extent of photoreactivation was enhanced. Following UV irradiation (to achieve a 5 log reduction) exposure to fluorescent light at intensity of 6 kLux resulted in > 50% recovery of two *E. coli* strains. At an intensity of 11.5 kLux, recovery exceeding 70% was observed. They also evaluated the effect of actual sunlight on photo-repair. Even at low light intensity (< 5 kLux) photoreactivation of > 60% was recorded. Again, when sunlight intensity was higher, even greater recovery was seen. This observation is important as it has been reported that the intensity of sunlight can reach 100 kLux in tropical regions (Neppolian *et al.*, 2002). These results thus indicate that the phenomenon of photo-repair could present serious implications for UV disinfection of irrigation water. In this regard, the intensity of the fluorescent light used in this study (3.5 kLux) was fairly low, yet meaningful recovery was observed in a 5 h period. Thus, depending on environmental factors, even greater repair is possible should UV disinfected water be exposed to sunlight prior to its application for fresh produce irrigation.

It was found that the extent of photoreaction (in terms of percentage recovery) observed in the current study was often lower than that reported by some researchers in the literature. The

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majority of investigations of photoreactivation, however, were performed using light of higher intensity in comparison to that used here (3.5 kLux). It was therefore expected that lower magnitudes of recovery would be observed. For example, Quek & Hu (2008a) investigated photo-repair using fluorescent light at intensity of 11.5 kLux and reported that recovery of up to 80.0% was observed following MP UV irradiation that initially resulted in a 5 log reduction in *E. coli.* In addition to the influence of light intensity, however, it was reported that the high levels of repair observed in their work could be attributed to the use of log phase cultures. It was suggested that these were in an energetically active state, which allowed them to very effectively repair UV-induced DNA damage (Quek & Hu, 2008a). Considering this, it is clear that the number of variables influencing photoreactivation can be vast and the comparison of data therefore becomes a tedious task. Factors such as light intensity, wavelength and temperature, for example, are well-known to influence the repair of DNA damage performed by the photolyase enzyme (Bohrerova & Linden, 2006; Hu & Quek, 2008). With regards to the literature, most researchers make use of pure *E. coli* cultures and media such as buffered saline or sterilised water when investigating photo-repair. In the current study, nonetheless, river water and its actual TC population were utilised for this purpose. Additional variability, referring to the influence of water quality and the diversity of the TC population, consequently becomes important.

In this regard, Guo *et al.* (2009) evaluated inactivation and photo-repair of TC in wastewater from different origins using MP UV doses up to 40 $mJ.cm^{-2}$ and photoreactivating light produced by a 20 W sunlight lamp (F20T12, Philips, USA). Although they did not report on the intensity of the light, it was expected to be comparable to that produced by the two 10 W fluorescent lamps used in the present study. It was found that a MP UV dose of 15 $mJ.cm^{-2}$ was sufficient to restrain photoreactivation to < 10%, irrespective of water quality. The results in Figure 7, however, indicate that even at a dose of 24 $mJ.cm^{-2}$, greater reactivation of TC was observed in this investigation. It could be possible that the study conducted by Guo *et al.* (2009) showed a lower degree of photoreactivation as a result of the better general quality of the treated water in comparison to that shown in Table 5. For instance, they recorded UV transmission up to 76% while the turbidity of the samples never exceeded 6.70 NTU. The difference in results may also be attributed to microbial factors as some coliform species may show greater UV resistance and/or greater levels of photolyase activity compared to others.

In conclusion, it has become clear that discrepancies in experimental variables may significantly influence investigations of UV disinfection potential and subsequent photo-repair. It may, therefore, be necessary to carefully consider the influence of environmental factors such as light intensity and water quality in such studies. Only then will it be possible to come to accurate conclusions regarding the influence of phototreactivation on purpose-driven UV disinfection of water. Nonetheless, in the current study MP UV doses of 13 and 24 $mJ.cm^{-2}$ resulted in fairly good initial TC reductions. These were calculated as 3.30 and 3.36 log, respectively. Taking photoreactivation into account, however, effective reductions were calculated as 1.69 and 2.17 log

as the TC population were reactivated to reach concentrations of 4.41 and 3.39 log cfu.100 mL^{-1}, respectively. The influence of photo-repair on MP UV disinfection of irrigation water may thus be vital and should be carefully considered when suggesting parameters for water disinfection.

Nevertheless, in this study photoreactivation was investigated under optimal conditions of lighting. To obtain a more realistic understanding of photo-repair it was important to determine the impact thereof under less optimal conditions. Also, it was important to determine whether the release of nutrients from UV-damaged microbial cells could possibly promote the growth of living microorganisms which could incorrectly be attributed to photo-repair.

Study C: Photo and dark-repair potential following varied conditions of MP UV irradiation

The effect of increased UV dosage and a varied treatment technique on the potential of photoreactivation were investigated. Filtered river water was exposed to MP UV doses of 40 mJ.cm^{-2} and half of that in a sequential manner (2 x 20 mJ.cm^{2}), respectively. Irradiated samples were subjected to photoreactivating light as in Study B (3.5 kLux) and were also kept in the dark for the same period (5 h). Furthermore, photoreactivation under less-optimal conditions (lower light intensity) was investigated by exposing the irradiated water samples to the ambient light found in the laboratory. The latter represented intensity in the range of 1.0 to 2.0 kLux.

Photoreactivation under 3.5 kLux light: 40 mJ.cm^{-2} vs 2 x 20 mJ.cm^{-2}

The results presented in Figure 8 show that, regardless of more extreme UV irradiation, inactivated coliforms were again capable of recovering upon exposure to fluorescent light. The TC population reached concentrations of 3.49 and 3.30 log cfu.100 mL^{-1}, respectively, following the 40 and 2 x 20 mJ.cm^{-2} treatments and exposure to 3.5 kLux light. Log recovery equated to 0.81 and 0.52 units for the respective treatments. The sequential treatment regime was evidently more effective in supressing photoreactivation (Fig. 8).

Figures 9 and 10 show the percentage recovery achieved under the different test conditions. Upon exposure to 3.5 kLux light, total recovery of 31.55% and 20.88% were achieved within 5 h in the 40 and 2 x 20 mJ.cm^{-2} irradiated samples, respectively. The latter thus repressed photo-repair to a greater extent with a significant difference ($p<0.05$) of 10.67% in log recovery being observed between the two treatments.

For the 40 mJ.cm^{-2} treatment, rapid reactivation within the first hour was observed and resulted in recovery of 25.81%. Thereafter, the curve levelled off with additional recovery of 6.67% occurring in the 1 to 3 h period (Fig. 9). During the 3 to 5 h period the percentage recovery decreased and the total was slightly lower after 5 h compared to 3 h. However, the difference in total percentage log recovery after 3 and 5 h of exposure to the 3.5 kLux light was statistically not significant ($p=0.78$) and possibly indicates that maximum recovery was already achieved after 3 h. This implies that MP UV irradiation also resulted in irreversible damage that could not be repaired

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through the action of photolyase. Quek & Hu (2008b) also came to the conclusion that UV irradiation may result in incurable damage of microbial DNA.

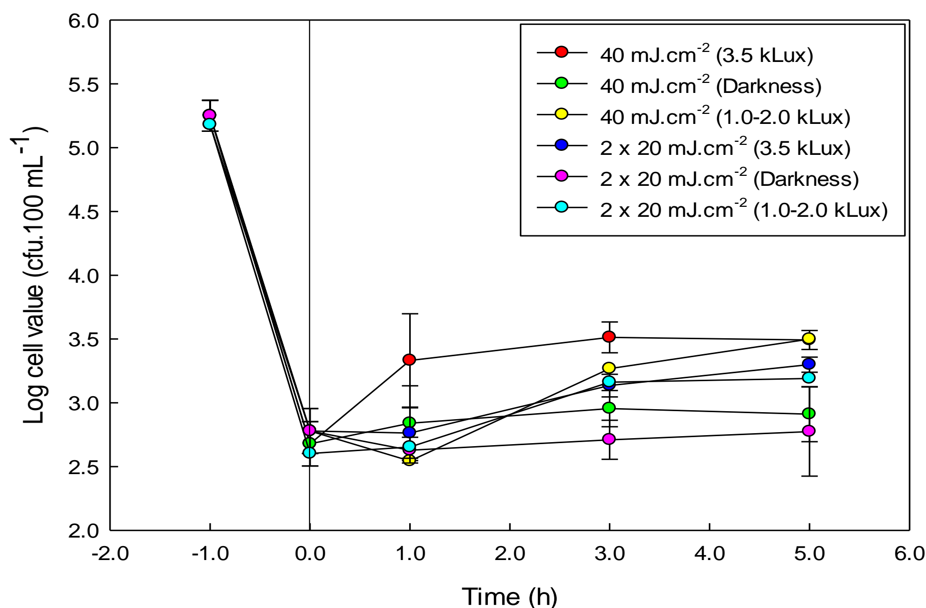

Figure 8 Total coliform (TC) population size before and 1, 3 and 5 h after exposure to different conditions of light and darkness following two respective MP UV treatments. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

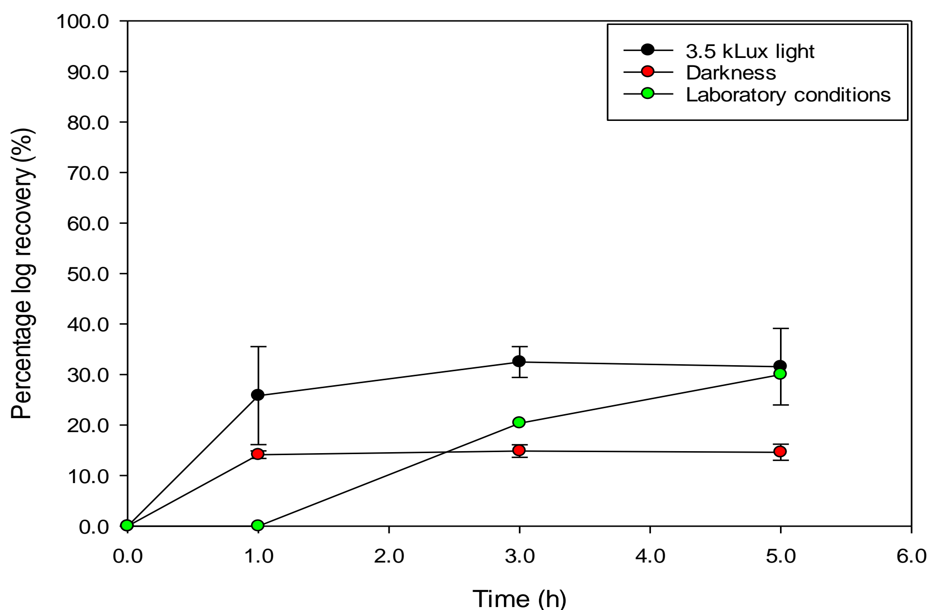

Figure 9 Percentage log recovery of TC with time of exposure to different conditions of light and darkness following MP UV treatment at a dose of 40 mJ.cm^{-2}. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

For the 2 x 20 $mJ.cm^{-2}$ treatment, reactivation of TC was delayed and was only observed from 1 h onwards (Fig. 10). Recovery of 14.39% was observed between 1 and 3 h, with an additional 6.49% recorded during the 3 to 5 h interval. These results indicate that the rate of dimer repair was much lower following the 2 x 20 $mJ.cm^{-2}$ treatment compared to 40 $mJ.cm^{-2}$. The sequential treatment technique therefore showed disinfection efficiency equivalent to a dose exceeding 40 $mJ.cm^{-2}$ as Hu & Quek (2008) reported that increased UV doses resulted in decreased dimer repair rates. However, it was observed that the two treatments resulted in very similar initial reductions of the TC population. Values of 2.57 and 2.47 log were recorded for the 40 and 2 x 20 $mJ.cm^{-2}$ treatments, respectively. The difference of 0.10 log was statistically insignificant ($p>0.05$). This can be seen in Figure 8 where the initial TC population and the population immediately post irradiation are represented by time -1.0 and 0.0 h, respectively. The lower degree of photoreactivation observed following the latter treatment (2 x 20 $mJ.cm^{-2}$) could thus be related to the effect of UV irradiation on photolyase as the difference in reductions were very slight. In other words, the similar reductions imply that the two treatments (40 and 2 x 20 $mJ.cm^{-2}$) resulted in comparable levels of DNA damage, while the difference in their effects on photolyase may have been more significant.

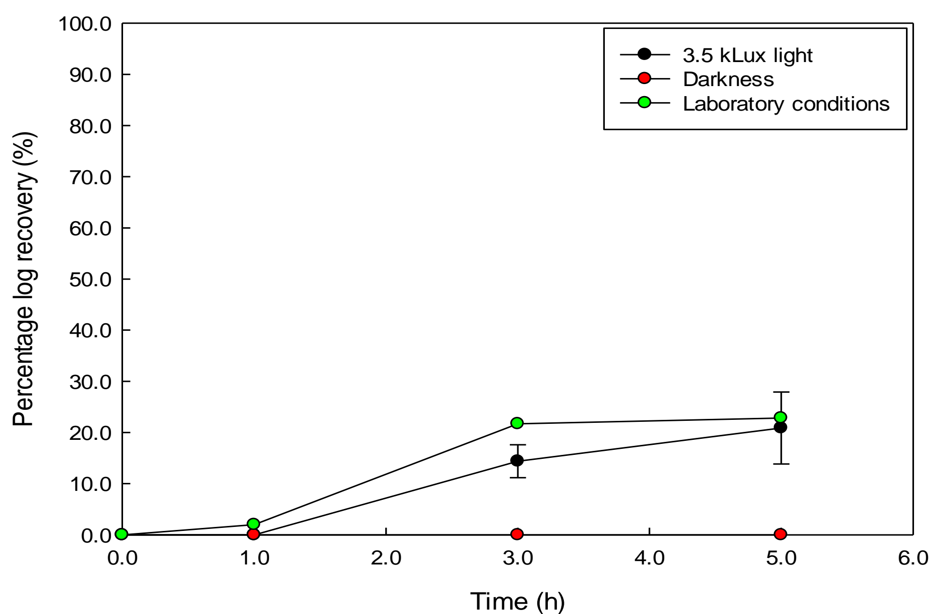

Figure 10 Percentage log recovery of TC with time of exposure to different conditions of light and darkness following MP UV treatment at two successive doses of 20 $mJ.cm^{-2}$. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

Photolyase in known to show high absorption of UV light at wavelengths of 280 and 384 nm (Hu & Quek, 2008). This could lead to reversible (structural) damage and irreversible damage (by means of oxidation) which would both result in decreased dimer repair ability (Hu & Quek, 2008). It is possible that the 2 x 20 $mJ.cm^{-2}$ treatment resulted in greater photolyase damage (compared

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to the 40 mJ.cm^{-2} treatment), consequently explaining the lower rate of repair. Taking the effect of photo-repair into account, effective reductions of 1.76 and 1.95 log for the same treatments were, respectively, achieved. In this sense, the 2 x 20 mJ.cm^{-2} treatment was slightly more effective.

Effect of increased UV dose on photoreactivation potential

In comparison to the 24 mJ.cm^{-2} treatment (Study B), lower degrees of photo-repair were observed following both treatments (40 and 2 x 20 mJ.cm^{-2}) and exposure to 3.5 kLux light in the present study. Photoreactivation was 3.82% and 14.49% lower following 5 h of incubation for the 40 and 2 x 20 mJ.cm^{-2} samples, respectively, compared to the 24 mJ.cm^{-2} sample. It was only for the 2 x 20 mJ.cm^{-2} treated sample that significantly lower repair ($p<0.05$) was observed compared to the 24 mJ.cm^{-2} treatment after 5 h. These results were in agreement with that found previously as greater repair was observed at 13 than 24 mJ.cm^{-2} in Study B.

Interesting, however, is the fact that the doses utilised in Study C initially produced log reductions that were 0.79 (40 mJ.cm^{-2}) and 0.89 (2 x 20 mJ.cm^{-2}) log lower compared to that reached at 24 mJ.cm^{-2} in Study B (3.36 log). This was not expected as intensified UV irradiation was utilised while water quality in Study C was also generally better compared to that reported for Study B (Table 5 & 6). These results, therefore, suggest that the restraining effect of higher UV doses on photo-recovery could be related to its influence on the photolyase enzyme, rather than on the genetic material of the irradiated microorganisms. In this regard, taking photoreactivation into account, the effective reductions achieved at 40 and 2 x 20 mJ.cm^{-2} were 0.41 and 0.22 log lower, respectively, compared to the 24 mJ.cm^{-2} treatment used earlier. Nevertheless, the important fact is that increased UV doses resulted in lower percentages of repair under the same experimental conditions. This was true for both the 40 and 2 x 20 mJ.cm^{-2} treatments in comparison to the 24 mJ.cm^{-2} treatment used in Study B. However, from these results it can be concluded that the sequential treatment technique, in particular, offers increased effectiveness, possibly due to increased damage of the photolyase enzyme.

Looking at literature, results of previous studies are often contradictory to that observed here. Guo *et al.* (2009) reported that a MP UV dose of 40 mJ.cm^{-2} was sufficient to restrain photoreactivation of TC in wastewater to below 1%. They suggested that such a dose resulted in the formation of dimers to an extent that could prevent repair within a specific time. Also, Hoyer (1998) found that the same dose (40 mJ.cm^{-2}) was sufficient to entirely prevent photo-repair. Other researchers have also reported that minimal photo-repair was observed when MP UV disinfection was employed (Oguma *et al.*, 2001; Zimmer & Slawson, 2002). In this regard, it is important to consider the influence of the properties of the irradiated liquid/water on the extent of photoreactivation. As previously mentioned, for instance, UV transmission of the treated water in the study of Guo *et al.* (2009) reached 76%, indicating better overall water quality in comparison to that dealt with in the current study. This may have contributed to greater degrees of UV induced microbial damage, consequently resulting in lower degrees of photo-repair. The authors

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unfortunately did not report the initial reduction of TC at a dose of 40 $mJ.cm^{-2}$. However, it was expected to be greater than the 2.57 log inactivation achieved in this study, consequently leaving less microorganisms capable of repairing UV induced DNA-damage. Bohrerova *et al*. (2015) investigated photoreactivation of two *E. coli* strains in sterilised drinking water and treated wastewater effluent. The authors reported that maximum recovery (0.70 log) was recorded in the wastewater following MP UV treatment at 40 $mJ.cm^{-2}$. This result was more in line with that observed in the current study as recovery of 0.81 log was observed following treatment at the same dose. Bohrerova *et al*. (2015) did not report the UVT% of the water samples but did record absorbance values of 0.051 and 0.087 cm^{-1} at 254 nm for the drinking and reuse water samples, respectively. The turbidity of the respective water samples were recorded as 0.30 and 1.10 NTU. Thus, although the irradiated water was again of better quality compared to the river water treated in this study the extent of photo-repair recorded in the two investigations were similar.

Due to the fact that in none of the above mentioned studies all experimental variables were similar, the different conclusions cannot be accurately compared. Factors such as water quality, the characteristics of irradiated microorganisms and the intensity of the photoreactivating light may be particularly important. Nonetheless, although it is often suggested in literature, it is evident that even high MP UV doses may not be entirely capable of preventing the phenomenon of photoreactivation.

Table 6 Physico-chemical and microbiological properties of river water before performing photo and dark-repair experiments

Quality Parameter	Measured value
UVT%	50.00
COD ($mg.L^{-1}$)	18.90
Turbidity (NTU)	11.50
TSS ($mg.L^{-1}$)	15.00
VSS ($mg.L^{-1}$)	10.00
pH	7.75
Alkalinity (mg $CaCO_3.L^{-1}$)	112.20
Conductivity ($mS.m^{-1}$)	36.30
Total coliforms (TC) (log cfu.100 mL^{-1})	5.25

Effect of light intensity on photoreactivation

According to Guo *et al*. (2013) limited work has been done to investigate the effect of light intensity on the extent of photo-repair. Nevertheless, the degree of photoreactivation induced at a lower light intensity (laboratory conditions) in the current study again allowed for meaningful regrowth (Fig. 8). In fact, following the 5 h incubation period, the TC population reached concentrations of 3.49 and 3.19 log cfu.100 mL^{-1} in the 40 and 2 x 20 $mJ.cm^{-2}$ treated samples, respectively.

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Evidently, it was only for the 2 x 20 $mJ.cm^{-2}$ sample that light of higher intensity (3.5 kLux) resulted in a slightly greater (0.11 log $cfu.100\ mL^{-1}$) final TC concentration after 5 h. Nevertheless, the differences in the percentage log recovery following 5 h incubation under 3.5 and 1.0 to 2.0 kLux light were insignificant (p=0.55 and p=0.40) for both of the respective treatments (40 and 2 x 20 $mJ.cm^{-2}$). As was observed for samples exposed to 3.5 kLux light, the 2 x 20 $mJ.cm^{-2}$ treatment again prevented photoreactivation to the greater extent (Fig. 8).

As indicated in Figures 9 & 10, photoreactivation under laboratory lighting showed somewhat different trends over a 5 h period in comparison to that observed under 3.5 kLux light. This was expected as it was suggested by Hallmich & Gehr (2010) that factors such as light source, the type of UV lamp used and the species of microorganisms present, for instance, may influence the initiation of photo-repair. It is therefore quite clear that the phenomenon of photoreactivation cannot be solely reported on a time-basis as several factors will influence the progression of the process.

For the sample treated at 40 $mJ.cm^{-2}$, repair was slower with no reactivation observed in the first hour (Fig. 9). In the 1 to 3 and 3 to 5 h periods recovery of 20.36% and 9.62%, respectively, were observed and a maximum of 29.98% was reached (Fig. 9). Thus, even though it was slightly delayed total photoreactivation was very close to the 31.55% reached under 3.5 kLux light. A similar observation was made by Quek & Hu (2008b) who evaluated the effect of light intensity on the reactivation of *Escherichia coli*. The authors reported that photoreactivation occurred at a faster rate when fluorescent light intensity was increased. Since photo-repair is a light-mediated, enzymatic process it was expected that increased light intensities would result in greater reactivation (Quek & Hu, 2008b). In addition, it was reported that this effect was only observed up until a certain intensity, indicating that MP UV irradiation also induced irreversible damage of microbial DNA (Quek & Hu, 2008b). Bohrerova & Linden (2007) investigated photoreactivation of *E. coli* using four different fluorescent lamps. They also reported that the rate of photo-repair increased with lamp intensity. Locas *et al.* (2008) confirmed this observation by reporting that photoreactivation of *E. coli* increased drastically following exposure to 5.6 kLux light compared to 1.6 kLux light.

For the 2 x 20 $mJ.cm^{-2}$ treated sample, photoreactivation under laboratory light (1.0 to 2.0 kLux) was more rapid than that observed under 3.5 kLux light. The major part of reactivation (19.71%) occurred during the 1 to 3 h period where after the curve levelled off. In the 3 to 5 h period additional reactivation of only 1.13% was recorded and a total of 22.82% was reached (Fig. 10). This was slightly higher than the 20.88% reached in the same sample exposed to 3.5 kLux light. The difference in percentage log recovery reached after 5 h under the different conditions of light, however, was not statistically significant (p=0.40). The result reported here was not expected and contradicts the work of previously mentioned researchers. It is thus possible that the effects of the 40 and 2 x 20 $mJ.cm^{-2}$ treatments on photolyase differed so that the importance of light intensity on reactivation rate was more profound following the former (40 $mJ.cm^{-2}$). Also,

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since the degree of photoreactivation is influenced by many factors, including temperature, the time and intensity of light exposure, UV dose and UV lamp type (Zimmer & Slawson, 2002; Salcedo *et al*., 2007; Hu & Quek, 2008) it is possible that other factors could also have influenced conditions and resulted in the unpredicted result.

Dark-repair following UV irradiation

In addition to the effect of photoreactivation, it has also been reported that UV induced damage in microorganisms may be reversed by means of dark-repair mechanisms (Jungfer *et al*., 2007; Locas *et al*., 2008; Guo *et al*., 2011). As opposed to photoreactivation, dark-repair is described as a light-independent process in which numerous enzymes are coordinated to remove DNA damage (Locas *et al*., 2008; Guo *et al*., 2013). The influence thereof, however, is generally regarded as being less important compared to that of photoreactivation over short term (Guo *et al*., 2011).

According to Bohrerova *et al*. (2015) dark-repair is a complex process which is difficult to investigate as a result of its delayed occurrence post UV irradiation. This implies that it might be challenging to distinguish between normal regrowth and actual dark-repair. Nevertheless, the most frequently described and encountered dark-repair mechanism is termed nucleotide excision repair (NER) (Zimmer-Thomas *et al*., 2007; Gáyan *et al*., 2014; Bohrerova *et al*., 2015). This process is capable of removing a variety of DNA lesions through the action of the UvrABC exinuclease, which initiates a range of cascade reactions. In the literature, however, the phenomenon of dark-repair is less extensively studied compared to photoreactivation, yet it may occur in distribution systems following disinfection. The investigation of dark-repair potential in the present study was therefore largely appropriate.

As observed in Figure 8, exposure of the UV irradiated samples to darkness had a significantly lower effect on microbial growth (in terms of an increase in log value) ($p<0.05$) compared to exposure to the different intensities of light following the 40 and 2 x 20 mJ.cm^{-2} treatments, respectively. After 5 h, dark-repair resulted in a final TC population reaching 2.91 log cfu.100 mL^{-1} following the 40 mJ.cm^{-2} treatment. This equated to a significant increase ($p<0.05$) of 0.23 log over the 0 to 5 h period. For the 2 x 20 mJ.cm^{-2} treatment no significant increase in cell concentration ($p=1.00$) was observed in darkness over the same period and the final TC concentration was recorded as 2.77 log cfu.100 mL^{-1} (Fig. 8). Thus, as was reported for photoreactivation, the sequential treatment technique was shown to better inhibit dark-repair.

The percentage recovery recorded for the dark-repair investigations following MP UV treatment are presented in Figures 9 & 10. For the 40 mJ.cm^{-2} treatment, dark-repair was clearly observed and maximum recovery of 14.62% was recorded (Fig. 9). No increase in population size was detected for the 2 x 20 mJ.cm^{-2} treated sample (Fig. 10). Looking at Figure 9, recovery following the 40 mJ.cm^{-2} treatment was initially rapid with a significant increase ($p<0.05$) of 14.13% in the number of culturable coliforms being recorded for the first hour of exposure to darkness. From 1 h onwards, no significant increase in the percentage recovery was seen ($p=0.86$ and

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p=0.91) and total recovery was recorded as 14.86% and 14.62% after 3 and 5 h, respectively. Thus, after 5 h of exposure to darkness recovery was 2.16 and 2.05 times lower than that achieved following exposure to visible light at intensities of 3.5 and 1.0 to 2.0 kLux, respectively, for the 40 mJ.cm^{-2} treatment.

In the literature, studies investigating dark-repair were often performed using LP UV irradiation (Jungfer *et al.*, 2007; Salcedo *et al.*, 2007) while others that utilised MP UV light were conducted in buffered saline or distilled water (Hu *et al.*, 2005; Zimmer-Thomas *et al.*, 2007; Quek & Hu, 2008a). Nevertheless, Quek & Hu (2008a) evaluated the dark-repair potential of seven *E. coli* strains following exposure to MP UV irradiation to achieve a reduction of 5 log. As observed in the present study, it was found that levels of dark-repair were much lower than the corresponding values detected for photoreactivation. Following a 4 h incubation period, maximum dark-repair was recorded as approximately 18% for *E. coli* strain ATCC 11775. Furthermore, the authors (Quek & Hu, 2008a) additionally indicated that levelling off of dark-repair, post MP UV irradiation, occurred after 1 h of incubation. In the current study, a comparable level of repair was achieved while reactivation also levelled off after the first hour (Fig. 9). Dark-repair to a certain extent was observed for all but one of the *E. coli* strains (strain 9481 from the National Collections of Industrial, Marine and Food Bacteria (NCIMB)) used in the 2008 study. Earlier research performed by Oguma *et al.* (2001) and Zimmer & Slawson (2002) produced contrasting results. They reported that an *E. coli* strain form the National Collections of Industrial, Marine and Food Bacteria (NCIMB 10083) and ATCC 11229 (also used by Quek & Hu, 2008a) showed no degree of dark-repair following MP UV disinfection. Thus, as was observed in the current investigation (Fig. 10) MP UV irradiation may be sufficient to entirely supress dark-repair.

In support of the latter statement, Locas *et al.* (2008) evaluated dark and light-repair of *E. coli* and enterococci in MP UV disinfected wastewater. Visible light at intensity of 5.6 kLux prompted the *E. coli* cell concentration to increase by 7 times within 6 h, whereas no significant increase (p>0.05) in the population size was seen following exposure to darkness. Dark-repair has also been investigated in reclaimed wastewater (Guo *et al.*, 2011) as well as drinking and reuse water (Bohrerova *et al.*, 2015) obtained from water treatment plants. Bohrerova *et al.* (2015) evaluated dark-repair of a wild type *E. coli* isolate, as well as a reference *E. coli* strain (ATCC 11229), in sterilised drinking water and wastewater effluent. Ultraviolet doses ranging from 40 to 120 mJ.cm^{-2} were utilised and dark-repair was investigated over a 48 h period. Importantly, the levels of repair were quantified and corrected with reference to the expected regrowth (in the absence of photo or dark-repair) occurring post-inactivation. Considering this, the authors reported that dark-repair was not at all detected. Likewise, Kollu & Örmeci (2015) reported that dark-repair was not detected for *E. coli*, nor faecal coliforms, in enriched phosphate-buffered saline (PBS), sterilised wastewater or natural wastewater following MP UV irradiation at a dose of 40 mJ.cm^{-2}.

Considering all of these results, dark-recovery observed following the 40 mJ.cm^{-2} treatment was somewhat unexpected as most studies could not detect repair under comparable experimental

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conditions. However, in addition to dark-reactivation, increments in population size may also be attributed to normal growth of viable cells resulting from the increased availability of nutrients following UV disinfection. The UV inactivated cells remaining in the water may serve as a source of biodegradable carbon which could be utilised by other microorganisms (Bohrerova *et al.*, 2015). In this regard, the same authors reported that the problem of regrowth of UV survivors was more influential than that of repair in the presence of UV inactivated cells. The use of extracellular DNA (available following UV irradiation) as nutrient by *Escherichia coli* has been reported in 2001 already (Finkel & Kolter, 2001). As conveyed by Bohrerova *et al.* (2015), Kollu & Örmeci (2015) also found that fair regrowth of faecal coliforms and *E. coli* were observed in the presence of UV damaged microorganisms.

In the present study, the level of dark-repair was not corrected with reference to the influence of regrowth. It could thus be that the 14.62% increase in population size resulted from rapid initial regrowth due to the increased availability of nutrients after disinfection. The surviving cells may have utilised such sources within the first hour where after regrowth was again limited. In the case of the 2 x 20 $mJ.cm^{-2}$ treatment, the lower successive doses may not have resulted in damage adequate for the release of cell constituents that could serve as source of nutrition. In that case, regrowth (observed as dark-repair in this study) would not be detected. Nonetheless, the increase in population size may also have resulted from actual dark-repair as Jungfer *et al.* (2007) have previously reported that UV doses of 40 $mJ.cm^{-2}$ (and higher) induced such mechanisms. They, however, only indicated the expression of some important repair genes (recA) but did not detect actual repair following cultivation experiments. Considering this, and the fact that repair was observed for the first hour only in this study, it is concluded that the percentage increase most probably resulted from regrowth due to a temporary increase in the availability of nutrients.

CONCLUSIONS

In this study, the potential of MP UV irradiation for the disinfection of microbiologically contaminated water from the Plankenburg River was investigated. It was observed in Study A that the river water never complied with guidelines set by DWAF (1996) for water intended to be used for fresh produce irrigation. This was true for both physico-chemical and microbiological properties of the analysed samples and the urgent need for effective disinfection became apparent. While it was difficult to correlate the efficiency of MP UV disinfection with water quality, it was evident that doses exceeding 24 $mJ.cm^{-2}$ would be required to achieve sufficient inactivation of coliform bacteria. While the maximum dose (24 $mJ.cm^{-2}$) was capable of achieving a 3 log reduction of FC, the limit suggested by DWAF (1996) (1 000 $cfu.100\ mL^{-1}$) was never met. The extreme levels of faecal contamination detected in the water (reaching 6.41 log $cfu.100\ mL^{-1}$) thus call for reductions nearing 3.50 log. Conclusively, MP UV irradiation was found to be incapable of achieving adequate disinfection of river water in the current study. Throughout the respective trials in Study A the effect of increased UV dosage only had a slight impact (0.00 to 1.18 log) on microbial

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inactivation. Differences in the log reductions achieved at 13, 17 and 24 $mJ.cm^{-2}$ were statistically insignificant ($p>0.05$) in some instances. Furthermore, reductions achieved for TC and FC were often very similar whereas the heterotrophic population showed much greater UV resistance. This implies that the use of commonly encountered indicator microorganisms (such as *E. coli*) in laboratory-scale investigations may not be the most accurate method for establishing/suggesting parameters for larger scale water disinfection.

In Study B, the phenomenon of photoreactivation was investigated following MP UV irradiation at the lowest and highest UV doses used in Study A i.e. 13 and 24 $mJ.cm^{-2}$. Water quality was generally better than that observed in the previously discussed investigation and correlated with increased disinfection. It was suggested that the lower levels of interfering substances in the treated water resulted in greater availability of UV energy for microbial inactivation. It was also shown that photoreactivation (post-disinfection) deserves attention as its influence on disinfection efficiency was clear. Each of the applied UV doses were capable of reducing TC from an initial concentration of 6.10 to below 3 log cfu.100 mL^{-1}. However, even the maximum UV dose allowed for log reactivation exceeding 35%. This equated to a final TC concentration of 3.93 log cfu.100 mL^{-1}. In agreement with the literature, nonetheless, the higher UV dose resulted in significantly lower degrees of photo-repair.

Subsequent investigations assessed the effect of harsher irradiation and a varied irradiation protocol on the incidence of photoreactivation as well as dark-repair. In comparison to 24 $mJ.cm^{-2}$, doses of 40 and 2 x 20 $mJ.cm^{-2}$ more effectively inhibited photo-repair. Interestingly, the application of a dose of 20 $mJ.cm^{-2}$ in a sequential manner (the 2 x 20 $mJ.cm^{-2}$ treatment) resulted in significantly lower levels of reactivation ($p<0.05$) under 3.5 kLux light compared to 40 $mJ.cm^{-2}$. This implies that the use of two smaller UV units/lamps, to deliver a dose adequate to prevent photoreactivation, may be more beneficial compared to the use of one stronger unit for the same purpose. Nevertheless, the UV doses tested here were again fairly ineffective and TC concentrations exceeding 3 log cfu.100 mL^{-1} were reached within 5 h of incubation. Dark-repair of TC was only observed in the sample treated with a dose of 40 $mJ.cm^{-2}$. This, however, was unexpected and the increase of 14.62% in population size possibly resulted from normal regrowth, rather than reactivation, following disinfection. From these results it is concluded that dark-repair does not show potential to significantly influence the efficiency of UV disinfection of irrigation water.

When the same UV irradiated samples were exposed to light of lower intensity (1.0 to 2.0 kLux) reactivation occurred at a slower rate in the 40 $mJ.cm^{-2}$ sample. This was in agreement with the findings of other researchers. The percentage recovery following 5 h of incubation, however, was not significantly less ($p=0.55$) under the low intensity light. Thus, under all conditions of the photoreactivation experiments, TC were reactivated to exceed a concentration of 3 log cfu.100 mL^{-1} of water. Since FC are expected to react similarly in the presence of light, the influence of photo-repair on the aptness of river water for the irrigation of fresh produce could be substantial. Even if FC could be reduced to below 1 000 cfu.100 mL^{-1}, exposure to visible light

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(post-disinfection) may easily allow reactivation of FC to exceed this limit, even within 1 h. Furthermore, it is concluded that in practice, UV disinfected irrigation water may be exposed to even greater light intensities. This indicates that photoreactivation is a critical factor when suggesting process parameters for water treatment. In this regard, the use of combination treatments, employing low doses of chlorine for instance, may be worthwhile to investigate as the residual chemical activity may aid in preventing reactivation.

If UV irradiation is to be used exclusively, it is advisable to apply the disinfected water immediately after treatment in order to minimise the time available for photo-repair. However, the potential of coliform bacteria to photoreactivate on plant surfaces, following irrigation, should be considered and investigated before conforming to such a technique. Furthermore, it is important to note that in the current study UVT% of the irradiated water never exceeded 50%. Since greater UV transmission could result in increased microbial inactivation (and a lower degree of reactivation) manipulation of process parameters could increase the credibility of UV disinfection. In practice, it would be possible to improve overall water quality by means of employing sand-filters that are more effective than those used in the current study. This would allow for improved UV transmission and ultimately greater effectiveness of the process. In addition, UV systems which are capable of delivering much higher doses than those generated by the system used here are commercially available. Importantly, such equipment is obtainable at reasonable cost making its use more feasible. It would, therefore, be possible to design and install larger scale disinfection systems that could effectively reduce microbial contaminants in irrigation water, irrespective of poor water quality. Higher UV doses would also restrain the extent of photoreactivation. Hence, while UV disinfection was accompanied by some limitations in this study the technique shows potential as alternative method for irrigation water decontamination. Under carefully optimised conditions irrigation water adhering to the DWAF (1996) guideline of 3 log cfu.100 mL^{-1} (set on FC) could be produced using MP UV irradiation.

REFERENCES

Ackerman, A. (2010). *Assessment of microbial loads of the Plankenburg and Berg Rivers and the survival of Escherichia coli on raw vegetables under laboratory conditions*. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

APHA (American Public Health Association) (2005). *Standard Methods for the Examination of Water and Wastewater,* 21st ed. Washington, DC: American Public Health Association Press.

Berney, M., Weilenmann, H.U., Ihssen, J., Bassin, C. & Egli, T. (2006). Specific growth rate determines the sensitivity of *Escherichia coli* to thermal, UVA, and solar disinfection. *Applied and Environmental Microbiology*, **72**(4), 2586-2593.

Bohrerova, Z. & Linden, K.G. (2006). Assessment of DNA damage and repair in *Mycobacterium terrae* after exposure to UV irradiation. *Journal of Applied Microbiology*, **101**(5), 995-1001.

Stellenbosch University https://scholar.sun.ac.za

Bohrerova, Z. & Linden, K.G. (2007). Standardizing photoreactivation: comparison of DNA photorepair rate in *Escherichia coli* using four different fluorescent lamps. *Water Research*, **41**(12), 2832-2838.

Bohrerova, Z., Rosenblum, J. & Linden, K.G. (2015). Importance of recovery of *E. coli* in water following ultraviolet light disinfection. *Journal of Environmental Engineering*, DOI: 10.1061/(ASCE)EE.1943-7870.0000922.

Brahmi, M., Belhadi, N.H., Hamdi, H. & Hassen, A. (2010). Modeling of secondary treated wastewater disinfection by UV irradiation: effects of suspended solids content. *Journal of Environmental Sciences,* **22**(8), 1218-1224.

Britz, T.J., Sigge, G.O., Huisamen, N., Kikine, T., Ackermann, A., Lötter, M., Lamprecht, C. and Kidd, M. (2013). Fluctuations of indicator and index microbes as indication of pollution over three years in the Plankenburg and Eerste Rivers, Western Cape, South Africa. *Water SA*, **39**(4), 457-465.

Bucheli-Witschel, M., Bassin, C. & Egli, T. (2010). UV-C inactivation in *Escherichia coli* is affected by growth conditions preceding irradiation, in particular by the specific growth rate. *Journal of Applied Microbiology*, **109**(5), 1733-1744.

Child, M., Strike, P., Pickup, R. & Edwards, C. (2002). *Salmonella typhimurium* displays cyclical patterns of sensitivity to UV-C killing during prolonged incubation in the stationery phase of growth. *FEMS Microbiology Letters,* **213**(1), 81-85.

Davies, C.M. & Bavor, H.J. (2000). The fate of stormwater-associated bacteria in constructed wetland and water pollution control pond systems. *Journal of Applied Microbiology*, **89**(2), 349-360.

Droppo, I.G., Liss, S.N., Williams, D., Nelson, T., Jaskot, C. & Trapp, B. (2009). Dynamic existence of waterborne pathogens within river sediment compartments. Implications for water quality regulatory affairs. *Environmental Science & Technology*, **43**(6), 1737-1743.

DWAF (Department of Water Affairs and Forestry) (1996). *South African Water Quality Guidelines*, 2nd ed. (edited by S. Holmes). *Volume 4: Agricultural Water Use: Irrigation*. Pp. 71-76. The Government Printer. Pretoria, South Africa.

Finkel, S.E. & Kolter, R. (2001). DNA as a nutrient: novel role for bacterial competence gene homologs. *Journal of Bacteriology*, **183**(21), 6288-6293.

Fredericks, I.N., Du Toit, M. & Krügel, M. (2011). Efficacy of ultraviolet radiation as an alternative technology to inactivate microorganisms in grape juices and wines. *Food Microbiology*, **28**(3), 510-517.

Fries, J.S., Characklis, G.W. & Noble, R.T. (2008). Sediment-water exchange of *Vibrio* sp. and fecal indicator bacteria: implications for persistence and transport in the Neuse River Estuary, North Carolina, USA. *Water Research*, **42**(4-5), 941-950.

Gabriel, A. (2012). Inactivation of *Escherichia coli* O157:H7 and spoilage yeasts in germicidal UV-C-irradiated and heat-treated clear apple juice. *Food Control*, **25**(2), 425-432.

Stellenbosch University https://scholar.sun.ac.za

Gayán, E., Condón, S. & Álvarez, I. (2014). Biological aspects in food preservation by ultraviolet light: a review. *Food and Bioprocess Technology*, **7**, 1-20.

Guo, M., Hu, H., Bolton, J.R. & El-Din, M.G. (2009). Comparison of low- and medium-pressure ultraviolet lamps: photoreactivation of *Escherichia coli* and total coliforms in secondary effluents of municipal wastewater treatment plants. *Water Research*, **43**(3), 815-821.

Guo, M., Huang, J., Hu, H. & Liu, W. (2011). Growth and repair potential of three species of bacteria in reclaimed wastewater after UV disinfection. *Biomedical and Environmental Sciences*, **24**(4), 400-407.

Guo, M., Huang, J., Hu, H., Liu, W. & Yang, J. (2012). UV inactivation and characteristics after photoreactivation of *Escherichia coli* with plasmid: health safety concern about UV disinfection. *Water Research*, **46**(13), 4031-4036.

Guo, M., Huang, J., Hu, H., Liu, W. & Yang, J. (2013). Quantitative characterisation and prediction modelling of photoreactivation of coliforms after ultraviolet disinfection of reclaimed municipal wastewater. *Water, Air, & Soil Pollution*, DOI: 10.1007/s11270-013-1774-z.

Hallmich, C. & Gehr, R. (2010). Effect of pre- and post-UV disinfection conditions on photoreactivation of fecal coliforms in wastewater effluents. *Water Research*, **44**(9), 2885-2893.

Harm, W. (1980). *Biological effects of ultraviolet radiation*. New York: Cambridge University Press.

Hijnen, W.A.M., Beerendonk, E.F. & Medema, G.J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Research*, **40**(1), 3-22.

Hoyer, O. (1998). Testing performance and monitoring of UV systems for drinking water disinfection. *Water Supply*, **16**(1/2), 424-429.

Hu, J.Y., Chu, X.N., Quek, P.H., Feng, Y.Y. & Tan, X.L. (2005). Repair and regrowth of *Escherichia coli* after low- and medium-pressure ultraviolet disinfection. *Water Science and Technology: Water Supply*, **5**(5), 101-108.

Hu, J.H. & Quek, P.H. (2008). Effects of UV radiation on photolyase and implications with regards to photoreactivation following low- and medium-pressure UV disinfection. *Applied and Environmental Microbiology*, **74**(1), 327-328.

Hu, X., Geng, S., Wang, X. & Hu, C. (2012). Inactivation and photorepair of enteric pathogenic microorganisms with ultraviolet irradiation. *Environmental Engineering Science*, **29**(6), 549-553.

Huisamen, N. (2012). *Assessment of microbial levels in the Plankenburg and Eerste Rivers and subsequent carry-over to fresh produce using source tracking as indicator*. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Stellenbosch University https://scholar.sun.ac.za

Jones, L.A., Worobo, R.W. & Smart, C.D. (2014). UV light inactivation of human and plant pathogens in unfiltered surface irrigation water. *Applied and Environmental Microbiology*, **80**(3), 849-854.

Jungfer, C., Schwartz, T. & Obst, U. (2007). UV-induced dark repair mechanisms in bacteria associated with drinking water. *Water Research*, **41**(1), 188-196.

Kalisvaart, B.F. (2004). Re-use of wastewater: preventing the recovery of pathogens by using medium-pressure UV lamp technology. *Water Science and Technology*, **50**(6), 337-344.

Kollu, K. & Örmeci, B. (2015). Regrowth potential of bacteria after ultraviolet disinfection in the absence of light and dark repair. *Journal of Environmental Engineering*, DOI: 10.1061/(ASCE)EE.1943-7870.0000905.

Lindenauer, K.G. & Darby, J.L. (1994). Ultraviolet disinfection of wastewater: effect of dose on subsequent photoreactivation. *Water Research*, **28**(4), 805-817.

Linden, K.G., Shin, G.A., Faubert, G., Cairns, W. & Sobsey, M.D. (2003). UV disinfection of *Giardia lamblia* cysts in water. *Environmental Science and Technology*, **36**(11), 2519-2522.

Locas, A., Demers, J. & Payment, P. (2008). Evaluation of photoreactivation of *Escherichia coli* and enterococci after UV disinfection of municipal wastewater. *Canadian Journal of Microbiology*, **54**(11), 971-975.

López-Malo, A. & Palou, E. (2004). Ultraviolet light and food preservation. In: *Novel Food Processing Technologies* (edited by G.V. Barbosa-Cánovas, M.S. Tapia & M.P. Cano). Pp. 405-421. USA: CRC Press.

Melidis, P., Athanasoulia, E., Parisis, A. & Koppmann, M. (2009). Microbiological effluent control by UV-disinfection of a small-scale SBR system. *Desalination*, **248**(1-3), 915-922.

Mezzanotte, V., Antonelli, M., Citterio, S. & Nurizzo, C. (2007). Wastewater disinfection alternatives: chlorine, ozone, peracetic acid and UV light. *Water Environment Research*, **79**(12), 2373-2379.

Moné, M.J., Volker, M., Nikaido, O., Mullenders, L.H., Van Zeeland, A.A. & Verschure, P.J. (2001). Local UV-induced DNA damage in cell nuclei results in local transcription inhibition. *EMBO reports*, **2**(11), 1013-1017.

Neppolian, B., Choi, H.C., Sakthivel, S., Arabindoo, B. & Murugesan, V. (2002). Solar light induced and TiO_2 assisted degradation of textile dye reactive blue 4. *Chemosphere*, **46**(8), 1173-1181.

Oguma, K., Katayama, H., Mitani, H., Morita, S., Hirata, T. & Ohgaki, S. (2001). Determination of pyrimidine dimers in *Escherichia coli* and *Cryptosporidium parvum* during UV light inactivation, photoreactivation and dark repair. *Applied and Environmental Microbiology*, **68**(12), 4630-4637.

Oguma, K., Katayama, H. & Ohgaki, S. (2002). Photoreactivation of *Escherichia coli* after low- or medium-pressure UV disinfection determined by an endonuclease sensitive site assay. *Applied and Environmental Microbiology*, **68**(12), 6029-6035.

Stellenbosch University https://scholar.sun.ac.za

Örmeci, B. & Linden, K.G. (2002). Comparison of UV and chlorine inactivation of particle and non-particle associated coliforms. *Water Science and Technology: Water Supply*, **2**(5-6), 403-410.

Oteiza, J.M., Giannuzzi, L. & Zaritzky, N. (2010). Ultraviolet treatment of orange juice to inactivate *E. coli* O157:H7 as affected by native microflora. *Food and Bioprocess Technology,* **3**(4), 603-614.

Paulse, A.N., Jackson, V.A. & Khan, W. (2009). Comparison of microbial contamination at various sites along the Plankenburg- and Diep Rivers, Western Cape, South Africa. *Water SA*, **35** (4), 469-478.

Poepping, C., Beck, S., Wright, H. & Linden K.G. (2014). Evaluation of DNA damage reversal during medium-pressure UV disinfection. *Water Research*, **56**, 181-189.

Qian, Y. (2011). *UV disinfection of total bacteria, E. coli and Salmonella in irrigation water*. MSc Thesis, Cornell University, USA.

Quek, P.H., Hu, J.Y., Chu, X.N., Feng, Y.Y. & Tan, X.L. (2006). Photoreactivation of *Escherichia coli* following medium-pressure ultraviolet disinfection and its control using chloramination. *Water Science and Technology*, **53**(6), 123-129.

Quek, P.H. & Hu, J. (2008a). Indicators for photoreactivation and dark repair studies following ultraviolet disinfection. *Journal of Industrial Microbiology and Biotechnology*, **35**(6), 533-541.

Quek, P.H. & Hu, J. (2008b). Influence of photoreactivating light intensity and incubation temperature on photoreactivation of *Escherichia coli* following LP and MP UV disinfection. *Journal of Applied Microbiology*, **105**(1), 124-133.

Quek, P.H. & Hu, J. (2013). Effects of wavelengths of medium-pressure ultraviolet radiation on photolyase and subsequent photoreactivation. *Water Science and Technology: Water Supply*, **13**(1), 158-165.

Rubin, L.B., Menshonkova, T.N., Simukova, N.A. & Budowsky, E.I. (1981). The effects of high-intensity UV-irradiation on nucleic acids and their components. *Photochemistry and Photobiology*, **34**(3), 339-344.

Salcedo, I., Andrade, J.A., Quiroga, J.M. & Nebot, E. (2007). Photoreactivation and dark repair in UV-treated microorganisms: effect of temperature. *Applied and Environmental Microbiology*, **73**(5), 1594-1600.

Sancar, A. (2003). Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors. *Chemical Reviews*, **103**(6), 2203-2237.

SANS (South African National Standards) (2007a). Method 4832. Microbiology of Food and Animal Feeding Stuffs: Horizontal methods for the enumeration of coliforms: Colony-count technique. Pretoria: South Africa.

Stellenbosch University https://scholar.sun.ac.za

SANS (South African National Standards) (2007b). Method 4833. 2nd ed. Microbiology: General guidance for the enumeration of microorganisms: Colony count technique at 30°C. Pretoria: South Africa.

Selma, M.V., Allende, A., López-Gálvez, F., Conesa, M.A. & Gil, M.I. (2008). Disinfection potential of ozone, ultraviolet-C and their combination in wash water for the fresh-cut vegetable industry. *Food Microbiology*, **25**, 809-814.

Sinton, L.W., Finlay, R.K. & Lynch, P.A. (1999). Sunlight inactivation of fecal bacteriophages and bacteria in sewage polluted seawater. *Applied and Environmental Microbiology*, **65**(8), 3605-3613.

Spellman, F.R. (2003). *Handbook of Water and Wastewater Treatment Plant Operations*, 3rd ed. USA: CRC Press.

Truglio, J.J., Croteau, D.L., Van Houten, B. & Kisker, C. (2006). Prokaryotic nucleotide excision repair: the UvrABC system. *Chemical Reviews*, **106**(2), 233-252.

Van der Veen, S. & Abee, T. (2011). Bacterial SOS response: a food safety perspective. *Current Opinion in Biotechnology*, **22**(2), 136-142.

Vélez-Colmenares, J.J., Acevedo, A. & Nebot, E. (2011). Effect of recirculation and initial concentration of microorganisms on the disinfection kinetics of *Escherichia coli*. *Desalination*, **280**, 20-26.

Walters, E., Graml, M., Behle, C., Müller, E. & Horn, H. (2014). Influence of particle association and suspended solids on UV inactivation of fecal indicator bacteria in an urban river. *Water, Air, & Soil Pollution*, **225**(1), 1822-1828.

Wang, X., Hu, X., Hu, C. & Wei, D. (2011). Sequential use of ultraviolet light and chlorine for reclaimed water disinfection. *Journal of Environmental Sciences*, **23**(10), 1605-1610.

WHO (World Health Organisation) (1989). *Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture.* WHO, Technical Report Series no. 778. Geneva, Switzerland.

WHO (World Health Organisation) (2003). Expert consensus. In: *Heterotrophic Plate Counts and Drinking-water Safety: The Significance of HPCs for Water Quality and Human Health* (edited by J. Bartram, J. Cotruvo, M. Exner, C. Fricker & A. Glasmacher). Pp. 1-11. London: IWA Publishing.

Whitby, G.E. & Palmateer, G. (1993). The effect of UV transmission, suspended solids, wastewater mixtures and photoreactivation on microorganisms in wastewater treated with UV light. *Water Science and Technology*, **27**, 379-386.

Xu, L., Tian, C., Lu, X., Ling, L., Lv, J., Wu, M. & Zhu, G. (2015). Photoreactivation of *Escherichia coli* is impaired at high growth temperatures. *Journal of Photochemistry and Photobiology B: Biology*, **147**, 37-46.

Stellenbosch University https://scholar.sun.ac.za

Zimmer, J.L. & Slawson, R.M. (2002). Potential repair of *Escherichia coli* following exposure to UV radiation from both medium- and low-pressure UV sources used in drinking water treatment. *Applied and Environmental Microbiology*, **68**(7), 3293-3299.

Zimmer-Thomas, J.L., Slawson, R.M. & Huck, P.M. (2007). A comparison of DNA repair and survival of *Escherichia coli* 0157:H7 following exposure to both low- and medium-pressure UV irradiation. *Journal of Water and Health*, **5**(3), 407-415.

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Chapter 5
GENERAL DISCUSSION AND CONCLUSIONS

In recent years, an increase in the consumption of fresh and minimally processed foods (MPFs) has been observed globally. At the same time, the number of foodborne disease outbreaks associated with these products have escalated drastically. *Escherichia coli* (*E. coli*) and *Salmonella* are often identified as causative agents of such outbreaks and have resulted in several illnesses and deaths. In this regard, research shows that microbiologically polluted irrigation water is a major source of concern and a key contributor to produce contamination.

In the South African context, a multitude of commercial and subsistence farmers utilise rivers as primary source of irrigation water. Unfortunately, these rivers are often the receivers of faecal and other forms of waste as the infrastructural requirements of urbanisation, population growth and informal settlements are overlooked. Research has consequently confirmed that Western Cape Rivers, in particular, carry faecal coliforms (*E. coli*) at concentrations exceeding the Department of Water Affairs (DWA) and World Health Organization (WHO) limit of 1 000 colony forming units (cfu).100 mL^{-1} (WHO, 1989; DWAF, 1996; Britz *et al.*, 2012). These extraordinarily high levels of faecal coliforms (FC) present a high risk for carry-over to irrigated crops, even if minimal processing would be applied. Coliform bacteria may survive and multiply once transferred to such products and will threaten the health of consumers. In a country such as South Africa, where a large percentage of the population is immunocompromised due to HIV/AIDS and other factors, this becomes a grave matter. Moreover, the multi-billion rand agricultural sector of the Western Cape could be exposed to financial pressure should irrigated products deteriorate in microbiological quality. Considering that this industry contributes a 21% share to the gross farming income of the country (WESGRO, 2010), the matter deserves attention.

The extent of pollution of Western Cape Rivers requires that the water be treated to reduce the high contaminant loads to within DWA and WHO guideline limits prior to irrigation. In the literature, a 3 – 4 log reduction of faecal coliforms is suggested (Britz *et al.*, 2012; Britz *et al.*, 2013; Giddey *et al.*, 2015) in order to meet these guidelines. Several techniques are available for the disinfection of contaminated water, each representing distinctive advantages and disadvantages. In this regard, ultraviolet (UV) light irradiation is a novel method of water treatment and has been proven to be effective against a range of pathogenic microorganisms. Also, the process does not produce harmful disinfection by-products (DBPs) and is cost-effective on the long-term. In South Africa, however, UV irradiation is underutilised and seldom implemented as the use of chemical disinfectants is a firmly established practice. The overall objective of this study was to evaluate the efficiency and practicality of UV irradiation for the disinfection of irrigation water considering the influence of water quality and microorganism-related factors.

The first phase of the research was conducted on laboratory-scale using low-pressure (LP) UV irradiation. The investigation initially focused on the inactivation efficiency of LP UV

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(4 mJ.cm^{-2}) and a UV/Hydrogen peroxide (H_2O_2) combination treatment (4 mJ.cm^{-2}/20 mg.L^{-1}) which was tested against six *E. coli* strains. From the data recorded for the UV treatment clear variation in sensitivity was observed between the six strains. Compared to the American Type Culture Collection (ATCC) strains, environmental *E. coli* strains generally showed greater resistance. This, too, was true for the 4 mJ.cm^{-2}/20 mg.L^{-1} combination treatment. Nevertheless, variation was observed between the reductions achieved for the environmental strains which indicate that certain strains are better adapted to withstand the effects of UV and UV/H_2O_2 treatments. It was also shown that resistance towards the UV/H_2O_2 treatment may be linked to higher levels of the Hydroperoxidase 1 (HP1) catalase activity. However, in addition to catalase activity, it was concluded that mechanisms such as non-specific efflux pumps may further enable *E. coli* to protect itself against the treatment. The target reduction (a minimum of 3 log) was reached for some *E. coli* strains while both of the initial treatments (4 mJ.cm^{-2} and 4 mJ.cm^{-2}/20 mg.L^{-1}) were largely ineffective against others. It was, therefore, concluded that greater UV doses and/or H_2O_2 concentrations would be required to achieve adequate disinfection. The results of the first study also indicated that the UV/H_2O_2 treatment could potentially be more effective compared to UV irradiation, singly.

Based on the above results, three *E. coli* strains were selected and exposed to higher UV doses (8, 10 and 13 mJ.cm^{-2}) and additional combination treatments (4 mJ.cm^{-2}/2.5, 100 and 200 mg.L^{-1} H_2O_2). These strains were: 1) the most resistant environmental strain; 2) an environmental strain sensitive to the initial combination treatment of 4 mJ.cm^{-2}/20 mg.L^{-1}; and 3) an ATCC reference strain which is often encountered in literature. Higher UV doses resulted in better disinfection and for the most resistant strain an UV dose of 8 mJ.cm^{-2} produced a near 3 log reduction. However, for the UV/H_2O_2 combination, even a 4 mJ.cm^{-2}/200 mg.L^{-1} treatment could not do the same. Following irradiation at the same UV dose (8 mJ.cm^{-2}), no growth was detected for either of the other two strains (MJ56 and ATCC 25922). Again, the degree of resistance to the different combination treatments were proven to be associated with the levels of catalase activity (Hydroperoxidase 1 and 2) represented by different *E. coli* strains.

The second part of the study investigated the influence of water quality (measured in terms of chemical oxygen demand (COD), ultraviolet transmission percentage (UVT%), turbidity, total suspended solids (TSS) and conductivity) on the efficiency of UV and UV/H_2O_2 treatments that were tested against an ATCC reference strain as well as the most resistant strain identified in the previous study. For both *E. coli* strains significantly poorer disinfection was achieved in sterilised river water compared to Sterile Saline Solution (SSS) at an UV dose of 10 mJ.cm^{-2}. Nevertheless, in river water, the 3 log target reduction was just about reached for the most resistant strain. For the same strain, a slightly enhanced water quality (following flocculation and filtration) did not result in improved inactivation, as opposed to the ATCC strain that was better inactivated in flocculated river water. A radical combination treatment (10 mJ.cm^{-2}/200 mg.L^{-1}) was not influenced by slight changes in water quality and resulted in complete inactivation of the resistant *E. coli* strain in both

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untreated and flocculated river water. From the results obtained here it is concluded that, depending on the characteristics of the irradiated microbial population, water quality may have an important influence on the efficiency of UV based disinfection of irrigation water.

An intermediate study was conducted to evaluate different methods and media for the enumeration of coliforms and total heterotrophic microorganisms (bacteria) in river water. Having considered the results, as well as the cost related to each of the methods, the pour plate technique was selected for enumeration purposes in subsequent studies. Also, Violet Red Bile Agar (VRBA) and Plate Count Agar (PCA) were proven to be appropriate media for the enumeration of coliforms and heterotrophic microorganisms, respectively, and were therefore used in all subsequent experiments.

Since a LP UV dose of 8 $mJ.cm^{-2}$ entirely inactivated two test strains (and produced a near 3 log reduction of a resistant environmental strain) in SSS the efficiency of UV disinfection of contaminated river water was investigated at both lower and higher UV doses (5, 7 and 10 $mJ.cm^{-2}$). The study included three trials, performed on different days, in order to assess the influence of changing water quality and a variable microbial population on disinfection efficiency. Poor water quality was observed throughout the three trials and FC levels consistently exceeded the 3 log $cfu.100\ mL^{-1}$ limit by a large margin. When faecal pollution was at its greatest (Trial 1) a reduction of 3.41 log was required to obtain water that could be safely used for irrigation. The conductivity of the water was always above the limit set by the DWA while all other physico-chemical quality parameters were within the set guideline. Although differences in water quality were observed on the different days, these were too small to be clearly correlated with UVT%. Nevertheless, UV transmission was always low (< 38%) and a 3 log reduction could not be reached in any of the trials. Consequently, while increased UV doses resulted in increased efficacy, the maximum UV dose (10 $mJ.cm^{-2}$) could not reduce FC levels to below 1 000 $cfu.100\ mL^{-1}$. The treated water did, therefore, not comply with DWA guidelines. The data also indicated that microbial characteristics, in addition to water quality, may largely influence UV efficiency. The heterotrophic population was always more resistant than the total coliform (TC) and FC populations but, importantly, variation was also observed between reductions achieved for coliforms on the respective days. Better inactivation was not always associated with better water quality and could be attributed to variation in the characteristics of the microbial population present in the water. From these results it was concluded that LP UV doses greater than 10 $mJ.cm^{-2}$ would be required to ensure adequate disinfection of river water of the discussed microbiological and physico-chemical quality.

The second phase of the research was conducted on pilot-scale using medium-pressure (MP) UV irradiation. Initially, the objective was to evaluate disinfection efficiency in river water at higher UV doses (13, 17 and 24 $mJ.cm^{-2}$) compared to that used in the laboratory-scale study (5, 7 and 10 $mJ.cm^{-2}$) discussed earlier. These two investigations (laboratory and pilot-scale) were conducted concurrently. As the MP UV dose was increased a very slight improvement in efficiency

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was generally observed. The suggested 3 log target reduction was attained for TC and FC on some days. However, the data also indicated that even a dose of 24 $mJ.cm^{-2}$ could not reduce FC to below 1 000 $cfu.100\ mL^{-1}$. Furthermore, it was again difficult to identify correlations between the efficiency of the applied treatments and the physico-chemical characteristics of the river water.

Subsequent studies investigated the influence of photo-repair on the efficiency of UV disinfection of river water at MP UV doses of 13 and 24 $mJ.cm^{-2}$. Compared to previous pilot-scale trials, greater inactivation was observed and clearly correlated with improved water quality. The UVT% of the water nearly reached 50% and even at 13 $mJ.cm^{-2}$ the 3 log target reduction was reached. While the treatments reduced TC concentrations to below 1 000 $cfu.100\ mL^{-1}$, photoreactivation allowed for recovery so that a final level of close to 4.5 log $cfu.100\ mL^{-1}$ was reached after 5 h for the 13 $mJ.cm^{-2}$ treatment. The higher UV dose, however, resulted in slightly delayed and lower total reactivation compared to that following irradiation at 13 $mJ.cm^{-2}$. These results were in agreement with those of other studies which indicated that higher UV doses will lead to increased formation of pyrimidine dimers as well greater damage to endogenous photolyase.

When the UV dose was further increased (to 40 $mJ.cm^{-2}$) and compared to the use of a sequential treatment regime (2 x 20 $mJ.cm^{-2}$), photoreactivation (in both instances) was lower than that recorded earlier (at 13 and 24 $mJ.cm^{-2}$). Nevertheless, both treatments again allowed for photo-repair of TC to a final concentration that exceeded 3 log $cfu.100\ mL^{-1}$, although the sequential irradiation method better inhibited the phenomenon. Dark-repair potential was also investigated in this study but results indicated that the influence thereof on the efficiency of the treatments was trivial. Furthermore, it was demonstrated that photoreactivation occurred at a slower rate under light of lower intensity (1.0 to 2.0 vs 3.5 kLux) following the application of the single UV treatment (40 $mJ.cm^{-2}$). However, the difference was insignificant and statistically similar ($p=0.55$) levels of recovery were reached after 5 h. For the sequential treatment (2 x 20 $mJ.cm^{-2}$), light of lower intensity did not impair photoreactivation potential.

From the results of this study a number of important implications should be highlighted. Initially it was observed that UV and UV based advanced oxidation processes (AOPs) could, potentially, be effective methods for water disinfection. However, when optimising treatment parameters it is important to consider strain-to-strain variation in sensitivity. Environmental *E. coli* were particularly resistant to the treatments executed in SSS in this study. Using only ATCC reference *E. coli* strains in future optimisation studies could, therefore, result in the selection of treatment conditions which will not be able to produce adequate disinfection of an environmental microbial population. Based on studies in SSS an UV dose of at least 8 $mJ.cm^{-2}$ would be required to reach a 3 log reduction should the most resistant *E. coli* strain be present in irrigation water.

Results of the current study also indicated that UV disinfection efficiency was significantly influenced by dissolved and suspended particulate matter present in river water. In this regard, it was demonstrated that a slightly improved water quality allowed for improved disinfection of an

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ATCC reference strain while a resistant environmental strain was oblivious to such changes. Therefore, although enhanced water quality could result in greater UV efficiency it would be important for farmers to compare the cost of pre-treatment to that of increasing the UV dose to achieve the same result. The range of UV/H_2O_2 treatments evaluated in SSS was fairly ineffective and the harshest combination (4 mJ.cm^{-2}/200 mg.L^{-1}) could not produce a 3 log reduction of a resistant *E. coli* strain. However, in sterilised river water a “shock” combination treatment (10 mJ.cm^{-2}/200 mg.L^{-1}) completely inactivated the same strain. Also, it was observed that for some strains the initial combination treatment (4 mJ.cm^{-2}/200 mg.L^{-1}) was more effective compared to UV light alone. Therefore, the efficiency of combination treatments using UV doses greater than 4 mJ.cm^{-2} and a maximum H_2O_2 concentration of 200 mg.L^{-1} should be investigated further to suggest optimal conditions for AOPs. Applying H_2O_2 at 200 mg.L^{-1}, however, may be costly and it would be important to carefully optimise UV/H_2O_2 treatment parameters to identify the most sensible combination.

In subsequent studies it was observed that water from the Plankenburg River was vastly contaminated and should, therefore, not be used for irrigation if not treated. Above all, it is important that the relevant authorities and role-players be informed about the significance of the issue in an attempt to reduce the extent of waste disposal into local rivers. Low-pressure UV irradiation, up to a maximum dose of 10 mJ.cm^{-2}, was not efficient in disinfecting the water. Poor water quality, in terms of physico-chemical and microbiological properties, largely impaired the efficiency of the process. It would, therefore, be necessary to apply LP UV doses > 10 mJ.cm^{-2} should the water to be treated on commercial-scale be polluted to the same extent. Low-pressure UV irradiation is, however, not commonly used on larger-scale and was chiefly investigated to gain insight into the effectiveness of UV irradiation against a microbial population in river water compared to isolated strains in SSS.

Due to the limitations usually associated with upscaling of disinfection processes higher UV doses (13, 17 and 24 mJ.cm^{-2}) compared to those used in the LP study were used in the pilot-scale (MP) study. Medium-pressure UV irradiation, even at a maximum dose of 24 mJ.cm^{-2}, could also not reduce FC in river water to within the 1 000 cfu.100 mL^{-1} limit. Water quality had a great impact on treatment efficiency and an increase in UVT% resulted in much improved disinfection. In the “cleaner” water, coliforms were easily reduced to below the 3 log cfu.100 mL^{-1} limit, even at a low MP UV dose of 13 mJ.cm^{-2}. In terms of its practical application, it would, therefore, be worthwhile to improve water quality before UV irradiation (to reach UVT% of at least 50%) to obtain better disinfection. Farmers could easily employ more effective filtration techniques prior to UV irradiation to remove a large quantity of substances that could interfere with UV transmission in the water. Nonetheless, the use of stronger UV lamps to deliver UV doses that would achieve sufficient disinfection should be considered as its cost could well be lower compared to that of pre-treatments such as filtration. Also, it could be advantageous to use multiple UV units rather than a single system to deliver these high doses as it would more effectively reduce the likelihood of

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photoreactivation. In this study, photoreactivation was proven to be a substantial concern, even in the presence of low-intensity light. It is, therefore, further recommended that the effect of combination treatments, such as UV/Chlorine or UV/Peracetic acid, be evaluated also to determine if chemical activity could suppress the extent of repair. It would also be interesting to investigate whether the application of exceptionally high UV doses would be able to limit the degree of photoreactivation that could, potentially, be reached post-irradiation. Furthermore, members of the heterotrophic population were generally more resistant to UV irradiation compared to coliform bacteria. The use of additional indicator microorganisms should thus be considered when optimising parameters for UV disinfection. Using *E. coli* as sole indicator could lead to dose recommendations that are incapable of inactivating more resistant gram-positive pathogens, for instance.

Finally, the extent of contamination observed in river water in this study called for FC reductions exceeding 3 log to produce a water fit for agricultural irrigation. Under carefully optimised conditions, UV irradiation will effortlessly reach such a target and shows great potential as an effective technique for irrigation water disinfection. However, the efficacy of UV treatments are largely influenced by the physico-chemical properties of water and the phenomenon of photo-repair. These limitations are not considered crucial as simple interventions should be able to enhance the effectiveness and the overall credibility of the process.

REFERENCES

Britz, T.J., Sigge, G.O., Buys, E.M., Schmidt, S., Potgieter, N. & Taylor M.B. (2012). Baseline study on extent (types and quantities) of contamination found in irrigation water at selected sites (K5/1773). In: *Quantitative investigation into the link between irrigation water quality and food safety* (Volume 2). WRC Report no. K5/1773/4, Pretoria, South Africa.

Britz, T.J., Sigge, G.O., Huisamen, N., Kikine, T., Ackermann, A., Lötter, M., Lamprecht, C. & Kidd, M. (2013). Fluctuations of indicator and index microbes as indication of pollution over three years in the Plankenburg and Eerste Rivers, Western Cape, South Africa. *Water SA*, **39**(4), 457-465.

DWAF (Department of Water Affairs and Forestry) (1996). *South African Water Quality Guidelines*, 2nd ed. (edited by S. Holmes). *Volume 4: Agricultural Water Use: Irrigation*. Pp. 71-76. The Government Printer. Pretoria, South Africa.

Giddey, K., Kidd, M., Britz, T.J., Sigge, G.O. & Lamprecht, C. (2015). Impact of hydrogen peroxide treatment on environmental *Escherichia coli* strains. *Journal of Applied Environmental Microbiology*, **3**(2), 49-57.

WESGRO (Western Cape Trade and Invesment Promotion Agency) (2010). *Agribusiness sector brief*. Western Cape Trade and Investment Promotion Agency, Cape Town. Pp. 1-46.

Stellenbosch University https://scholar.sun.ac.za

WHO (World Health Organisation) (1989). *Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture.* WHO, Technical Report Series no. 778. Geneva, Switzerland.