EVALUATION OF THE EFFICACY OF CHEMICAL, ULTRAVIOLET (UV) AND COMBINATION TREATMENTS ON REDUCING MICROBIAL LOADS IN WATER PRIOR TO IRRIGATION

By

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DECLARATION

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ABSTRACT

The investigation of Western Cape Rivers has highlighted the importance of the implementation of cost-effective, on-farm disinfection treatments solutions. Irrigation water, if used untreated, has the potential to be a serious health hazard as faecal coliform (FC) levels often far exceed the allowable limit of 1 000 FC per 100 mL water. Chlorine (Cl), peracetic acid (PAA) and hydrogen peroxide (H_2O_2) are popular chemical disinfectants that have been used in water disinfection over the years. On-farm ultraviolet (UV) irradiation, a less conventional water treatments option, can also prove to be advantageous for water treatment. The aim of this study, therefore, was to investigate the application of chemical treatments in combination with UV irradiation in the disinfection of river water used for irrigation.

Initially, the efficacy of Cl, PAA and H_2O_2 in combination with low-pressure (LP) UV (Cl+UV; PAA+UV; H_2O_2+UV) required evaluating the stand-alone efficacy of each treatment first. Environmental *Escherichia coli (E.coli)* strains, F11.2 and MJ58 when exposed to Cl (6 $mg.L^{-1}$) and H_2O_2 (2.5 $mg.L^{-1}$) showed much resistance to disinfection. Strain F11.2, showed much greater sensitivity to PAA (4 $mg.L^{-1}$), recording > 3 log reductions for both 15 and 25 min contact times. However, LP-UV doses of 13 $mJ.cm^{-2}$ proved more effective than any of the chemical disinfectants for the *E. coli* strains. Combination treatments did not show much evidence on the initiation of advanced oxidation processes (AOPs) as the sum of the individual treatments more clearly justified the log reductions recorded.

An additional study investigated the impact of river on disinfection treatments whilst keeping the chemical and UV doses the same as in the first study. Considering the variability in the physico-chemical properties of the river water, Cl most effectively reduced the TC and FC groups, recording no less than 2.9 log reduction for TC and well over 3 log reduction for FC. PAA and H_2O_2 showed highly compromised disinfection and were unable, as stand-alone treatments, to offer adequate defence against the naturally present microorganisms in the river water. However, residual Cl levels of > 1 $mg.L^{-1}$ measured, post-treatments is of concern, as the formation of disinfection by-products (DBPs) is unwelcomed. UV treatments showed to be greatly influenced by poor ultraviolet transmission percentages (UVT%) and turbidity, which greatly decreased its effectiveness. Assessing the benefits of combination treatments, if any, through the initiation of AOP proved redundant as UV treatments were so effective.

The efficiency of medium-pressure (MP) UV irradiation (25 – 30 $mJ.cm^{-2}$) at pilot-scale, was able to, in some instances, successfully reduced FC levels by over 3 log. However, significantly poorer ($p<0.05$) disinfection was reported for all the chemical treatments. UV irradiation was again directly affected by poor optical water characteristics measured for the river water.

Cl disinfection, dosed at 3 $mg.L^{-1}$, half that of the dose used in previous trials, still proved to be the most effective of the chemical treatments investigated. Regardless thereof, Cl was only able to reduce FC by 1.58 log at best, which was insufficient, considering the > 6.0 log initial FC levels. Positively, when dosing Cl at 3 $mg.L^{-1}$, residual levels never exceeded 0.50 $mg.L^{-1}$. In most instances, no significant differences ($p>0.05$) were observed between stand-alone UV treatments and combination treatments, thus, insignificant contributions were made by advanced oxidation processes (AOPs). Investigating the effects of photo-repair revealed up to 13.72% and 15.86% photo-recovery for TC and FC, respectively, after UV irradiated river water was subjected to visible light at 3.5 kLux intensity for 3 h. Considering the importance of UV irradiation for the microbial reduction in combination treatments in this study, a 15.86% recovery rate for FC would, in many instances, result in the target 1 000 colony forming units (cfu). 100 mL^{-1} not being met.

As the efficacy of the disinfection treatments was influenced by varying microbial and physico-chemical properties of river water, the ability of biochar to improve the initial microbial and physico-chemical quality of river water was investigated. Significant improvements ($p<0.05$) to river water quality were observed for the eucalyptus biochar filter columns, with significantly less effective filtration recorded for pine biochar filter columns. No microbiological growth was detected after eucalyptus biochar filtration. And with significant improvements to UVT% from 49.60% to 88.00% after filtration. However, previously ‘used’ eucalyptus filter columns proved to be ineffective if left unused for $\geq$ 48 h, recording a > 3 log washout for TC and FC.

From the current study, combination treatments did not produce irrigation water of consistent acceptable standards for fresh produce. This was a results of UV irradiation being the main contributor to disinfection for the combination treatments and being greatly influenced by poor and varying water quality. Secondly, the poor contributions made by chemical disinfectants to the overall disinfection resulted in the dependence on UV irradiation for acceptable water disinfection. More effective filtration processes, combined with increased chemical and UV doses should be investigated to further optimise UV disinfection and ultimately combination treatments.

UITTREKSEL

Die belangrikheid van die implimentering van koste effektiewe, plaasvlak onstmetting-behandlings oplossings is tydens die ondersoek van die Wes-Kaapse riviere uitgelig. Onbehandelde besproeiingswater wat gebruik word het die potensiaal om ernstige gesondheidsriskos te verhoog, omdat fekale kolivorm (FK) vlakke dikwels die toelaatbare limiet van 1 000 FK per 100 mL oorskry. Chloor (Cl), Perasynsuur (PAA) en Waterstofperoksied (H2O2) is gewilde chemiese ontsmettingsmiddels, wat oor jare al gebruik word vir ontsmetting van water. Plaasvlak ultraviolet (UV) bestraling, ‘n minder konvensionele keuse om water te behandel, is ook bewys om voordelig te wees in die ontsmetting van water.

Aanvanklik vereis die behandeling van Cl, PAA en H_2O_2 in kombinasie met laedruk (LD) UV (Cl+UV; PAA+UV; H2O2+UV) eers die evaluering van elke behandeling se doeltreffendheid op sy eie. Omgewings *Escherichia coli* (*E. coli*) isolate F11.2 en MJ58 het meer weerstand teen ontsmetting getoon wanneer dit aan Cl (6 $mg.L^{-1}$) blootgestel is. Isolaat F11.2 wys ‘n hoër sensitiwiteit teenoor PAA (4 $mg.L^{-1}$), waar log verminderings van > 3 log vir beide 15 en 25 min kontaktyd waargeneem is. LP-UV dosisse van 13$mJ.cm^{-2}$ was egter meer doeltreffend as enige van die ander chemiese ontsmettingsmiddels gebruik vir *E. coli* isolate. Gekombineerde behandelings het nie meer bewyse getoon op die inisiasie van gevordered oksidasie prosesse (GOPs) nie, aangesien die som van die individuele behandelings die log reduksies beter aangedui het.

‘n Addisionele studie het die impak van die rivier op ontsmetting behandelings ondersoek, terwyl die chemiese en UV dosisse dieselfde gehou is as die eerste studie. In ag genome die variëring in die fisies-chemiese eienskappe van die rivierwater het Cl die FK en TK groepe die effektiefste verminder, waar nie minder as 2.9 log reduksie vir TK en vêr oor 3 log reduksie vir FK aangeteken is. PAA en H_2O_2 het hoogs gekompromitteerde ontsmetting aangedui en was nie in staat, as ‘n losstaande behandeling, om voldoende beskerming teen die natuurlik teenwoordige mikro-organismes in die rivierwater te bied nie. Alhoewel residuele Cl vlakke van > 1 $mg.L^{-1}$ gemeet is, is die post-behandeling ‘n bekommernis, omdat die vorming van ontsmetting bymiddels onwelkom is. UV behandelings is sterk beïnvloed deur swak ultraviolet oordrag persentasies (UVO%) en troebelheid, wat dus die effektiwiteit in ‘n groot mate laat afneem. Die evaluering van die voordele van kombinasiebehandelings, indien enige, deur die aanvang van GOP was oorbodig aangesien UV-behandelings so effektief was.

Die doeltreffendheid van medium-druk (MP) UV-bestraling (25 - 30 mJ.cm-2) op loodskaal was in sommige gevalle in staat om die FK-vlakke suksesvol te verminder met meer as 3 log. Daar is egter aansienlik swakker ($p < 0.05$) ontsmetting gerapporteer vir al die chemiese behandelings. UV-bestraling is weer direk beïnvloed deur swak optiese water eienskappe wat gemeet vir die rivierwater.

Cl ontsmetting gedoseer teen 3 $mg.L^{-1}$, die helfte van die dosis wat in vorige proewe gebruik is, blyk steeds die mees doeltreffendste van die chemiese behandelings wat ondersoek is.

Ongeag daarvan kon Cl net FK op die beste met 1,58 log verminder, wat onvoldoende was in die lig van die aanvanklike > 6.0 log FK-vlakke. Positief, wanneer die Cl by 3 $mg.L^{-1}$ toegedien word, het residuele vlakke nooit 0,50 mg L-1 oorskry nie. In die meeste gevalle is geen beduidende verskille (p> 0.05) waargeneem tussen alleenstaande UV-behandelings en kombinasiebehandelings nie, dus is onbeduidende bydraes deur gevorderde oksidasieprosesse (GOP's) gemaak. Ondersoek na die effekte van fotoreparasie het tot 13,72% en 15,86% fotoherwinning vir onderskeidelik TK en FK gewys, na UV-bestraalde rivierwater vir 3 uur lank blootgestel aan 3,5 kLux-intensiteit. Met die oog op die belangrikheid van UV-bestraling vir die mikrobiese reduksie in kombinasiebehandelings in hierdie studie, sal 'n 15,86% herstelvermoë vir FK in baie gevalle veroorsaak dat die teiken van 1 000 cfu 100 mL^{-1} nie bereik word nie.

Aangesien die doeltreffendheid van die ontsmettingsbehandelings beïnvloed is deur wisselende mikrobiese en fisies-chemiese eienskappe van rivierwater, is die vermoë van ‘biochar’ om die aanvanklike mikrobiese en fisies-chemiese kwaliteit van rivierwater te verbeter, ondersoek. Aansienlike verbeterings ($p < 0.05$) tot die rivierwaterkwaliteit is waargeneem vir die ‘biochar’ filterkolomme van bloekom, met aansienlik minder doeltreffende filtrasie aangeteken vir pynappel biochar filterkolomme. Geen mikrobiologiese groei is waargeneem ná die bloekom ‘biochar’ filtrasie nie, en met beduidende verbeteringe aan UVT% van 49,60% tot 88,00% na filtrasie. Maar, voorheen 'ebruikte bloekom filterkolomme was oneffektief as dit vir > 48 uur gelaat, met ‘n uitwassing van 'n> 3 log aangetekne vir beide TK en FK.

Met die huidige studie het kombinasiebehandelings nie besproeiingswater nie konsekwente, aanvaarbare standaarde vir vars produkte gelewer nie. Dit was omdat UV-bestraling die belangrikste bydraer tot ontsmetting vir die kombinasiebehandelings was, en uitermate beïnvloed word deur swak en wisselende watergehalte. Tweedens het die swak bydraes deur chemiese ontsmettingsmiddels tot die algehele ontsmetting, gelei tot die afhanklikheid van UV-bestraling vir aanvaarbare waterdesinfeksie. Meer effektiewe filtrasieprosesse, gekombineer met verhoogde chemiese en UV dosisse, moet ondersoek word om UV-ontsmetting, en uiteindelik kombinasiebehandelings verder te optimaliseer.

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In the words of William Louw Burger; ‘If you do something, do it properly, Klaar!’

CONTENTS

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.

ABBREVIATIONS

ANOVA:	Analysis of Variance
AOP:	Advanced Oxidation Process
$Ca(OCl)_2$:	Calcium hypochlorite
CES:	Chromocult® Coliform Agar Enhanced Selectivity
cfu:	Colony Forming Units
COD:	Chemical Oxygen Demand
CPD:	Cyclobutane Pyrimidine Dimer
DBPs:	Disinfection By-products
DWA:	Department of Water Affairs
DWAF:	Department of Water Affairs and Forestry
E. coli:	*Escherichia coli*
EC:	Electrical Conductivity
FC:	Faecal Coliforms
H_2O_2:	Hydrogen Peroxide
HPC:	Heterotrophic Plate Count
•OH:	Hydroxyl radical
kLux:	Kilolux
L-EMB:	Levine's Eosin Methylene-Blue Lactose Sucrose Agar
LP:	Low-pressure
LPM:	Litres Per Minute
LSD:	Least Significant Difference
MP:	Medium-pressure
NaOCl:	Sodium hypochlorite
NTU:	Nephelometric Turbidity Units
PAA:	Peracetic acid
PCA:	Plate Count Agar
SANS:	South African National Standards
SSS:	Sterile Saline Solution
TC:	Total Coliforms
TDS:	Total Dissolved Solids
TSS:	Total Suspended Solids
UV:	Ultraviolet
UVT%:	Ultraviolet Transmission Percentage
VRBA:	Violet Red Bile Agar

VSS:	Volatile Suspended Solids
WHO:	World Health Organisation

Chapter 1

INTRODUCTION

Water is considered one of the vital components for all basic metabolic activities. The increasing demand for fresh water, coupled with poor waste management, has consequently led to diminishing water sources often contaminated with alarming levels of pollution (DWAF, 2004; Hanjra & Qureshi, 2010). Additionally, due to the exponential rate of population growth, increased water consumption has been reported yearly (Rijsberman, 2006; Namara *et al.*, 2010). On average 70% of all freely available fresh water is consumed by the agricultural sector and only about 30% for domestic and industrial use (FAO, 2013). Critically, increased pollution of fresh water sources is threatening the ever demanding agricultural sector in South Africa. Africa is considered a developing continent and it has been estimated that 80% of illnesses and deaths reported can be related to poor water quality (Schaefer, 2008). Most fresh water in South Africa is found in river systems, which often pass through various areas that contribute to the pollution of already volatile rivers in South Africa. Multiple studies done on South African river water quality has revealed high levels of microbial pollution, often of a pathogenic nature (Paulse *et al*., 2009; Britz *et al*., 2013; Gemmell & Schmidt, 2013; Lamprecht *et al*., 2014). Disease outbreaks have been linked to microbiologically contaminated fresh-produce irrigated with water highly polluted with microorganisms of a faecal origin. Disease causing microorganisms most closely associated with fresh produce are *Escherichia coli* (*E.coli*) and *Salmonella* spp. (Warriner *et al.*, 2009; Benjamin *et al.*, 2013). However, other viruses and bacteria have also shown to cause illness when present in irrigation water (Harris *et al.*, 2003).

Many researches have reported on the seriousness of faecal contamination of South African rivers, specifically those in the Western Cape (Paulse *et al.*, 2009; Huisamen, 2012; Britz *et al.*, 2013, Bester, 2015; Olivier, 2015). Paulse *et al*. (2009) found very high levels of *E. coli* contamination from the Berg River, reporting 6.2 log colony forming units (cfu) per 100 mL^{-1}. Furthermore, Olivier (2015) reported similarly high levels of Coliforms in the Plankenburg River, reporting 5.25 and 6.41 log cfu per 100 mL^{-1} for Total and Faecal Coliforms (TC and FC) respectively. The Department of Water Affairs (DWAF, 1996) has thus established guidelines limiting the amount of FC allowable for irrigation water to $\leq$ 1 000 colony forming units (cfu) per 100 mL.

The treatment of water intended to be used for irrigation purposes for fresh or minimally processed crops is thus vital, and considered a priority. An approach in alleviating this concern is to ensure proper disinfection of irrigation water (Lewis Ivey & Miller, 2013; Van Haute *et al.*, 2013). Disinfection treatments employed include chemical, physical and photochemical methods. The different treatment options are dependent on a variety of factors, thus selection of an appropriate water treatment method becomes imperative, as not all methods allow for the same disinfection potential.

In water treatment chlorine has been the most frequently and long standing disinfectant used. Its use dates back to the early 1900's, as its ability to successfully remove bacteria, viruses and protozoa is undeniable (Schoenen, 2002; Macauley *et al*., 2006; Mezzanotte *et al*., 2007; Lewis Ivey & Miller, 2013; Bester 2015). Specifically *E. coli*, a Gram-negative bacteria, displays less resistance to chlorine than Gram-positive bacteria (Veschetti *et al*., 2003; Van Haute *et al*., 2013). Various forms of chlorine are available. Chlorine in hypochlorite forms are the more preferred, as they are considered safer to use than the chlorine gas in water treatment (Clasen & Edmondson, 2006; Fukuzaki, 2006; Lewis, 2010). The two main forms of hypochlorite are in either a powder form, providing 65 – 70 % ($m.v^{-1}$) available chlorine or a liquid form, usually in a solution with 12 – 15% ($m.v^{-1}$) available chlorine (Newman, 2004; Momba, 2008; Deborde & von Gunten, 2008). As chlorine is a chemical oxidant it has a direct and damaging effect on the cell membranes of microorganisms, as it affects the lipids and proteins within the membrane (Cho *et al*., 2010). Furthermore, intercellular chlorine is also known to affect enzymes and other lipid structures (Cho *et al*., 2010). Enzymes affected include dehydrogenase and catalase (Vitro, 2005). The damage chlorine induces on the cell membranes can also lead to leaking of genetic materials (DNA, RNA), directly interfering with transcription and translation (Cho *et al*., 2010; Bitton, 2011). Various studies report on the effectiveness of chlorine, when applied as a disinfectant in water and wastewater treatment. Wang *et al*. (2011) reported a dose of 1.5 – 3 $mg.L^{-1}$ (using a 6% NaOCl solution) was able to achieve a 4 log reduction for *E. coli* strain ATCC15597 and near complete inactivation (> 5 log reduction) when using 5 $mg.L^{-1}$ with 30 min contact time. Bester. (2015) found effective microbial reductions using 12 $mg.L^{-1}$ of chlorine with > 15 min contact times. Chlorine disinfection is still used today due to its low cost and its residual disinfection effect (Lewis, 2010). Residual levels are, however, not always advantageous, as they promote the formation of disinfection by-products (DBPs) when reacting with organic matter. These DBPs are often mutagenic and/or carcinogenic by nature.

Another chemical disinfectant that has application as an effective water disinfectant is peracetic acid (PAA). PAA has gained attention due to its antimicrobial activity displayed towards a variety of microorganisms, including bacteria and fungi (Gehr *et al*., 2003; Kitis, 2004; Rossi *et al*., 2007). PAA, as chlorine, has the ability to reduce indicator microorganisms present in wastewater, but with the added benefit of less formation of DBPs (Gehr *et al*., 2003; Veschetti *et al*., 2003; Koivunen & Heinonen-Tanski, 2005a; Rossi *et al*., 2007). Similar microorganisms are affected by PAA as with Cl disinfection, which include FC and TC groups, *E. coli* and *Salmonella* spp. (Veschetti *et al*., 2003). Koivunen & Heinonen-Tanski (2005b) reported using PAA at a concentration of 3 $mg.L^{-1}$ were able to achieve a 2 – 3 log reduction for *E. coli*, thus proving PAA to be effective in water treatment. Gehr *et al*. (2003) reported that allowing less than 1 hour contact time, together with low concentrations of PAA, of 4 $mg.L^{-1}$, initial microbial levels of 4 – 5 log $cfu.100\ mL^{-1}$ were able to be reduced to satisfy the recommended guideline for FC (1 000 $cfu.100\ mL^{-1}$) (DWAF, 1996). A pilot-scale study by Caretti & Lubello (2003) reported using PAA (8 $mg.L^{-1}$)

and allowing a 30 min contact time was sufficient to reduce initial microbial levels by 3.99, 4.21 and 4.42 log for TC, FC and *E. coli,* respectively (Caretti & Lubello, 2003). PAA offers clear advantages and potential to be used as an irrigation water treatment for fresh produce, however, it is more costly than Cl. Furthermore, because PAA has a higher oxidation potential than Cl and H_2O_2, adequate disinfection, even when dosed at low concentrations and allowing short contact times, is possible (Veschetti *et al*., 2003; Rossi *et al*., 2007).

Nevertheless, H_2O_2 has proved to be effective in controlling a large variety of microorganisms, specifically those of a pathogenic nature (Newman, 2004; Koivunen & Heinonen-Tanski, 2005a; Sherchan *et al*., 2014). H_2O_2 solutions generally are able to control bacteria, fungi and yeasts, less so for viruses (Newman, 2004; Sherchan *et al*., 2014). H_2O_2 has successfully been implemented in the water and wastewater industries (Ksibi, 2006; Vargas *et al*., 2013), as well as effectively improving water quality by reducing the chemical oxygen demand (COD) and biochemical oxygen demand (BOD) (Ksibi, 2006). Using H_2O_2 at a concentration of 2.5 $mg.L^{-1}$, Ksibi (2006) achieved a 3 log FC reductions, after a 2 h contact time. Regarding water quality effective COD reductions from 322 $mg.L^{-1}$ to 44 $mg.L^{-1}$ were also reported after a 2 h contact time at a H_2O_2 concentration of 2.5 $mg.L^{-1}$ (Ksibi, 2006). Ronen *et al*. (2010) allowed a 56 min contact time, whilst using a H_2O_2 concentration of 125 $mg.L^{-1}$, and was able to achieve 99% reduction in faecal indicator microorganisms. H_2O_2 displays good disinfection potential at higher doses to achieve the desired disinfection efficacy (Koivunen & Heinonen-Tanski, 2005a). Therefore, researches have incorporated H_2O_2 with other treatments like flocculation or filtration as to optimise its efficacy. Advantageously, H_2O_2 can be used in combination with UV irradiation, further highlighting its versatility and potential disinfection capabilities (Labas *et al*., 2008; Linley *et al*., 2012a).

UV irradiation is considered a non-thermal disinfection treatment. UV wavelengths are produced in the range of 100 – 400 nm making use of medium pressure (MP) or low pressure (LP) mercury vapour lamps (Koutchma, 2009). UV treatments have shown to be affective in eliminating a large variety of microorganisms, including those of a pathogenic nature. However, not all microorganisms display similar sensitivity towards UV treatments, as greater UV-resistance is displayed by bacterial spores and viruses (Caretti & Lubello, 2003; Koivunen & Heinonen-Tanski, 2005; Hijnen *et al.*, 2006; Gayán *et al.*, 2014). Selecting a single UV dose can, therefore, be challenging when considering the variations of microorganisms, that are likely to be present within a body of water (Hijnen *et al.*, 2006; Santos *et al.*, 2013). Olivier. (2015) found significantly better disinfection observed at a UV dose of 14 $mJ.cm^{-2}$ compared to that at 10 $mJ.cm^{-2}$, suggesting improved microbial reductions were possible at increased UV doses. Genetic material is damaged when the UV light is absorbed by genetic material of the targeted microorganisms. The two main photoproducts formed are Cyclobutane Pyrimidine Dimers (CPDs) and less damage incurring, pyrimidine 6 – 4 pyrimidone photoproducts (6-4PPs) (Bolton & Linden, 2003; Poepping *et al*.,

2014). Both photoproducts result in cell damage and ultimately cell death (Rastogi *et al*., 2010; Rodriguez *et al*., 2014; Premi *et al*., 2015).

UV irradiation has its drawbacks. Microorganisms have shown to reverse/repair genetic material damage. The most common repair mechanism are referred to as photo-repair. Furthermore, UV transmittance (UVT%), turbidity and the presence of suspended solids are important optical water characteristics influencing the overall efficacy of UV irradiation (Gayán *et al*., 2011; Abdul-halim & Davey, 2016). Unfavourable water quality parameters result in reflecting, absorbing and scattering of UV light waves, ultimately reducing UV treatments' effectiveness. Thus, UV lethality is highly dependent on the target microorganisms' ability to repair damaged genetic material, as well as environmental influences regarding water quality. Ultimately, as UV irradiation shows promising disinfection potential, the possibility of microbial repair must be investigated and better understood. Therefore, the development of more effective, less environmentally damaging disinfection treatments must be considered (Sharp *et al.*, 2006).

One such approach involves the combination of UV light and chemicals such as PAA, Cl and H_2O_2 (Rosenfeldt *et al.*, 2006; Oturan & Aaron, 2014). These combination treatments initiate a phenomenon, advanced oxidation process (AOPs) which involve the production of Hydroxyl Radicals (•OH) which are considered powerful and effective oxidisers of organic pollutants (Sherchan *et al.*, 2014a). These AOPs have shown to be able to cause a greater disinfection effect than simply summing the effects of the individual treatments. Sherchan *et al.* (2014a) reported complete microbial inactivation (> 7 log) when H_2O_2 was applied in combination with UV, which was likely due to the effects of AOPs. Studies involving PAA and Cl in combination with UV have also shown successful microbial reductions and evidence of AOPs (Koivunen & Heinonen-Tanski, 2005; Rosenfeldt *et al*., 2006; Montemayor *et al*., 2008). As combination treatments possess the potential to reduce organic matter present in water, assessing the effect chemical treatments applied in combination with both UV irradiation would prove to be valuable.

As most of the treatment methods show good disinfection potential, varying water quality effect both chemical and UV treatments. Specifically, UV treatments are greatly comprised by increasing levels of organic and inorganic matter that may be present in water which aid in reflecting and scattering the UV waves, thus reducing the overall efficacy of the treatment. Other water quality parameters such as UVT% and turbidity also have a direct impact and are responsible for variations in the lethality of UV treatments. Physico-chemical parameters such as pH, temperature, Chemical Oxygen Demand (COD) has shown to specifically effect the disinfection potential of chemical disinfectants.

Filtration methods are employed to improve the physico-chemical quality of water. When implemented, although they can be effective, their viability as a sole disinfection treatment remains greatly subjective. Herein lies the potential of alternative filtration methods such as biochar filtration. Biochar is produced by pyrolysis of different carbon-rich, organic materials at different temperatures in the absence of oxygen, producing a substance with a porous charcoal-like

appearance (Hunt *et al.*, 2010; Ahmad *et al.*, 2014). Biochar has shown potential to successfully remove both organic and inorganic unwanted pollutants present in water and wastewater (Mohan *et al.*, 2014; Tan *et al.*, 2015; Baltrenaite, 2016). Biochar types have shown to be extremely effective in removing dyes, pesticides, chemicals and phenolic compounds from water (Han *et al.*, 2013). Thus, the potential of retaining pathogens by the biochar should be considered (Dempster *et al.*, 2012; Mohanty *et al.*, 2014). There can, however, be considerable variation in the final composition of different biochar types (Chen *et al.*, 2008; Cui *et al.*, 2016). Pyrolysis temperature is important as it influences the sorbent characteristics displayed by the final biochar type.

The overall aim of this study was to evaluate the potential of chemical disinfectants, applied in combination with UV irradiation for the disinfection efficacy of microbiologically contaminated irrigation water. The individual disinfection potentials of the stand-alone chemicals and UV treatments also had to be determined. Several studies were performed focusing on the disinfection potential of Cl, PAA, H_2O_2, as well as, low and medium-pressure UV, evaluated as individual treatments and in combination with UV (Cl+UV; PAA+UV and H_2O_2+UV) against *E. coli* environmental strains at laboratory-scale and applying the same treatments at pilot-scale using MP-UV irradiation for the reduction of the naturally occurring microorganisms in the river water; the possibility of DNA repair, following UV irradiation at pilot-scale was also investigated when using MP-UV irradiation. In addition, the filtration efficacy of self-made biochar filters was also tested in their ability to improve physico-chemical and microbial quality of microbiological contaminated river water.

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Chapter 2

LITERATURE REVIEW

2.1 BACKGROUND

A statement by Ismail Serageldin: “The wars of the twenty-first century will be fought over WATER.” should be reason enough to strongly consider the current global water situation, especially that of South Africa. Water is not only beneficial, but vital for life to continue as we know it. Large quantities of fresh water are required by humans for daily activities; including health and sanitation, generating electricity, irrigation of commercial and agricultural land, as well as sustaining livestock and crops of subsistence farmers and their families (Namara *et al.*, 2010). Ultimately, water can thus directly and indirectly be accounted to a large portion of income earned by a country each year (DEAT, 2006). Worryingly fresh water sources are currently falling under threat. The increasing demand for fresh water, coupled with poor waste management, has consequently led to diminishing water sources often contaminated with alarming levels of pollution (DWAF, 2004; Hanjra & Qureshi, 2010). Africa is considered a developing continent and it has been estimated that 80% of illness and deaths reported can be related to poor water quality (Schaefer, 2008).

There are pre-dominantly two forms of water available on earth, namely fresh water and saline water (Pachepsky *et al.*, 2011). Fresh water is associated with surface and ground water, whereas saline water, the dominant form of water on earth (more than 90% of water available), is of an oceanic origin. It is estimated that < 3% of water on earth is fresh water. Currently it is speculated that there is a 64 billion m^3 increase in fresh water demand per year (Wada *et al.*, 2011). Due to the exponential rate of population growth, coupled with increased energy demands, increased water consumption has been reported each year, especially for irrigation purposes (Rijsberman, 2006; Namara *et al.*, 2010). An average of 70% of all fresh water used is consumed by the agricultural sector, followed by only 30% for industrial and domestic use (FAO, 2013). The population of the world is increasing by approximately 80 million people a year. Rising trends also indicate that more people are consuming foods that require more water to produce (Jung *et al.*, 2014). The global water demand is therefore higher than the global water supply, threatening life as we know it.

South Africa is currently in a phase of immense urban and industrial growth, which is positive for the short term economic standing of the country. These developments are, however, threatening food security, as increasing amounts of water are being allocated to support these non-agricultural ventures (De Bon *et al.*, 2010). Availability of fresh water is becoming an ever increasing concern due to climate change, as well as pollution of current water supplies (Hanjra & Qureshi, 2010). Currently Africa is experiencing an unusually warmer climate, increasing the demand for fresh, clean water, further worsened by diminishing water sources and decreased

rainfall (Jorgensen *et al.*, 2009). The large dependence on fresh water is not only important for urban settlements, but greatly important in rural areas as well. High levels of unemployment and increased living costs in rural areas, together with the lack of basic sanitation and waste removal further highlights the dependence on safe, fresh water in rural communities (Obi *et al.*, 2004). People are therefore becoming ever more reliant on nearby water sources for daily activities, often being exposed to microbially unsafe water (Gemmell & Schmidt, 2012).

Multiple studies done regarding the water quality of South African rivers has revealed high levels of microbial pollution of pathogenic nature (Paulse *et al.*, 2009; Britz *et al.*, 2013; Gemmell & Schmidt, 2013; Lamprecht *et al.*, 2014). When considering the reliance the agricultural sector in South Africa has on fresh water from rivers, especially for irrigation purposes, high levels of pathogens can present a serious problem (Paulse *et al*., 2009; Ijabadeniyi & Buys, 2012). Guidelines have been established by the World Health Organisation (WHO), as well as the Department of Water Affairs (DWA) regarding irrigation water quality intended for fresh or minimally processed crops. A guideline of $\leq$ 1 000 Faecal Coliforms (FC) for every 100 mL of water is considered a safe range for irrigation water (DWAF, 1996). This microbial guideline however, more often than not, is significantly exceeded by many rivers in South Africa (DWAF, 1996; Paulse *et al*., 2009; Gemmell & Schmidt, 2012; Olivier, 2015).

Global trends promoting healthier lifestyles, supported by eating more “natural” and less processed foods are gaining acceptance, placing emphasis on fresh or minimally processed crops (Jung *et al.*, 2014). Fresh produce is most susceptible to poor irrigation water quality and has been linked to numerous outbreaks of foodborne illnesses reported over the past few years (Ijabadeniyi & Buys, 2012; Benjamin *et al.*, 2013). Untreated river water, contaminated with faecal waste and pathogenic microorganisms, can act as a vector responsible for the transfer of pathogens to crops (Teklehaimanot *et al.*, 2014). These contaminated crops, if consumed fresh or only with minimal processing, can ultimately be responsible for causing disease outbreaks in human populations (Pachepsky *et al.*, 2011; Ijabadeniyi & Buys, 2012). Many disease outbreaks have been reportedly caused due to consumption of *Salmonella* spp. and *Escherichia coli (E. coli)* O157:H7, found on fresh fruits and vegetables (Warriner *et al.*, 2009; Benjamin *et al.*, 2013). A large variety of other pathogenic microorganisms have also been associated with fresh fruits and vegetables, including *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Shigella* spp., *Giardia* spp., *Cryptosporidium* spp., as well as a large range of viruses (Harris *et al.*, 2003).

Thus, when considering South Africa specifically, available research has shown a strong trend highlighting the possible dangers associated with using water directly from rivers for irrigation purposes (Paulse *et al.,* 2009; Bester, 2015; Olivier, 2015). Considering the ever increasing demand on fresh water sources, action must be taken to ensure safe water is available for all South Africans.

2.2 FRESH WATER SITUATION IN SOUTH AFRICA

A report compiled by the DEAT (2006) stated that water availability was one of the main factors influencing South African's well-being and economic development. South Africa, as a country, is currently exceeding its ecological carrying capacity, placing its inhabitants in a vulnerable position (DEAT, 2006). Natural resources are greatly under threat, as the South African population has grown more than eight times in the last 100 years. The population has increased from only 5 million inhabitants at the start of the 1900's, to just under 55 million a 100 years later. Thus immense stress has been placed on the already limited resources, as to sustain this exponential population growth (DEAT, 2006). The demand for fresh water to sustain this population increase, has become a growing concern over the last few years (Teklehaimanot *et al.*, 2014). This immense population growth has directly been linked to an exponential increase in the quality of waste water generated and untimely released back into the environment. Trends seeing the migration of people from rural to urban settlements further increase the demand for fresh water significantly for urban domestic uses. Indirectly, due to increased domestic water needs, the needs of industrial and commercial water use are consequently also on the rise (DWAF, 2004).

Alternative factors, other than already mentioned, have also been reported to affect the availability of fresh water, contributing to the current global water circus. The country experiences on average a low rainfall of approximately 450 mm annually, classing it in a water scarce bracket (DEAT, 2006). Rainfall is also not evenly distributed throughout the country, due to the various climatic regions found (DEAT, 2006; DWA, 2013). It has been estimated that by the year 2025 there will be a national deficit of available fresh water, emphasising the need to secure the current water sources (DEAT, 2006). Adding to the already poor distribution of rainfall, climate change is considered to further alter the rainfall and its distribution (DEAT, 2006; Hanjra & Qureshi, 2010). Increased rainfall is expected in the Eastern region of the country, with a decrease expected in the Western regions.

To maximise water availability, dams and rivers have been well developed to conserve the water made available via rainfall. Approximately 60 – 70% of runoff water made available from rainfall is captured in dams throughout, greatly relied upon for fresh water sources (DWAF, 2004). The safety of these water sources cannot always be ensured, as mining waste water, irrigation return flows, as well as improper treatment of sewage water often pollute these fresh water sources (DEAT, 2006; Teklehaimanot *et al.*, 2014). A significant contributor of fresh water is due to return flows. Adequate treatment must therefore be guaranteed to prevent unsafe water entering rivers and dams (DEAT, 2006; Schaefer, 2008).

In order to help manage the water sources 19 Water Management Areas have been created to help assess and manage potential water crises. Currently it is reported that ten of these Water Management areas have water deficits. This alone should be reason enough to strongly consider the potential impact of poor water management in South Africa.

Irrigation water is considered the largest consumer of fresh water, followed by basic usage in urban areas. According to DEAT (2006) it is vital to ensure proper management of fresh water resources to ensure the safety and availably of water in the future. However, estimating the demands for fresh water can be difficult to manage, due to differences in water quality required for different sectors, as well as seasonal variation requirements (Namara *et al.*, 2010). As agricultural irrigation is the largest consumer of fresh water, a significant portion of the country's income is generated by exporting crops (Ijabadeniyi & Buys, 2012). More importantly, the agricultural sector is vital as to sustain the inhabitants of South Africa in terms of food security (Fauchereau *et al.*, 2003; Namara *et al.*, 2010).

2.3 CURRENT WATER QUALITY IN SOUTH AFRICA

Limited research is available regarding microbiological contamination of most South African rivers. Information that has, however, been made available concerning the water quality of a few rivers is alarming. It is suggested that the lack of proper sanitation facilities and overloaded sewage treatment plants have led to improperly treated sewage and wastewater being released back into the environment (DEAT, 2006; Schaefer, 2008; Britz *et al.*, 2013). When considering the South African population is growing exponentially each year, little has been done on improving and upgrading wastewater treatment facilities (Bryan *et al.*, 2009; Gemmell & Schmidt, 2013). This consequently has resulted in improperly treated faecal waste being released into the already limited fresh water sources available (Teklehaimanot *et al.*, 2014).

As a result high levels of faecal contamination are consistently recorded in river water and is becoming a major public health concern (Paulse *et al.*, 2009; Britz *et al.*, 2013; Bester, 2015; Olivier, 2015). Recently the South African Water Research Commission (WRC) has adopted a hands on approach, with the goal to determine the extent of microbial contamination of South African rivers. In order to establish the current water quality situation in South Africa, the water contamination levels recorded were compared to guidelines established by the World Health Organisation (WHO), as well as the South African Department of Water Affairs (DWA). A major indication of the likely presence of pathogenic bacteria in water is indicated by quantifying the presence of *Escherichia coli (E. coli)*, a bacteria that is strongly associated with faecal contamination and consequently the likelihood of sewage entering the water system (Nnane *et al.*, 2011; Britz *et al.*, 2013; Odonkor & Ampofo, 2013).

E. coli forms part of the Coliform group, specifically the Faecal Coliform group (Odonkor & Ampofo, 2013). A guideline established by the DWA and WHO suggest a limit of $\leq$ 1 000 Faecal Coliforms (FC) per 100 mL in water, intended to be used for irrigation purposes (WHO, 1989; DWAF, 1996). Gemmell & Schmidt (2013) strongly enforced the idea that South African rivers, specifically the Msunduzi River in KwaZulu-Natal, is no exception to other South African rivers as high levels of microbial pollution were recorded. Microbial levels, recorded over a 13 month period, found that there was on average 3 000 MPN.100 mL^{-1} *E. coli* present in the river water, with Faecal

Coliforms averaging 8 300 MPN.100 mL^{-1} and Total Coliforms 22 000 MPN.100 mL^{-1} (Gemmell & Schmidt, 2013). Therefore the $\leq$ 1 000 cfu.100 mL^{-1} FC limit was far exceeded by the 8 300 MPN.100 mL^{-1} reported (Gemmell and Schmidt, 2013). Similar trends have been reported by a number of studies done across South Africa on the microbial status of river water (Germs *et al.*, 2004; Paulse *et al.*, 2009; Gemmell & Schmidt, 2012; Olivier 2015). A study completed by Germs *et al.* (2004) found that the Chunies River in Limpopo, was unacceptable for domestic and agricultural usage due to poor microbial and water quality. Obi *et al.* (2004) found that multiple rivers presented alarmingly high amounts of *E. coli* of a pathogenic nature, directly associated with human faecal waste. These are just a few studies that highlight the probability of poor water quality of many South African rivers, strengthening the belief of faecal waste entering the environment.

2.4 THE MICROBIAL POLLUTION SITUTION OF WESTERN CAPE RIVERS

The river systems in the Western Cape region are no exception, as multiple studies done over the past few years have shown that there are major concerns regarding microbial pollution in various rivers investigated (Paulse *et al.*, 2009; Huisamen, 2012; Britz *et al.*, 2013, Bester, 2015; Olivier, 2015). These high levels of microbial pollution consequently increases the possibility for disease outbreaks, when using this contaminated water for agricultural purposes (Ijabadeniyi & Buys, 2012). Britz *et al.* (2013) reported the presence of undesirable indictor microorganisms in the Plankenburg and Eerste Rivers, concluding that the water was not safe to be used for irrigation purposes. Paulse *et al.* (2009) found high levels of *E. coli* contamination from the Berg River, reporting 6.2 log cfu.100 mL^{-1}. Barnes & Taylor (2004) reported Faecal Coliforms numbers of 7.2 log cfu.100 mL^{-1} for the Plankenburg River. Huisamen (2012) reported high levels of *E. coli* in water collected from the Eerste and Plankenburg Rivers, with *E. coli* levels as high as 6.8 log cfu.100 mL^{-1} recorded.

Overall, these levels of contamination are quite alarming when considering the proposed guideline for FC of $\leq$ 1 000 cfu.100 mL^{-1}, which was greatly exceeded by most rivers brought under investigation (DWAF, 1996). Furthermore, Olivier (2015) reported similar high levels of Coliforms in the Plankenburg River, reporting 5.25 and 6.41 log cfu.100 mL^{-1} for Total and Faecal Coliforms respectively. One of the sources of these high levels of contamination reported, can be informal settlements such as Kayamandi informal settlement, situated upstream from many of the water sampling sites on the Plankenburg River (Britz *et al.*, 2013). Informal settlements are known to be less than sufficient in providing adequate sanitation facilities, as well as waste removal services (Nyenje *et al.*, 2010; Ijabadeniyi & Buys, 2012). Nearby industrial and agricultural establishments can also contribute to the polluted water of the Plankenburg River (Paulse *et al.*, 2009; Britz *et al.*, 2013).

2.5 DISEASE OUTBREAKS ASSOCIATED WITH FRESH PRODUCE

Due to social changes in consumer behaviour over the past few years, there has been an increase in foodborne outbreaks related to fresh or minimally processed crops (Lee *et al.*, 2014). One of the contributing factors to this exponential increase in foodborne outbreaks, is the increase in consumption of fresh or minimally processed fruits and vegetables (Jung *et al.*, 2014). This shift in consumer preference sees populations supporting healthier lifestyles, often accompanied by short preparation times of foods (Jung *et al.*, 2014). Through this minimal processing practice consumers are hoping to retain beneficial macro and micro nutrients from the specific produce, thus seeking maximum health benefits the food has to offer (Qadri *et al.*, 2015).

However, the health benefits sought may be jeopardised, as foodborne outbreaks have been linked to a variety of fresh produce, including spinach, tomatoes, seed sprouts, fresh herbs and lettuce, just to mention a few (Jung *et al.*, 2014). Waterborne pathogens that are more frequently associated with fresh produce include bacteria, viruses and parasites. Specifically pathogenic strains of *E. coli*, *Salmonella* spp. and *Listeria monocytogenes* have been closely associated with disease outbreaks (Warriner *et al.*, 2009; Pachepsky *et al.*, 2011; Lee *et al.*, 2014). Outbreaks have also been strongly associated with green leafy vegetables, often consumed with only minimal processing, if any (Khalil & Frank, 2010; Olaimat & Holley, 2012). Due to the large surface area made available by leafy vegetables, the attachment of pathogens is more likely to occur when overhead irrigation is used (Luo *et al.*, 2011). Pathogenic, as well as the non-pathogenic strains of *E. coli* also have the ability to adhere to plant roots when applied via soil irrigation. Root vegetables can thus also be potential carriers of pathogenic microorganisms, further highlighting the concerns associated with microbially contaminated irrigation water (Benjamin *et al.*, 2013).

South Africa has yet to establish more strict and accurate protocols documenting the presence of pathogens on minimally processed crops, as well as reporting more reliable data on illnesses induced by microbially unsafe produce. Benjamin *et al.* (2013) highlighted that multiple foodborne outbreaks, associated with minimally processed crops, have been reported over the last 20 years. In these studies irrigation water was identified as the main vector responsible for the transfer of pathogenic microorganisms from faecal waste, causing illness in humans. The majority of the outbreaks were due to ingestion of *E. coli* O157:H7 and O145:7 (Bernstein *et al.*, 2007; Jay *et al.*, 2007; Zhang *et al.*, 2009). Jay *et al.* (2007) reported a large outbreak of *E. coli* O157:H7 strain that was present on bagged baby spinach, causing multiple deaths. Over the last ten years multiple outbreaks related to fresh produce have been documented globally (Table 1).

Table 1 Global foodborne outbreaks associated with fresh produce (Lynch *et al.*, 2009; Warriner *et al.*, 2009)

Pathogen detected	Number of cases	Affected areas	Produce affected
Salmonella	1442 cases	North America	Fresh peppers
E. coli (O157:H7)	203 cases, 3 deaths	North America	Fresh spinach
Salmonella	32 cases	United Kingdom	Basil
E. coli (O157:H7)	134 cases	North America	Lettuce
E. coli (O104:H4)	3950 cases, 50 deaths	Europe	Fresh Sprouts
E. coli (O157:H7)	14 cases, 1 death	North America	Strawberries
L. monocytogenes	15 cases, 5 deaths	North America	Celery

2.6 POSSIBLE SOURCES OF CONTAMINATION

Sources of pathogenic microorganisms, present on fresh produce, are not only limited to irrigation water, although it is of primary concern (Benjamin *et al.*, 2013). There have been multiple reports of foodborne illness originating from soil and soil amendments, contaminated harvesting equipment, workers handling the crops, processing plants and even retail handing (Jung *et al.*, 2014).

Diseases with a water origin are roughly estimated to be responsible for a third of intestinal infections across the world. Faecal contamination has been strongly associated to be a leading cause of waterborne disease (Odonkor & Ampofo, 2013). The most dominant contributors to faecal pollution of surface waters are made by humans, livestock and wild animals, with the largest contributor being human faecal waste (Parajuli *et al.*, 2009; Benjamin *et al.*, 2013). The introduction of faecal matter is aided by rainfall that carries the faecal matter to rivers, streams and dams. Cooley *et al.* (2007) reported increased numbers of *E. coli* O157:H7 in surface water after increased rainfall, resulting in more rapid flow rates of rivers. Microorganisms are more likely found in surface waters such as rivers and streams, as these aid in their distribution. However, wells have also been reported to support pathogenic microorganisms. When factors are favourable, such as temperature and exposure to sunlight, microorganisms, once introduced into surface or ground water, have the ability to survive days or even months (Benjamin *et al.*, 2013). As confirmed by multiple reports, contaminated irrigation water has been the leading cause for multiple disease outbreaks over the last 20 years (Zhang *et al.*, 2009).

Furthermore, a report by Bernstein *et al.* (2007) stated microorganisms have the ability to adhere to plant roots when applied to soil via irrigation. Soil samples taken at a farming site responsible for producing spinach infected with *E. coli* O157:H7, confirmed that soil was able to harbour these pathogenic microorganisms for a undetermined period of time (Jay *et al.*, 2007).

Therefore, irrigating with microbially unacceptable water not only effects the crops being irrigated, but has the potential to contaminate the soil, effecting future crops planted in the contaminated soil.

Indicator microorganisms used to establish water quality

Water of poor microbial quality used for irrigation of crops has been associated with increased occurrence of pathogen microorganisms and foodborne disease outbreaks. This is largely because water has the potential to contain a large variety of microorganisms, as it is often exposed to human and livestock faecal contamination prior to irrigation (Parajuli *et al.*, 2009; Odonkor & Ampofo, 2013). Furthermore, water has the potential to transfer and distribute a large number of pathogens to many people in a relatively short period of time (Qadri *et al.*, 2015). It is therefore of utmost importance to monitor the microbial quality of water used for irrigation, by establishing confident estimates of microorganisms and specifically pathogens that are likely to be present (Odonkor & Ampofo, 2013). Testing for all the possible microorganisms present in a water sample will be an endless task associated with costly and lengthy testing procedures (Britz *et al*., 2013). Therefore 'indicator' or 'index' microorganisms have been identified and are tested for.

Firstly, the presence of an indicator microorganism suggests that water has been exposed to conditions that has increased the risk of it containing pathogenic microorganisms, granted that conditions are favourable for pathogenic growth (Savichtcheva & Okabe, 2006). Testing for these indicator microorganism is considered an accurate and well accepted method in determining water quality. It gives accurate insight in the potential presence of pathogens and the degree of microbial pollution in a water sample. Indicator microorganism often tested for include Total and Faecal Coliforms, *E. coli* and *Pseudomonas aeruginosa* (Savichtcheva & Okabe, 2006). Specifically *E. coli*, being a member of the Faecal Coliform group, can give a strong indication of faecal contamination and the possibility of pathogenic strains being present in the irrigation water (Savichtcheva & Okabe, 2006; Odonkor & Ampofo, 2013). Furthermore, the presence of *E. coli* may also be indicative of other pathogenic microorganisms present, for example *Salmonella* spp. Index microorganisms are similar to indicator microorganism. They vary because specific index microorganisms are tested for to quantify and to qualify the presence of specific individual pathogenic microorganisms, via specific models that have been established (Parajuli *et al.*, 2009). For example, *E. coli* can be tested for and be an index for *Salmonella* (Gemmell & Schmidt, 2013).

Escherichia coli

The Gram-negative, rod shaped bacterium, *E. coli*, forms part of the Faecal Coliform group (Ijabadeniyi & Buys, 2012; Odonkor & Ampofo, 2013; Teklehaimanot *et al.*, 2014). *E. coli* have been associated with faecal contamination, as they are predominantly found in the large intestine of warm blooded animals and don't easily reside outside these environments (Gemmell & Schmidt, 2013; Odonkor and Ampofo, 2013). Typically they are considered non-sporulating, facultative anaerobes that produce gas from the fermentation of nutrients in the intestine (Odonkor & Ampofo,

2013). As *E. coli* generally resides in the digestive tract of warm blooded animals, they can even prove to be beneficial to their host. They are able to produce nutrients that can be utilised by the host, as well prevent the growth of pathogenic bacteria within the digestive tract (Brennan *et al.*, 2010).

Testing for the presence of *E. coli* is a well-accepted, reliable and accurate indicator of possible pathogenic microorganisms that may be present in a body of water (Brennan *et al.*, 2010; Odonkor & Ampofo, 2013). There are however limitations when using *E. coli* as an indicator microorganism. Not all *E. coli* strains detected, when using standard methods, will be of a pathogenic nature and generally the quantified numbers of *E. coli* are much higher than the pathogenic strains present in a sample (Warriner, 2011). Alternatively, Faecal Coliforms as a collective microbial group are also used as indicators of possible faecal pollution. Not all microorganisms detected in the Faecal Coliform group will, however, be of a faecal origin (Odonkor & Ampofo, 2013). *E. coli* is thus considered to be a superior indicator of microorganisms when compared to the Faecal Coliform group (Brennan *et al.*, 2010).

When considering the pathogenic strains of *E. coli*, the well-known *E. coli* O157:H7 forming part of the (EHEC) group, has been well-documented and accepted as an illness causing *E. coli* (Kaper *et al.*, 2004; Gabriel, 2012). Six different groups have been established for the different pathogenic strains of *E. coli* (Table 2). The different groups vary in terms of the degree of severity of illness caused and the infection dose required to cause illness.

Table 2 Six different pathogenic microbial *E. coli* groups established, varying in terms of illness severity caused as well as infectious dose requirements (Kaper *et al*., 2004)

Pathogenic *E. coli* group	**Abbreviation**	**Infectious dose**	**Illness induced**
Enterotoxigenic	ETEC	$10^6 – 10^9$	Vomiting, diarrhoea, fever
Enteropathogenic	EPEC	$10^8 – 10^{10}$	Diarrhoea, pain
Enteroinvasive	EIEC	$10^6 – 10^{10}$	Bloody diarrhoea
Enterohemorrhagic	EHEC	> 100	Diarrhoea, vomiting
Enteroaggregative	EAEC	Unknown	Diarrhoea, pain
Diffusely Adherent	DAEC	Unknown	Diarrhoea

Controversially, some *E. coli* are believed not to be of a faecal origin, as they are able to exist in soil, provided temperatures are favourable for their survival. Benjamin *et al.* (2013) concluded that when using *E. coli* as an indicator for microorganisms to predict pathogenic strains that may be present in water, caution must be taken to ensure that this measurement is a reliable option (Odonkor & Ampofo, 2013). Alternative indicator microorganisms have been suggested to indicate faecal pollution. However, *E. coli* is still considered to be the superior indicator of faecal contamination, as it meets most of the requirements to be considered a good indictor microorganism (Britz *et al.*, 2012; Odonkor and Ampofo, 2013).

2.7 MICROBIAL SAFETY OF FRESH PRODUCE

As established, the presence of *E. coli* contamination on fresh produce is often associated with irrigation water being faecal contaminated (Pachepsky *et al.*, 2011). In order to establish whether or not irrigation water is safe to use, the absence of microorganisms of faecal origin is determined (Benjamin *et al.*, 2013). There have been multiple studies published dealing with reducing microbial contamination in river water, all with the end goal to prevent fresh produce causing illness in humans. *E. coli* has been identified as a major cause of multiple deaths after ingestion of contaminated crops (Warriner *et al.*, 2009).

There are various disinfection techniques that are available to reduce the initial load of microorganisms, however, variation of the efficiency of each treatment is highly dependent on the specific microbial species present. Because the individual efficacy of different disinfection treatment is highly dependent on the type and strain of microorganisms targeted, it is difficult to establish one specific treatment that will be effective across a broad range of microorganisms, whilst still ensuring significant reduction in number of pathogenic strains (Lee *et al.*, 2014).

South Africa, being a developing country, is strongly faced with the challenge of improving the microbial quality of fresh fruits and vegetables that reach the consumer, by implementing a plan of action that will ensure the safety of the consumer (Jung *et al.*, 2014).

2.8 ON-FARM DISINFECTION TREATMENTS FOR IRRIGATION WATER

The treatment of water intended to be used for irrigation purposes, for fresh or minimally processed crops, is of vital importance, as already highlighted. An approach in alleviating this concern is to ensure proper disinfection of irrigation water before it is used to irrigate the crops in question (Lewis Ivey & Miller, 2013; Van Haute *et al.*, 2013). Over the years various disinfection techniques have been used and tested, all with the common goal to reduce microbial loads present in water intended for irrigation purposes (Comninellis, 2008; Meneses *et al.*, 2010). There are a number of disinfection techniques available, each offering a unique set of advantages and disadvantages. Table 3 represents different disinfection treatments that have closely been associated to successful water treatment, each treatment assigned to one of the three main groups (Momba *et al.,* 2008; Yao *et al.*, 2012). Nevertheless, considerable variation regarding the disinfection efficacy of the different treatments, listed in Table 3, has been reported (Lubello *et al.*, 2002; Veschetti *et al.*, 2003; Koivunen & Heinonen-Tanski, 2005a; Al-Juboori *et al.*, 2015). A variety of factors have a considerable impact on these treatments (Table 3).

Understanding these efficacy altering factors is vital when selecting a suitable disinfection treatment (Montemayor *et al.*, 2008; Hallmich & Gehr, 2010). Unique advantages and limitations associated with each treatment, as well as varying physico-chemical and water quality parameters, must also be considered. According to Qadri *et al.* (2015) in order to understand the unique

properties of each of the different disinfection treatments, Table 3, each treatment should be critically evaluated in terms of advantages offered, as well as possible limitations.

Table 3 Three groups each presenting different treatments associated with effective water treatment (Momba *et al.,* 2008; Yao *et al.*, 2012)

PHYSICAL	CHEMICAL	PHOTOCHEMICAL
	Peracetic acid	
Sand filtration	Chlorine	Ultraviolet light (UV)
Biochar filtration	Hydrogen Peroxide	
	Bromine	
	Ozone	

2.9 PHYSICAL TREATMENT OF WATER INTENDED FOR IRRIGATION PURPOSE

Various physical filtration treatments can be applied to ultimately improve water quality (Table 3). These methods operate primarily by means of physical retention, or descriptively 'holding back' organic and inorganic matter that may be present in water (Schijven *et al.*, 2013). Filtration methods can rely solely on gravity, or alternatively mechanical intervention to promote the flow of water through the filter media, ultimately with the goal to improve water quality (Langenbach *et al.*, 2010; Gottinger *et al.*, 2011). Furthermore, filtration methods are unique, as they are not only effective in reducing the occurrence of microorganisms in water, but are also capable of retaining suspended solids, phenolic compounds, dyes and a variety of chemical compounds (Salgot *et al.*, 2002; Mukherjee *et al.*, 2007; Qiu *et al.*, 2009; Yu *et al.*, 2011). Employing filtration treatments hold potential in improving irrigation water quality, as better physico-chemical and microbial could be expected post-filtration. Although various filtration methods have been developed over the years, considerable variation has been shown to exist between the different types (Parish *et al.*, 2003). Evaluating different filtration methods, such as traditional slow bed and filtration to a more novel filtration concept such as biochar filtration, would provide a better overall understanding of the different potential advantages and limitations offered.

Gravity assisted slow bed sand filtration

In the middle 1900's slow sand filters had become a popular method used across the world by many municipalities in treating drinking water. Sand filtration, also referred to as slow bed sand filtration, has successfully been implemented as a treatment to remove suspended solids, ultimately improving the turbidity of water (Langenbach *et al.*, 2010; Gottinger *et al.*, 2011). The disinfection capabilities of slow bed sand filters were only fully realised a number of years after their successful application as filters improving water quality (Schijven *et al.*, 2013), by successfully removing pathogens from water (Gottinger *et al.*, 2011; Haig *et al.*, 2011; Hunter *et al.*, 2013). Because this filtration process incorporates a physical and biological aspect of filtration, it is

therefore able to improve filtered water on a physico-chemical and microbial level (Haig *et al.*, 2011). Slow bed sand filtration has been suggested to be the most simple and effective water treatment method available, given that the sand filters are fully functional (Langenbach *et al.*, 2010).

Mode of disinfection

As derived from the name, sand is predominantly the main filtration medium used in these filters, in combination with a gravel layer. Typically, water is directed to the top of the slow bed filter. Aided only by gravity, the water slowly passes through the sand and gravel layers (Figure 1) (Ludwig, 2013). The complete filtration process has been reported to take on average 3 – 15 h. However, filtration time is highly dependent on the initial quality of the water to be filtered, as well as the layout and design of the sand filter itself (Gottinger *et al.*, 2011). As the water passes between the sand particles, microorganisms as well as suspended solids present in the water are retained by the sand (Hunter *et al.*, 2013; Corral *et al.*, 2014). Salgot *et al.* (2002) reported successful removal of suspended solids and improvement of turbidity of water of up to 43% post-filtration. Additionally, the COD of the water was reportedly improved by 12.45%. Less success was, however, reported for Faecal Coliform removal, as only a reduction of 0.12 log cfu.100 mL^{-1} was reported by Salgot *et al.*, (2002). The type and quantities of retained organic and to a lesser extent inorganic matter, can be directly related to particle vs pore size of the sand grains used in each unique filter (Linlin, 2010).

Secondly, further decontamination of the water occurs through a biological process accredited to the formation of biologically active layers that are formed over the sand grains after a certain amount of operation time (Zheng & Dunets, 2014). These biological layers, also closely related to biofilms, are known as *schmutzdecke* (Schijven *et al.*, 2013). With increased operation time, suspended solids filtered out of the water by the filter start to accumulate within the filter. This accumulation of suspended solids is considered essential with regards to the formation of these biofilms or s*chmutzdecke*. The s*chmutzdecke* is primarily comprised of beneficial microorganism such as bacteria, fungi and protozoa (Langenbach *et al.*, 2010). These microorganisms tend to outcompete the water borne microorganisms and are ultimately responsible for disinfection properties attributed to the slow bed sand filtration systems (Willis *et al.*, 2011).

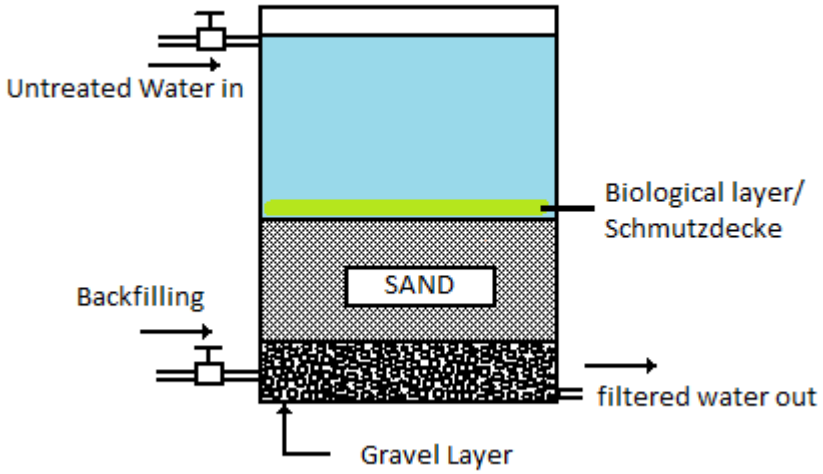

Figure 1 Simple graphical representation of a slow bed sand filter (Ludwig, 2013).

Advantages and limitations

As established, slow bed sand filtration has proven success by improving water quality and reducing the presence of microorganisms in water, especially the removal of pathogens. Furthermore, these filters have been reported to be capable of removing a variety of bacteria, viruses, (oo)cysts, algae and parasites, including those of a pathogenic nature (Langenbach *et al.*, 2010; Hunter *et al.*, 2013; Schijven *et al.*, 2013). However, nematodes have shown difficulty in being removed from irrigation water by sand filters.

Positively, no harmful chemicals are required to operate the filtration system, consequently limiting the environmental damage often caused by the formation of disinfection by-products (DBPs) associated with some chemical disinfection treatments (Doederer *et al.*, 2014; Sayyah & Mohamed, 2014; Zhang *et al.*, 2014). As relatively little expert experience is required to run and maintaining this filtration system, costs are kept low (Haig *et al.*, 2011). Traditionally there are multiple advantages surrounding the introduction of a sand filtration process in a water treatment plant. However, due to the variation of the pore sizes between the sand grains, it is recommended that filtration methods be used in combination with other treatments that exhibit better disinfection consistency. Viruses are generally less effectively removed by sand filters due to their relatively small molecular sizes, compared to that of other microorganisms (Hunter *et al.*, 2013). Parasites are also generally less easily removed by these filters. Due to the success of sand filtration systems, researches have recommended filtration as a pre-treatment step in combination with other treatments, such as chemical and UV irradiation to reduce costs and improve water quality (Corral *et al.*, 2014).

Biochar filtration

For many years carbonaceous materials have been used successfully as a sorbent for organic and inorganic substances present in water (Mukherjee *et al.*, 2007; Ahmad *et al.*, 2014). Currently activated carbon is widely used as a carbonaceous sorbent. However, there is an alternative. Biochar has been gaining increasing application as a successful sorbent (Ahmad *et al.*, 2014). Biochar is produced by pyrolysis of different carbon-rich, organic biomass materials (a large variety of plant materials have successfully been used) at different temperatures, in the absence or only in the presence of minimal levels of oxygen. This process of pyrolysis yields an end product with a fine, porous charcoal-like appearance (Hunt *et al.*, 2010; Ahmad *et al.*, 2014). Biochar has shown potential to successfully remove unwanted pollutants present in water and wastewater, both of an organic or inorganic nature (Mohan *et al.*, 2014; Tan *et al.*, 2015; Baltrenaite, 2016). Ultimately activated carbon and biochar are produced via similar processes of pyrolysis. However, biochar and activated carbon are different and should not be confused. Activated carbon, as the name suggests, is charred biomass (obtained from the pyrolysis of organic matter) enriched with oxygen. This enrichment process ultimately improves the sorbent properties of activated carbon, although being a costly process. The production of biochar, however, does not incorporate the process of oxygen enrichment (Tan *et al.*, 2015). The process of producing biochar allows for more Hydrogen and carbon to be 'fixed' and not expelled into the atmosphere when compared to activated carbon (Hale *et al.*, 2012; Mohan *et al.*, 2012).

However, due to a variety of factors, there can be considerable variation in the final composition of different biochar types (Chen *et al.*, 2008; Cui *et al.*, 2016). The selection of a pyrolysis temperature is important, as it will determine the sorbent characteristics displayed by the final biochar product. Furthermore, the original biomass used will also play a vital role in the final characteristics displayed by biochar (Chen *et al.*, 2008; Spokas and Reicosky, 2009). It has been found that certain biochar types have shown to be extremely effective in removing dyes, pesticides, chemicals, pharmaceuticals and phenolic compounds from water (Han *et al.*, 2013). Thus, the potential of retaining microorganisms by the biochar, even more so pathogens, should not be overlooked (Dempster *et al.*, 2012; Mohanty *et al.*, 2014).

Mode of disinfection/adsorption

The fine granular nature of biochar is advantageous when incorporated as a filter media, as it increases the surface area for improved adsorption characteristics (Anyika *et al.*, 2015). There are various adsorption mechanisms described for the possible retention of organic contaminants; these include electrostatic interactions, hydrophobic effect, Hydrogen bonds and possibly the most important, pore-filling, as displayed by Figure 2 (Tan *et al.*, 2015).

Varying surface properties are associated with different biochar variants (Mukherjee *et al.*, 2011). Ultimately the specific surface properties of biochar will have a direct influence on the

micropore surface area available, which directly determines the specific adsorption capabilities or surface pore filling abilities of the biochar (Han *et al.*, 2013). Variations in the micropore formation and adsorption abilities is due to different raw biomasses used, as well as the pyrolysis temperature selected to produce the biochar at (Hunt *et al.*, 2010; Han *et al.*, 2013). The high pore-filling affinity of biochar for organic matter is a direct result of the occurrence of pores found within the biochar due to its carbonaceous nature (Zhu *et al.*, 2014).

Lastly, adsorption properties of biochar are also affected by the rate at which pyrolysis takes place (Chen *et al.*, 2008). When pyrolysis is done rapidly it is referred to as fast pyrolysis. Alternatively biochar can be made by more a gradual heating process, known as slow pyrolysis (Bruun *et al.*, 2012; Baltrenaite, 2016). Biochar produced via the slow pyrolysis process is the more commonly used and accepted method when used as a sorbent in waste water treatment, and is generally the more favoured pyrolysis process (Han *et al.*, 2013; Mohan *et al.*, 2014). Temperatures ranging from 400 – 1200°C are typically required during the production process. Biochar types produced at higher pyrolysis temperatures generally display improved adsorption and retention capabilities. These improved adsorption properties are a direct result of increased formation of pores or cavities within the biochar at higher temperature (Alam *et al.*, 2009; Hunt *et al.*, 2010).

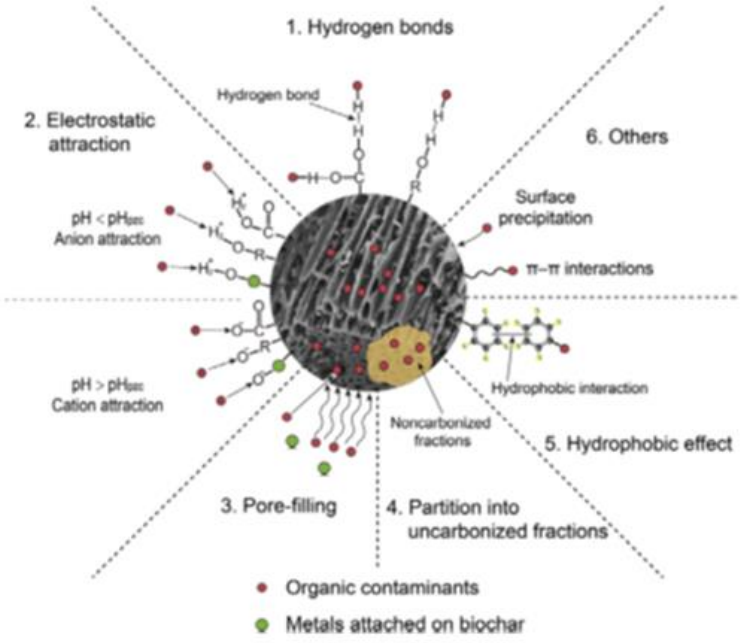

Figure 2 Possible adsorption mechanisms involved in the retention of organic and inorganic matter by a biochar particle (Tan *et al.*, 2015).

Advantages and limitations

Typically biochar can be made from any carbon-rich biomass, including all types and forms of wood, straw, leaves and even animal manure (Masters *et al.*, 2011; Ahmad *et al.*, 2012). However, making use of waste produced from agricultural practices makes sense in terms of economic and environmental sustainability (Chen *et al.*, 2008; Spokas and Reicosky, 2009). These waste materials include fruit tree branches, waste from wood and paper production and ultimately all forms of normally unusable agricultural by-products (Tan *et al.*, 2015). Through the process of pyrolysis, a large portion of the carbon of the organic materials is converted to a 'fixed' or better described, more stable form of carbon. The result thereof ultimately allows less greenhouse gasses to be released back into the atmosphere, compared to that released by the natural decaying of organic materials (Ahmad *et al.*, 2014).

The pyrolysis process can, however, be costly considering the price of electricity and the indirect impact on the environment when producing electricity and charring organic matter (Meyer *et al.*, 2011). Biochar types produced at higher temperatures display improved long term stability, when compared to low-temperature alternatives (Hale *et al.*, 2012). Negatively, higher pyrolysis temperatures will further add to the costs involved when producing effective biochar. In conclusion, although biochar filtration is a novel concept, it shows great potential as a feasible adsorbent for multiple pollutants that could be present in water, for both organic or inorganic nature pollutants (Dempster *et al.*, 2012; Mohan *et al.*, 2014; Tan *et al.*, 2015).

2.10 ULTRAVIOLET LIGHT IRRADIATION

Background

Forming part of the electromagnetic spectrum, ultraviolet (UV) irradiation produces wavelengths that can be classed between x-rays and visible light with a range from 100 – 400 nm (Fig. 3) (Koutchma, 2009). UV light is further divided into four different spectral groups, namely UV produced under vacuum, UV-C, UV-B and UV-A classified according to the wavelengths 100 – 200, 200 – 280, 280 – 315 an 315 – 400 nm respectively (Newman, 2004; Dai *et al*., 2012). Although the different groupings vary considerably with regard to germicidal action, UV-C wavelengths (200 – 280 nm) are considered most effective concerning microbial inactivation/reduction.

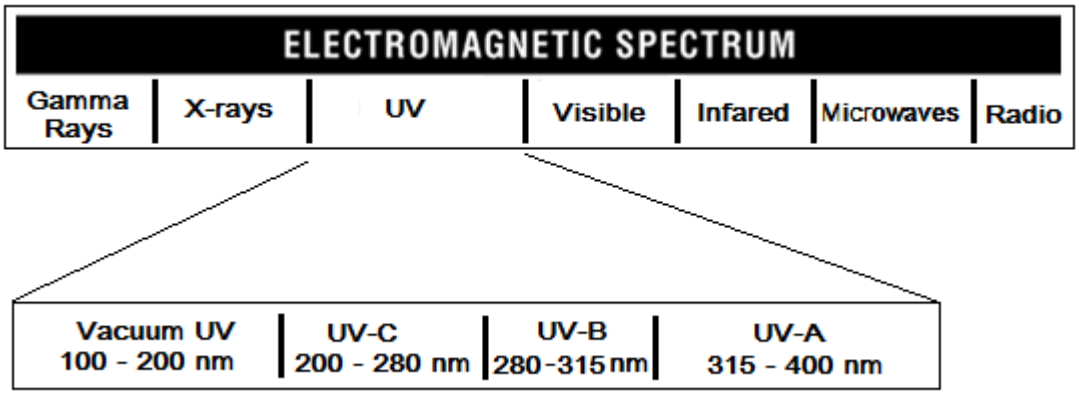

Figure 3 Ultraviolet light forming part of the electromagnetic spectrum with the different sub-groupings (USEPA, 2006).

UV light was first discovered to have disinfection properties in the late 1800's (Bolton & Cotton, 2008a). Throughout the years, UV light irradiation has become a well-accepted, multipurpose disinfection treatment (Gayán *et al*., 2014; Abdul-halim & Davey, 2016). In general UV irradiation is classed as a non-thermal disinfection treatment that alters the normal functioning of microbial cells (Koutchma, 2009). UV irradiation is considered effective in eliminating a large variety of microorganisms, including those of a pathogenic nature. These include enteric bacteria, viruses, moulds, yeast, fungi as well as *Cryptosporidium* and *Giardia*, which form part of the protozoa group (Koutchma, 2009). However, not all microorganisms display similar sensitivity towards UV irradiation, as greater UV-resistance is displayed by bacterial spores and viruses, especially Adeno-viruses and parasitic cysts, such as protozoon *Acanthamoeba* (Caretti & Lubello, 2003; Koivunen & Heinonen-Tanski, 2005; Hijnen *et al.*, 2006; Gayán *et al.*, 2014). Because of the disinfection abilities induced by UV irradiation exposure, it has become a well-accepted treatment used in the water industry (Poepping *et al.*, 2014).

Modes of disinfection

Primarily, microbial disinfection induced by UV irradiation is based on the belief that different cellular components have the potential to absorb UV light through photochemical processes (Bolton & Linden, 2003; Koutchma, 2009). Upon UV light exposure complex mechanisms are involved, ultimately responsible for the damage and destruction of vital biochemical processes (Bolton & Linden, 2003; Freese & Nozaic, 2004; Koutchman, 2009a). These mechanism, however, can be considered multifaceted, as extensive research in often complex photochemical processes will be necessary to gain a complete understanding of their functioning. Ultimately, although multiple cellular components are effected by UV irradiation, genetic material damage and alteration is highlighted as UV light induced damage (Bolton & Linden, 2003; Gayán *et al*., 2014; Premi *et al*., 2015).

When referring to the photochemistry, understanding that light energy is transferred to microbial cells as photons, thus placing the receiving molecules in an excited state, is of core importance (Gayán *et al*., 2014). There is a wide spectral range involved when referring to photochemistry (100 – 1 000 nm), however, UV wavelengths are specifically focused on the range of 100 – 400 nm (Koutchma, 2009), which are further divided into UV-A, UV-B, UV-C and UV produced under vacuum (Newman, 2004; Dai *et al*., 2012). Although the different sub-groups all fall within the UV electromagnetic spectrum, different chemical reactions have been documented for each of the different groupings. Focus has been placed on specifically UV-C wavelengths, as they are considered the most germicidal wavelengths of the UV spectrum (Bolton & Linden, 2003; Koutchma, 2009). Optimal absorption of UV light by genetic material of microorganisms is suggested to occur at the wavelengths emitted by the UV-C wavelengths (200 – 280 nm). Irrespective of that the UV-C light is considered the optimal wavelength range to cause a germicidal effect. Its efficacy is, however, still greatly dependent on the type of microorganisms predominately present in the body of water. Factors such as UV emission, transmission and absorption must also be considered, as these may have a direct influence on the overall efficacy of UV to cause a germinal effect (Gayán *et al*., 2011; Abdul-halim & Davey, 2016).

Wavelengths are absorbed by microorganisms as photons, which possess the ability to cause oxidative damage via a process, often referred to as photolysis. (Bolton & Linden, 2003; Koutchma, 2009). Optimal photon absorption by the nucleotide bases (genetic material) of microorganisms occurs in the germicidal range 254 – 260 nm. At lower UV wavelengths (< 230 nm) poorer disinfection is often observed due to water's ability to absorb UV light at lower UV doses. Furthermore, microorganisms' proteins are generally more effected at UV wavelengths < 230 nm, and to a lesser extent the genetic material (Bolton & Linden, 2008c; Koutchman, 2009a). Therefore, higher UV doses (>230 nm) will be required at lower UV wavelengths (UV-C) to enable successful disinfection (Fig. 4).

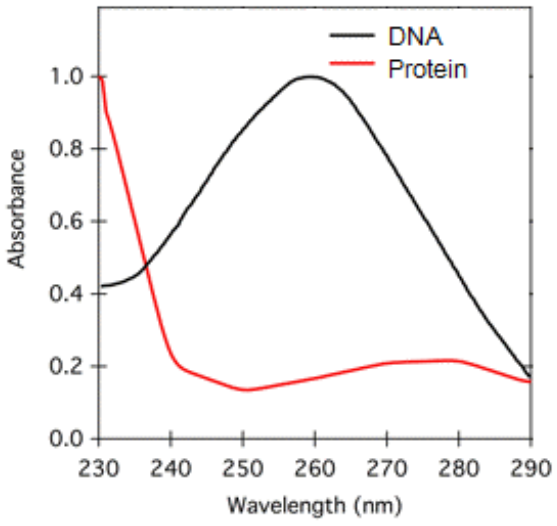

Figure 4 Wavelengths responsible for optimal UV absorption for proteins and nucleotides.

UV irradiation on microorganisms

Specific mechanisms are involved in damaging and ultimately the inactivation of microorganisms. These specific photochemical mechanisms, referred to as the process of photolysis, act both on microbial DNA and RNA, as well as cellular components such as proteins (Gayán *et al*., 2014). Placing focus on the destruction of DNA and RNA through the absorption of UV light, in the forms of photons, is of great importance. UV irradiation is responsible for the disruption of microbial genetic material at wavelengths > 210 nm, as sugar and phosphate molecules do not effectively absorb UV light above those wavelengths (Newman, 2004; Bolton & Linden, 2008c; Koutchman, 2009a). As mentioned, UV-C wavelengths are considered the most germicidal wavelengths, although affected nucleotides are not only limited to the wavelengths produced in the UV-C region (Newman, 2004; Gayán *et al*., 2011; Abdul-halim & Davey, 2016).

Nevertheless, pyrimidine and purine nucleic acid bases easily absorb photons produced within the UV-C wavelength region, resulting in the formation of photoproducts (Bolton & Linden, 2003; Poepping et al., 2014). Pyrimidines nucleic acids are comprised of thymine and cytosine and alternatively purine nucleic acids including adenine and guanine (Bolton, 2003; Rodriguez e*t al*., 2014). Upon absorption of photons by these nucleic acid bases the formation of photoproducts is initiated (Bolton & Linden, 2003; Gayán *et al*., 2014; Premi *et al*., 2015). Photoproducts have been directly linked to impaired microbial replication, associated with the inability of pathogens to cause illness in humans, ultimately causing cell death (Bolton, 2003; Rodriguez *et al*., 2014). Increased formation of photoproducts have been associated with pyrimidine bases in comparison to purine bases. Pyrimidine bases are therefore of greater importance when understanding the disinfection mechanism of UV irradiation.

The two main photoproducts formed by pyrimidine nucleic acids are cyclobutane pyrimidine dimers (CPDs) (Fig. 5) and pyrimidine 6 – 4 pyrimidone photoproducts (6-4PPs) (Bolton & Linden, 2003; Poepping *et al*., 2014). Although the dominant photoproduct responsible form nearly 75% of microbial DNA, damage during UV irradiation is through the action of CPDs, both photoproducts are of importance as both result in cell damage and ultimately cell death (Rastogi *et al*., 2010; Rodriguez *et al*., 2014; Premi *et al*., 2015).

CPD photoproducts are formed when two adjacent pyrimidines become covalently linked. This linking process is initiated when the carbon number 5 (C-5) and carbon number 6 (C-6) atoms of two opposite pyrimidines become saturated by UV waves (photons) (Rastogi *et al*., 2010). Furthermore, the formation of CPDs can be formed between two thymine bases, two cytosine bases or between cytosine and thymine bases. However, dimers containing thymine are proposed to be the dominant types formed by CPDs (Eischeid & Linden, 2007; Rastogi *et al*., 2010; Gayán *et al*., 2014).

Regarding the formation of 6-4PP photoproducts, a similar process is followed, although the additional formation of adducts must be considered. The formation of 6-4PPs can ultimately be ascribed to the formation of unstable oxetane and azetidine products. These products are further

rearranged by the transfer of a carboxyle or amino group. This process of rearrangement is thus responsible for the formation of 6-4PPs. Controversial to the formation of CPDs, 6-4PPs are formed more frequently between two cytosine bases and also between a cytosine and thymine base, much less frequently formed between two thymine bases (Rastogi *et al*., 2010; Douki, 2013; Gayán *et al*., 2014).

A third photoproduct of significance, namely spore photoproducts (SPs), (Fig. 5) affect bacterial spores when exposed to UV-C light (Wang *et al*., 2011). SPs are produced when a methyl group of a thymine base attaches to a carbon on an adjacent thymine base. Spore photoproducts lead to similar cellular disruptions as those induced by CPDs and 6-4PPs, ultimately affecting replication (Rastogi *et al*., 2010; Wang *et al*., 2011; Gayán *et al*., 2014). Similar mechanisms that are responsible for causing disruptions in the DNA are also responsible for causing RNA damage, the main difference being thymine bases being replaced by uracil.

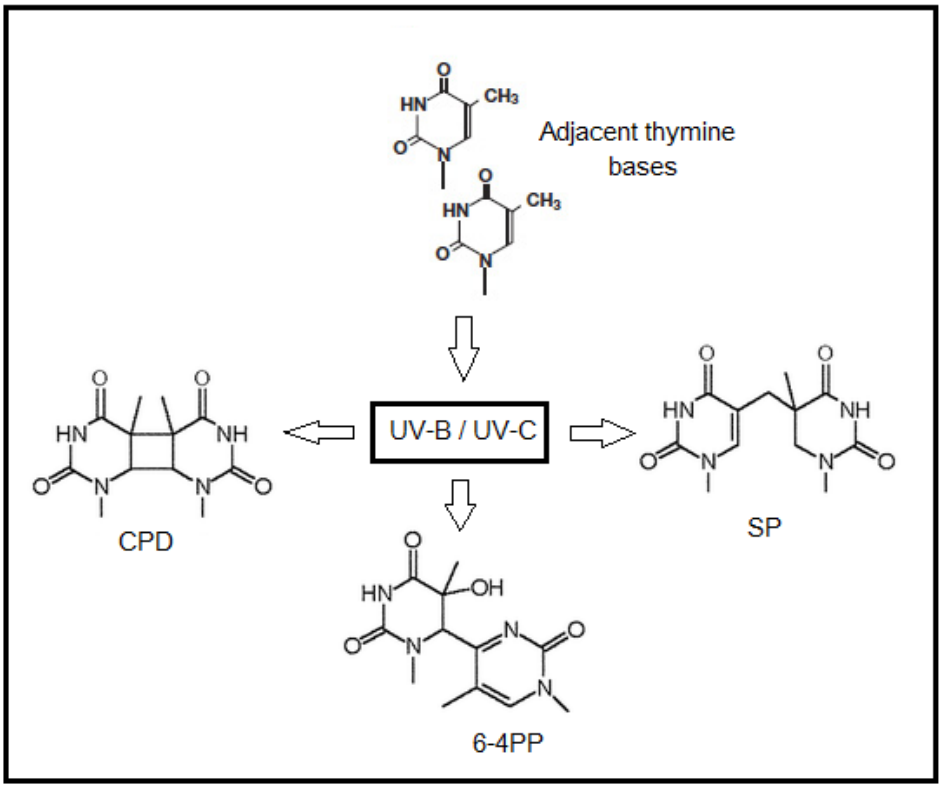

Figure 5 Photoproducts formation due to UV irradiation exposure (Bolton & Linden, 2003).

UV disinfection apparatus

Generally UV disinfection apparatus can be either in the form of open-channel or a closed-pipe systems, making use of mercury vapour lamps emitting light at either a low or high intensity, described as low-pressure (LP) or medium-pressure (MP) respectively (Bolton & Linden, 2003; Bolton & Cotton, 2008d; Koutchma *et al*., 2009b; Howe *et al*., 2012). Closed-pipe systems are described as disinfection units that are placed directly into pipelines responsible for carrying water to be disinfected (Bolton & Cotton, 2008d). There are generally three different closed-pipe lines

configurations used, namely single-lamp annular, multi-lamps parallel to water follow or multi-lamps perpendicular to flow (Bolton & Cotton, 2008d). These closed-pipe systems are usually employed for the treatments on drinking water for small and large scale purposes. Open-channel systems primarily make use of mercury lamps that are placed perpendicular to the flow of the water. Channels directing the flow of water can vary in shape. These UV systems are more often associated with the disinfection of wastewater (Bolton & Cotton, 2008d).

Apropos of the LP and MP-mercury vapour lamps used in the different disinfection apparatus, both lamps effectively have the ability to cause a germicidal effect (Eischeid & Linden, 2007; Sakai *et al*., 2007; Gayán *et al*., 2014). UV light is produced by LP-UV lamps, predominantly emitting 85% of their wavelengths at 253.7 nm, is considered to be the most germicidal wavelength (Bolton & Linden, 2003). Secondly, MP-UV lamps emit a much broader range of wavelengths, producing UV-B and UV-C light waves ranges (Eischeid & Linden, 2007; Guo *et al*., 2009). LP-UV disinfection systems are more often used in a laboratory environment, as the apparatus are generally smaller and more easily transported. MP-UV systems are associated with larger scale disinfect plants where water is passed over the lamps, with the lamps perpendicular to the water flow effectively treating large volumes of water. Hu *et al*. (2005) found that at the same UV dose MP-UV irradiation showed less reactivation potential compared to the alternative LP-UV irradiation. This was supported by Guo *et al.* (2009), showing that *E. coli* exposed to LP-UV irradiation produced significantly greater potential for regrowth compared to those exposed to MP-V irradiation. Furthermore, Guo *et al.* (2009) reported that a UV dose of 40 mJ.cm-2 was adequate to significantly reduce Coliforms in water, regardless if MP- or LP-UV lamps were used.

Influences on UV efficacy and microbial inactivation

There are several factors that may influence the efficacy of UV irradiation with regards to pathogen reduction. UV transmittance, turbidity and the presence of suspended solids are considered important optical characteristics that may influence the overall efficacy of UV irradiation for water treatment (Gayán *et al*., 2011; Abdul-halim & Davey, 2016). UV lethality towards microorganisms is greatly reduced when these water characteristics are considered less than adequate. Unfavourable water characteristics are responsible for reflecting, absorbing and scattering UV light waves, ultimately reducing the amount of UV light reaching the target microorganisms within the water.

Gilboa & Friedler (2008) suggested that with the increased occurrence of suspended solids in water, reduced UV disinfection efficacy could be expected, as suspended solids act to shield microorganisms from UV irradiation. Other water quality parameters that also have an effect on UV irradiation efficacy include the COD (Chemical Oxygen Demand) and electrical conductivity (EC) of the water to be treated. Wastewater with a COD range of 32 – 60 $mg.L^{-1}$ produced substantially better bacterial reduction after UV irradiation than wastewater with a COD range of 61 – 148 $mg.L^{-1}$ (Gilboa & Friedler, 2008). Freese & Nozaic (2004) reported that water with a COD

of 27 mg.L^{-1} exposed to a UV dose of between 40 – 70 mJ.cm^{-2} was able to produce an adequate 2 – 3 log reduction in indicator microorganisms.

Due to a variety of factors, both direct and indirect, microorganisms have shown differences in sensitivity towards UV irradiation. Therefore, selecting a single UV dose can be challenging when considering the variations of microorganisms that are likely to be present within a body of water (Hijnen *et al.*, 2006; Santos *et al.*, 2013). Caretti & Lubello. (2003) showed that there was definite variation in disinfection recorded among different microorganisms at a single UV dose. UV dose-response curves can help when selecting a UV dose, as they display the variations in sensitivity of different microorganisms to a specific UV dose.

Hijnen *et al.* (2006) reported adequate microbial reductions were attained at a UV dose of < 20 mJ.cm^{-2}, producing a bacterial reduction of 3 log. Koivunen & Heinonen-Tanski (2005a) noted less effective reductions of *Escherichia coli (E. coli)* at a UV does of 14 mJ.cm^{-2}, achieving a log reduction of 1.44 log. Furthermore, there was a significantly better disinfection observed at a UV dose of 14 mJ.cm^{-2} compared to that at 10 mJ.cm^{-2}, suggesting improved microbial reductions were possible at increased UV doses (Olivier, 2015).

Although UV irradiations efficacy as a disinfectant treatment is strongest linked to the ability of UV light to penetrate a body of water, there are, however, biological aspects that can also play a contributing role in the lethality expressed by UV irradiation (Gayán *et al.,* 2011; Wang & Xu, 2012; Abdul-halim & Davey, 2016). Researchers have found that photo reactivation and dark-repair of microorganisms post-UV disinfection, could also be of concern by essentially reducing the efficacy and reliably of UV irradiation as a disinfection treatment (Hu *et al.,* 2005; Albarracín *et al*., 2014).

DNA repair mechanisms

When evaluating the true disinfection capabilities of UV irradiation as a whole, repair mechanisms employed by microorganisms once exposed to UV light can be of significance. Microbial DNA can be repaired by one of two main mechanisms, namely (1) reverse damage repair and (2) excision repair. Both of these repair mechanisms are employed before replication takes place within the cell and are therefore considered to be accurate in repairing or replacing damaged genetic material (Guo *et al*., 2011; Gayán *et al*., 2014; Kneuttinger *et al*., 2014).

Reverse damage repair is carried out by DNA lyases, which are responsible for the repair of damaged genetic material without the synthesis of new DNA. There are broadly two types of DNA lyases responsible for this repair, namely CPD lyases and SP lyases (Kneuttinger *et al*., 2014; Premi *et al*., 2015). CPD lyases, also known as photolyases, are responsible for the phenomena referred to as photo-repair or photo reactivation within bacterial cells. These enzymes make use of visible light falling in the wavelengths of 350 – 500 nm in order to reverse the damage induced, specifically by CPD photoproducts (Rastogi *et al*., 2010; Gayán *et al*., 2014). Alternatively, SP lyases do not require visible light and this enzyme is responsible for the epair in spores upon spore germination. It has been shown that there has however been effective removal

of spores using UV irradiation, regardless of repair mechanisms and this was ascribed to the effective damage induced by UV irradiation on the bacterial DNA (Panitz *et al*., 2015).

Secondly, excision repair mechanisms are considered error-free, as they function by replacing damaged DNA by synthesising new nucleotides using the parental strand as a template (Rastogi *et al.*, 2010; Douki, 2013; Premi *et al*., 2015). This process is also referred to as dark-repair, as repair is not dependent on the availability of visible light. When considering microorganisms have the ability to repair damage induced by UV irradiation, the potential of unsatisfactory UV doses leading to undesirable microbial reduction is imminent. Furthermore, prolonged exposure to sunlight and increased storage times post-UV irradiation can add to this potential hurdle when using UV irradiation in the disinfection of water.

Guo *et al*. (2009) reported regrowth of *E. coli* after the exposure of both to LP and MP- UV light irradiation. Hu *et al*. (2005) showed that regardless of the UV lamp used (MP or LP) each allowed the regrowth of microorganisms after exposure to UV irradiation. When considering the time that is required for regrowth to take place, Gilboa & Friedler (2008) suggested that the majority of reactivation of microorganisms took place in the first 3 h post-UV exposure, thereafter regrowth took place at a much slower rate.

2.11 CHEMICAL DISINFECTION TREATMENTS

Peracetic acid

Background

As early as in the 1950's research has shown the effectiveness of PAA as a disinfectant for a variety of microorganisms, including bacteria and fungi (Kitis, 2004). Peracetic acid (PAA) has successfully been implemented as a disinfectant in the food and beverage industries for a number of years (Kitis, 2004; Rasimus *et al.*, 2011; Luukkonen *et al.*, 2014). Recently its application as an effective water disinfectant has been gaining attention, largely due to its antimicrobial activity displayed towards a large variety of microorganisms (Gehr *et al.*, 2003; Rossi *et al.*, 2007).

Current research done has proved PAA to be effective in reducing indicator microorganisms present in wastewater, thus satisfying safety guidelines regarding microbial levels for irrigation water (Gehr *et al.*, 2003; Veschetti *et al.*, 2003; Koivunen & Heinonen-Tanski, 2005a; Rossi *et al.*, 2007). Considering PAA as an alternative to chlorine (Cl) disinfection Veschetti *et al.* (2003) reported comparable microbial reductions between the two chemicals, highlighting the ability of PAA to be an effective alternative disinfectant. Furthermore, PAA showed adequate disinfection, even when dosed at low concentrations and allowing short contact times (Veschetti *et al.*, 2003; Rossi *et al.*, 2007). Microorganisms that were similarly affected by PAA when compared to Cl disinfection included Faecal Coliforms (FC), Total Coliforms (TC), *Escherichia coli* (*E. coli)*, *Salmonella* spp. and *Pseudomonnas* spp. (Veschetti *et al.,* 2003). Gehr *et al.* (2003) reported that low concentrations of PAA, of no more than 4 $mg.L^{-1}$, were able to reduce initial microbial levels (4

– 5 log cfu.100 mL) present in municipal wastewater, to the recommended guideline for FC (1 000 cfu.100 mL^{-1}) in less than 1 hour (DWAF, 1996). Furthermore, Koivunen & Heinonen-Tanski (2005b) reported PAA at a concentration of 3 $mg.L^{-1}$ and was able to produce a 2 – 3 log reduction for *E. coli*, thus reinforcing PAA as an effective water disinfectant. A study completed by Mezzanotte *et al.* (2007) found that PAA at a concentration of 15 $mg.L^{-1}$ was able to successfully reduce initial microbial levels of 4.5 log to < 1 log, whilst maintaining a contact time of 36 min for TC, FC as well as *E. coli*. A pilot-scale study completed by Caretti & Lubello (2003) reported on PAA dosed at concentrations 2, 4 and 8 $mg.L^{-1}$ allowing contact times of 10, 20 and 30 min. Poorest log reductions were reported for all microbial groups evaluated when dosing with PAA at a concentration of 2 $mg.L^{-1}$ for a contact time of 10 min (Caretti & Lubello, 2003). However, increasing the concentration of PAA, together with longer contact times, revealed improved microbial reductions for TC, FC and *E. coli* (Caretti & Lubello, 2003). When allowing a 30 min contact time, PAA (8 $mg.L^{-1}$) was successful in reducing initial microbial levels by 3.99, 4.21 and 4.42 log for TC, FC and *E. coli* respectively (Caretti & Lubello (2003).

Mode of action

PAA is considered a strong water soluble oxidant, capable of disinfecting a large variety of microorganisms (Kitis, 2004). Research has suggested it to possess greater oxidising potential than alternative chemical disinfectants such a Cl, thus establishing itself as a disinfectant in the water industry (Rossi *et al.*, 2007). In general commercially available PAA is considered a clear liquid solution, comprised of acetic acid, peracetic acid and hydrogen peroxide. Figure 6 displays the quaternary equilibrium mixture of commercially available PAA solution (Gehr *et al.*, 2003; Lubello *et al.*, 2002; Kitis, 2004; Luukkonen *et al.*, 2014):

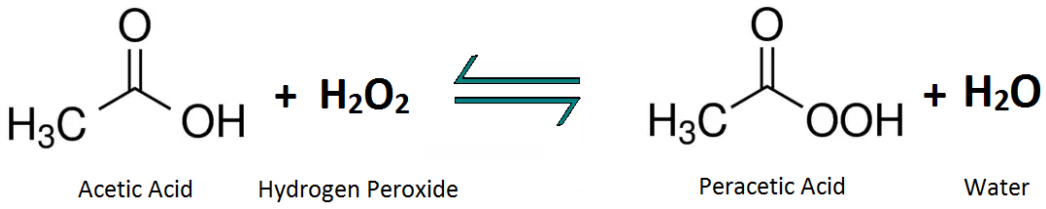

Figure 6 Quaternary equilibrium composition of commercially available PAA in solution.

Commercial PAA solutions, however, seldom exceed a PAA concentration of 15% ($m.v^{-1}$), as higher concentrations have been reported to be instable and is considered unsafe to handle (Luukkonen *et al.*, 2014). Other chemicals present in the PAA solution, such as H_2O_2, can also contribute to the anti-microbial action of commercially available PAA solutions (Higashi *et al.*, 2005). Regarding the disinfection capabilities, bacterial species exhibit least resistance to PAA disinfection when compared to viruses, bacterial spore and protozoan cysts, all of which show

increased resistance to PAA disinfection (Stampi *et al.*, 2002; Koivunen & Heinonen-Tanski, 2005a; Park *et al.*, 2014). Although the exact disinfection mechanism of PAA is not yet fully understood, it is suggested that germicidal action is induced in a similar way as most other oxidising agents.

PAA is suggested to affect the normal functioning of the lipoproteins in the cytoplasmic membrane of microorganisms, thus inducing compromised transport across membranes due to damaged cell walls, ultimately leading to cell death (Gehr *et al.*, 2003; Russell, 2003; Kitis, 2004). Furthermore, these damaging effects are not only limited to Gram-positive microorganisms which possess lipoprotein membranes, but also effect the outer membrane lipoproteins of Gram-negative microorganisms (Leaper, 1984; Kitis, 2004). Additionally intercellular PAA exposure induces enzyme denaturation, consequently impairing the functioning of important biochemical pathways within microbial cells (Gehr *et al.*, 2003; Lenntech, 2014).

Furthermore, catalase have been associated with removing or nullifying the effects of Hydroxyl Radicals (•OH), responsible for inducing a germicidal effect on microorganisms (Kitis, 2004; Wagner *et al.*, 2014). Thus, the ability of PAA to inactivate catalase within microbial cells is greatly advantageous for microbial inactivation. Lastly, the genetic material of microorganisms are also suggested to be affected by PAA, often resulting in improper transcription and translation, leading to cell mutations or death (Kitis, 2004).

Factors influencing disinfection efficacy

With regards to water quality certain physico-chemical parameters have shown to be influential regarding the disinfection potential of PAA (Lubello *et al.*, 2002; Koivunen & Heinonen-Tanski, 2005b; Olivier, 2015). Optimal PAA disinfection occurs in solutions at pH < 9, as at increased pH PAA easily dissociated (Block, 1991; Kitis, 2004). PAA in the undissociated form (CH_3COOOH) is considered more effective inducing microbial damage than in the dissociated form (CH_3COOO^-) (Kitis, 2004). Regardless thereof, variations in pH are not considered to have a major implication on PAA disinfection abilities (Block, 1991).

Research has suggested that high volumes of organic matter (related to TSS and COD) have shown to ultimately reduce the overall disinfection efficacy of PAA (Gehr *et al.*, 2003; Koivunen & Heinonen-Tanski, 2005a; González *et al.*, 2012). Regarding COD, Caretti & Lubello (2003) highlighted that elevated COD levels ($\geq$ 75 $mg.L^{-1}$) required substantially higher PAA doses to successfully remove bacteria present in water (500 $mg.L^{-1}$ PAA with a contact time of 30 min). González *et al.* (2012) also reported increases in COD were met by a decrease in residual PAA, indicating decreased disinfection potential at increased COD levels. Therefore, considering pre-treatment methods prior to PAA disinfection is recommended. Pre-treatments such as filtration, reduce the initial levels of suspended solids, aiming to produce more consistent and reliable disinfection (Luukkonen *et al.*, 2014). With regard to water temperature PAA has proven to be effective over a broad range of temperature (< 100°C), even proving more effective than

alternative chemical disinfections at increased temperatures such as chlorine (Cl) (Stampi *et al.*, 2002).

Advantages and disadvantages

Studies have suggested the use of PAA over other chemical disinfectants, such as Cl, when aiming to avoid the formation of harmful disinfectant by-products (DBPs) (Veschetti *et al.*, 2003; Kitis, 2004; Koivunen & Heinonen-Tanski, 2005b). PAA decomposes to form acetic acid, oxygen and water, considered non-toxic (Wagner *et al.*, 2002). However, the potential formation of DBPs when using PAA as a disinfectant should not be completely ruled out (Zanetti *et al.*, 2007). The formation of carboxylic acids, which can be regarded as a form of DBP, have been documented when using PAA as a disinfectant. These carboxylic acids formed, however, are not considered toxic or dangerous compounds (Monarca, 2001). Isolated reports have suggested the formation of potential toxic by-products, but only when using feasibly unsound doses of PAA.

Although the formation of DBP is minimal, PAA has proven to be successful at low concentrations, thus further reducing the potential of DBPs formation (Kitis, 2004). Therefore, when considering PAA as an alternative to Cl disinfection positively, Freese & Nozaic (2004) reported similar disinfection capabilities for PAA compared to that of Cl. Furthermore, Veschetti *et al.* (2003) stated that similar disinfection efficacy can be observed between sodium hypochlorite and PAA for *E. coli*, *Salmonella* spp. and *Pseudomonas* spp., highlighting the feasibility of PAA to be used as an alternative to the traditionally used chlorine treatments. Adding to its ease of use PAA generally shows low levels of degradation over time, granted it is kept in solution and not in a diluted form (Freese & Nozaic, 2004).

Some negative aspects have been linked to the use of PAA. As PAA readily decomposes, forming Acetic Acid, although considered non-toxic, can contribute directly to the increase of organic matter present in water (Wagner *et al.*, 2002; Kitis, 2004). Acetic acid specifically has been reported to lead to the increase in COD (Monarac, 2002). These organic compounds formed can also serve as a growth medium, promoting the regrowth of microorganisms (Zanetti *et al.*, 2007). Furthermore, organic molecules provide a shield-like protection for microorganisms against disinfection treatments (Kitis, 2004; Koivunen & Heinonen-Tanski, 2005a). Acetic acid is also less effective in controlling microorganisms post-treatment, further promoting the regrowth of microorganisms (Kitis, 2004; Zanetti *et al.*, 2007), thus, ensuring adequate residual levels in water could act as a preventative measure against the regrowth of microorganisms after disinfection (Kitis, 2004).

Increased cost have been associated with high doses of PAA used for water disinfection purposes (Freese & Nozaic, 2004). However, lower chemical concentrations generally produce satisfying disinfection results (Kitis, 2004). Low dose requirements can also provide an explanation as to why less DBPs have been detected using PAA disinfection, compared to that of other chemical treatments (Monarca *et al.*, 2004; Koivunen & Heinonen-Tanski, 2005b; Zanetti *et*

al., 2007; Luukkonen *et al.*, 2014).

Overall, PAA as a chemical disinfection, even when dosed at low concentrations together with short contact times, has successfully been employed as a disinfectant in the water industry, producing minimal DBPs (Caretti & Lubello, 2003; Gehr *et al.*, 2003; Veschetti *et al.*, 2003; Koivunen & Heinonen-Tanski, 2005b; Bester, 2015).

Hydrogen peroxide

Background

Hydrogen peroxide is known as a versatile disinfectant with a high oxidation potential. It is composed of Hydrogen and oxygen atoms bound together with single bonds to form the molecule H_2O_2 (Newman, 2004). Over the years H_2O_2 has found multiple applications in industries proving effective in controlling odours, colours, taste as well as corrosion (Ksibi, 2006; Labas *et al.*, 2008). H_2O_2 has proved to be effective in controlling a large variety of microorganisms, specifically those of a pathogenic nature that may be present in irrigation water (Newman, 2004; Koivunen & Heinonen-Tanski, 2005a; Sherchan *et al.*, 2014). Although variations of H_2O_2 solutions are available, they generally are able to control bacteria, viruses, fungi, yeasts, as well as algae (Newman, 2004). Sherchan *et al.* (2014), however, reported poor disinfection of viruses when using H_2O_2. Adding to its versatility it has found successful application as a stand-alone oxidant, as well as in combination with other treatments (Lubello *et al.*, 2002; Koivunen & Heinonen-Tanski, 2005a; Linley *et al.*, 2012b; Vargas *et al.*, 2013; Sherchan *et al.*, 2014b, Olivier, 2015). Furthermore, it has proven its effectiveness at improving water quality by reducing the COD and biochemical oxygen demand (BOD) (Ksibi, 2006). As H_2O_2 has successfully been implemented in the water and wastewater industries (Ksibi, 2006; Vargas *et al.*, 2013) its potential as an effective alternative disinfectant has been brought under investigation due to advantages that it might offer (Koivunen & Heinonen-Tanski, 2005a; Vargas *et al.*, 2013, Olivier 2015). Ksibi (2006) suggested that H_2O_2 was a strong enough oxidiser of organic matter, at a concentration of 2.5 $mg.L^{-1}$, to achieve sufficient removal of organic matter. Ksibi (2006) reported 3 log FC reductions after allowing a 2 h contact time using H_2O_2 at a low concentration of 2.5 $mg.L^{-1}$. Furthermore, effective COD reductions from 322 $mg.L^{-1}$ to 44 $mg.L^{-1}$ were also reported after a 2 h contact time and a H_2O_2 concentration of 2.5 $mg.L^{-1}$ (Ksibi, 2006). Giddey *et al.* (2015) reported that increasing concentrations of H_2O_2 were met with increased microbial inactivation, when using environmental and reference *E. coli* strains. Furthermore Giddey *et al.* (2015) suggested that environmental *E. coli* strains displayed increased resistance compared to the standard reference *E.coli* strains used in the study. Ronen *et al.* (2010) reported H_2O_2 concentrations of 125 $mg.L^{-1}$ were required to reduce 99% of faecal indicator microorganisms, with a 56 min contact time. Being such a versatile disinfectant, applying H_2O_2 in combination with another disinfection method could provide a suitable option if unsatisfactory disinfection is achieved by only using H_2O_2 (Vargas *et al.*, 2013).

Mode of action

H_2O_2 functions is a strong oxidising agent, as the bonds holding the two oxygen atoms together in the molecule are easily broken, consequently releasing Hydroxyl Radicals (•OH) (Raffellini *et al.*, 2011). To a lesser degree, the formation of SuperOxide (O_2^-) molecules have also been reported. However, they are considered much less damaging (Labas *et al.*, 2008; Linley *et al.*, 2012a; Vargas *et al.*, 2013). H_2O_2 acts directly on organic matter, consequently affecting microorganisms as well (Lenntech, 2014). The Hydroxyl Radicals are directly responsible for inducing microbial inactivation, by targeting cellular components (Linley *et al.*, 2012a; Vargas *et al.*, 2013). These molecules affect the cell membranes, lipids and proteins of microorganisms, often leading to abnormal cell structure and functioning. They are also able to induce damage to the DNA of the microorganisms, ultimately inhibiting transcription and translation leading to mutagenesis and cell death (Labas *et al.*, 2008; Raffellini *et al.*, 2011; Linley *et al.*, 2012b; Vargas *et al.*, 2013). H_2O_2 has proven effective, reducing Gram-positive as well as Gram-negative microorganisms (Koivunen & Heinonen-Tanski, 2005a).

However, there are many factors that have a direct effect on the disinfection potential of H_2O_2. These include variations in pH, temperature contact times as well as the concentrations of H_2O_2 used (Raffellini *et al.*, 2011). Defence mechanisms within microbial cells must not be overlooked, catalase enzymes within microbial cells can act as a defence against oxidising agents, lowering the overall efficacy of H_2O_2 as a disinfectant (Wagner *et al.*, 2014).

H_2O_2 resistance

The basic functioning of certain protection mechanisms, specifically those found in *E. coli*, involve multigene systems. Once triggered they aid in repair and prevention of oxidative damage incurred by oxidising agents such as H_2O_2 (Chapman, 2003). The systems responsible for this protection include the *sox*RS and *oxy*R systems (Demple, 1996; Dukan & Toutati, 1996; Chapman, 2003). Once activated, they are responsible for the formation of catalases, superoxide dismutase and alkyl hydroperoxidases. Specifically the oxyR radical defence system is induced by hydrogen peroxide as well as hypochlorous acid (chlorine dissolved into water). In a study completed by Dukan & Toutati (1996) it showed that the effect of 300 – 700 $mg.L^{-1}$ H_2O_2, once the *oxy*R system was activated, was greatly reduced and significantly less cell damage was reported to when the system was not active. Therefore these systems, once activated, enable better defence against chemical disinfectants, especially against H_2O_2.

Advantages and disadvantages

To be considered a viable disinfectant treatment a single chemical disinfectant should have the ability to target a large variety of microorganisms, which in the case of H_2O_2, is true. H_2O_2 has proven effective against bacteria, bacterial spores and viruses, as well a variety of other

microorganisms (Newman, 2004). Thus, H_2O_2 displays good capabilities as a chemical disinfection, however, higher doses concentrations are required to achieve desired removal of pathogens (Newman, 2004). Wagner *et al.* (2002) found that much higher concentrations of H_2O_2 were required to achieve similar levels of disinfection to those achieved by PAA. Similar findings were supported by Koivunen & Heinonen-Tanski (2005a), who reported less effective disinfection for H_2O_2 compared to its direct competitor chemical disinfectants. This could be disadvantageous, as higher chemical doses will be required, ultimately increasing costs (Lubello *et al.*, 2002). A possible reason why higher doses of H_2O_2 are generally needed for effective disinfection is that H_2O_2 reacts easily with organic matter present in the water, thus lowering the overall disinfection efficacy (Ksibi, 2006). Unfortunately H_2O_2 is not very stable and correct storage and handling procedures must be followed, further adding to the overall costs.

Due to the unstable nature of pure hydrogen peroxide, stabilisers are often added to the solutions that are used at an industrial scale (Ronen *et al.*, 2010). Stabilisers ensure better storage life as well as enables H_2O_2 to have a post treatment residual effect. Thus, even after the initial disinfection treatment was performed, low levels of unreacted H_2O_2 will remain in the water (through the action of the stabilises), consequently controlling re-growth of microorganisms (Ronen *et al.*, 2010). Positively there are limited amounts of toxic chemicals released into the environment upon complete degradation of H_2O_2 (Linley *et al.*, 2012a), as hydrogen peroxide is degraded to form oxygen, hydrogen and water, leaving minimal residual. Correct safety precautions must be taken when using H_2O_2, as it is an oxidising agent. It can be damaging and dangerous if handled incorrectly (Ksibi, 2006; Vargas *et al.*, 2013).

In conclusion, H_2O_2 displays good capabilities as a chemical disinfection, however, higher doses are required to achieve desired disinfection of pathogens (Koivunen & Heinonen-Tanski, 2005a). Therefore it is advisable to incorporate H_2O_2 with other treatments like flocculation or filtration, as to optimise the efficacy of H_2O_2. Advantageously H_2O_2 can be used in combination with UV irradiation, further highlighting its versatility and potential disinfection capabilities (Labas *et al.*, 2008; Linley *et al.*, 2012a).

Chlorine

Background

Carl W. Scheele was said to prepare chlorine in its pure form (Cl_2) in the late 1700's (Momba, 2008). In the 1800s chlorine had become a well-accepted chemical disinfectant and was implemented shortly thereafter as a water disinfectant (Schoenen, 2002). Chlorine is currently the most widely accepted water disinfectant used across the world, due to its effectiveness in removing a large variety of microorganisms as well as its affordability (Koivunen & Heinonen-Tanski, 2005a). However, problems have arisen associated with chlorine usage as a water disinfectant (Krasner *et al.*, 2006; Van Haute *et al.*, 2013).

Over the years chlorine has been used in various applications, as it has proven to be

effective in removing pathogenic microorganisms, especially bacteria (Macauley *et al.*, 2006; Mezzanotte *et al.*, 2007; Lewis Ivey & Miller, 2013; Bester 2015). It is to a lesser extent effective in the reduction of viruses and protozoa, which require higher concentrations of chlorine to be inactivated (Schoenen, 2002). Furthermore, chlorine has found extensive application as a bleaching agent, reducing the presence of odours and unwanted tastes, as well as reducing the occurrence of a variety of other compounds in water, including organic matter (Rajkumar & Kim, 2006; Wisniak, 2009). Chlorine is dominantly found in three forms: chlorine gas, chlorine dioxide and as a hypochlorite. Two forms of hypochlorite exist, namely sodium hypochlorite, which is in a liquid form and calcium hypochlorite, which is in a solid, granular form (Newman, 2004; Momba, 2008; Deborde & von Gunten, 2008). Recently chlorine in the hypochlorite form has gained much acceptance (Veschetti *et al.*, 2003; Fukuzaki, 2006) as the safer options to use, compared to the traditional chlorine gas (Cl_2) and chlorine Dioxide (ClO_2). Regardless of the type of chlorine used, they all are regarded to produce similar disinfection, however, they react slightly differently when used in water disinfection as displayed in Table 4.

Table 4 Different forms of chlorine when reacted with water (Newman, 2004)

Chlorine forms	**Shortened formula**	**Reactions when added to water (H_2O)**
1. Chlorine gas	**Cl_2**	$Cl_2 + H_2O \rightarrow HCl + OCl$
2. Sodium hypochlorite	**NaOCl**	$NaOCl + H_2O \rightarrow NaOH + HOCl$
3. Calcium hypochlorite	**$Ca(OCl)_2$**	$Ca(OCl)_2 + 2H_2O \rightarrow Ca(OH)_2 + 2HOCl$
4. Chlorine dioxide	**ClO_2**	$HOCl + HCl + 2NaClO_2 \rightarrow 2ClO_2 + 2NaCl + H_2O$

Chlorine is an effective disinfectant for many strains and species of microorganisms, including *Escherichia coli (E. coli)* (Veschetti *et al.*, 2003; Van Haute *et al.*, 2013). When applied to water prior to irrigation, adequate reductions in Faecal Coliform (FC) are often recorded, complying with recommended guidelines (Lewis, Ivey & Miller, 2013). Momba (2008) reported up to a 3 log reduction in FC when chlorine was dosed at a concentration of 3 $mg.L^{-1}$. However, more resistant microorganisms, like Oomycetes, require higher concentrations (4 $mg.L^{-1}$) of chlorine, requiring a

contact time of up to 8 min to produce satisfactory disinfection (Hong *et al.*, 2003). Likewise, Nematodes display an increased degree of resistance to chlorine disinfection and therefore require high concentrations of chlorine to ensure removal (Hong *et al.*, 2003). Researchers suggested that increasing the chlorine concentrations will lower the contact time necessary to cause a germicidal effect, similarly lowering the concentration will therefore increase the contact time needed for effective disinfection (Stanton & O'Donnell, 1994; Hong *et al.*, 2003).

Calcium hypochlorite ($Ca(OCl)_2$)

Calcium Hypochlorite (($Ca(OCl)_2$) is generally available in granular or a tablet forms (Lewis, 2010). The most freely available in a granular form is known as High Test calcium hypochlorite, also commonly referred to as HTH, which is well-known for its ease of application in water treatment (Freese & Nozaic, 2004). Calcium hypochlorite is made via a process that involves chlorine gas (Cl_2) being exposed to a solution of sodium hydroxide and calcium oxide (lime solution) (Lewis, 2010). $Ca(OCl)_2$ is relatively water soluble at room temperature and is estimated to have about 70% available chlorine in dry form (Lewis, 2010). When calcium hypochlorite is dissolved in water it results in the formation of two (2) parts, hypochlorous acid (HOCl) to one (1) part calcium hydroxide. Thus, due to a stronger oxidising potential of HOCl compared to $Ca(OH)_2$ and a 2 : 1 ratio when calcium hypochlorite is added to water, effective removal of pathogenic microorganisms can be expected (Estrela *et al.*, 2002; Fukuzaki, 2006). However, pH has shown to affect the production of hypochlorous acid, thus altering the efficacy of calcium hypochlorite (Newman, 2004).

Therefore, $Ca(OCl)_2$ has proven to be very effective in removing bacteria, fungi and algae as well as a host of other microorganisms, especially those related to water (Newman, 2004). Proper storage of calcium hypochlorite is vital due to it's the hydroscopic nature. It is able to absorb moisture from the surrounding air, often leading to decomposition and decreased effectiveness over time (Lewis, 2010). Furthermore, $Ca(OCl)_2$ is known to react vigorously with a large variety of acids, solvents, organic matter as well as a larger range of materials. Brought into contact with any of these materials, spontaneous combustion has been reported (Lewis, 2010).

Sodium Hypochlorite NaOCl

The second hypochlorite, sodium hypochlorite (NaOCl), is pre-dominantly found in liquid from and is a well-known bleaching agent, present in many cleaning and disinfecting agents (Clasen & Edmondson, 2006; Fukuzaki, 2006). Sodium hypochlorite is the most commonly used form of the two hypochlorites and has proven its success in water disinfection by removing unwanted pathogens (Fukuzaki, 2006). The production of sodium hypochlorite is initiated when one part chlorine gas (Cl_2) is exposed to two parts sodium hydroxide. This reaction is of an exothermic nature and forms by-products of salt (NaCl) and water (Newman, 2004; Lewis 2010).

The formation of hypochlorous acid (HOCl), a weak acid, is expected when sodium hypochlorite reacts in water (Dukan & Toutati, 1996; Deborde & von Gunten, 2008). HOCl is believed to cause a germicidal action in water, and further dissociates into hypochlorite ions and protons (Fukuzaki, 2006). Sodium hypochlorite, although an effective disinfectant for bacterial species, is not very effective in inactivating viruses and protozoa, with little efficacy on *Cyptosporidium* (Lewis, 2010). However, there are factors that affect the disinfection capabilities of sodium hypochlorite. Fukuzaki (2006) stated that the disinfection potential of sodium hypochlorite is dependent on the pH of the water and the Chlorine concentration used. Sodium hypochlorite is most stable at a pH of 11 – 13 (Fukuzaki, 2006; Lewis, 2010). A negative aspect associated with NaOCl is shelf-life deterioration. Over time deterioration is accelerated by exposure to air, light, heat as well as organic material. However, improved storage has been reported for diluted forms of NaOCl, stored under refrigerated conditions (Lewis, 2010). Using a 6% solution of NaOCl, Wang *et al.* (2011) reported a dose of 1.5 – 3 $mg.L^{-1}$ was able to achieve a 4 log reduction for *E. coli* strain ATCC15597 and near complete inactivation (> 5 log reduction) when using 5 $mg.L^{-1}$ with 30 min contact time. Koivunen & Heinonen-Tanski (2005a), however, reported poor reductions in *E. coli* when using NaOCl (18 $mg.L^{-1}$) reporting < 0.5 log inactivation. Bester (2015) found that regardless of increasing contact times, using low concentrations of NaOCl ($\leq$6 $mg.L^{-1}$) was not met with adequate disinfection of *E. coli* strains, as < log reductions were recorded. However, higher concentrations of NaOCl (12 $mg.L^{-1}$), with longer contact times, produced improved disinfection (Bester, 2015). Sodium hypochlorite is therefore widely accepted by consumers and is considered relatively safe to use and transport (Lewis, 2010).

Mode of action

There are some specific injuries or damages induced by chlorine on microorganisms. Pathogens are readily controlled through the action of chlorine, such a *Salmonella, E. coli, Shigella* spp., *Yersinia*, to mention a few (Bitton, 2011). Specifically *E. coli*, a Gram-negative bacteria, displays less resistance to chlorine than Gram-positive bacteria (Veschetti *et al.*, 2003; Van Haute *et al.*, 2013). This is largely due to the increased protection provided by peptidoglycan layers in Gram-positive microorganisms, ultimately shielding them against chlorine damage (Cho *et al.*, 2010). Gram-negative bacteria, however, even when containing a minimal peptidoglycan layer, are less protected against chlorine induced damage, as in the case of *E. coli*. (Van Haute *et al.*, 2013). It is suggested that chlorine has a direct and damaging effect on the cell membranes of microorganisms, as it affects the lipids and proteins within the membrane (Cho *et al.*, 2010). These disruptions of the membrane leads to decreased cell permeability, improper transport across membranes and overall interfering with normal functioning of the cell (Virto, 2005; Bitton, 2005). Furthermore, these damages affect the nutrient transport, leading to improper cellular respiration and oxidation of sulfhydryl groups (Bitton, 2005).

Secondly, intercellular chlorine is also known to affect enzymes and other lipid structures

(Cho *et al.*, 2010). Enzymes affected include dehydrogenase and catalase, therefore interfering with the normal biochemical functioning of the cells (Vitro, 2005). The damage chlorine induces on the cell membranes can also lead to leaking of genetic materials (DNA, RNA), directly interfering with transcription and translation (Cho *et al.*, 2010), thus resulting in important genes not being expressed, which are responsible for protein and enzyme production, as well as maintaining normal functioning of the cell (Bitton, 2011).

Chlorine has the greatest disinfection potential against pathogens at pH 6 (Lang et al., 2008), as pH has shown to affect the production of hypochlorous acid (Newman, 2004). When the chlorine demand of a body of water has been satisfied, the unreacted chlorine exists in equilibrium as HOCl and OCl^-. At a pH < 7 the dominant form present is HOCl, which is known to induce a greater germicidal effect than OCl^-, which is more abundantly found at a pH > 7 (Newman, 2004; Bitton, 2005). The chlorine demand is increased through the presence of organic and inorganic matter. Furthermore, free chlorine readily reacts with organic matter, producing undesirable by-products (Sayyah & Mohamed, 2014).

Post-treatment disinfection by-products (DBP)

As established, there are minimal toxic by-products formed when treating water with PAA or H_2O_2, but the same cannot be said for chlorine (Diehl *et al.*, 2000; Freese & Nozaic, 2004). The toxic compounds that are formed are known as disinfection by-products (DBPs) (Kitis, 2004; Crebelli *et al.*, 2005; Sayyah & Mohamed, 2014; Al-Juboori *et al.*, 2015). Once Chlorine has been introduced as a disinfectant there is often a residual or 'left over' amount of unreacted Chlorine post-disinfection, given the dose requirement, ensuring adequate disinfection, is exceeded (Allende *et al.*, 2009).

Unreacted chlorine that remains in the water, even after disinfection has taken place is known as residual chlorine which carries both a positive and negative connotation. Residual chlorine is beneficial, as it prevents the regrowth of microorganisms, including pathogens. Furthermore, it prevents the build-up of slime within irrigation pipelines. Luo *et al.* (2011) suggested to ensure the inactivation of pathogenic *E. coli* (O157:H7), a free chlorine concentration of 0.5 $mg.L^{-1}$ should be present in the water after initial disinfection. On the negative side, the formation of by-products is promoted by residual chlorine, post-disinfection (Diehl *et al.*, 2000). As chlorine is often used to treat surface or wastewater, the naturally present organic and inorganic matter may provide the base to form potentially toxic and/or carcinogenic disinfection by-products (DBPs) (Crebelli *et al.*, 2005; Wang *et al.*, 2013; Sayyah & Mohamed, 2014).

There are various DBPs that can be formed. The dominant types are suggested to be formed due to interactions with naturally present organic matter (NOM). NOM are usually non-polar/hydrophobic molecules, although polar organic matter may also produce DBPs, although in much lower quantities (Liang & Singer, 2003). A large variety of DBPs have been reported, the most dominant types being in the form of Trihalomethanes (THMs) and Haloacetic acids (HAA5)

(Dickenson *et al.*, 2008; Bond *et al.*, 2009). A specific THMs that have been relatively well documented are Chloroforms ($CHCl_3$), which are generally considered the most common type of THMs formed through chlorine treatment (Freese & Nozaic, 2004).

Researchers have suggested possible carcinogenic properties regarding Chloroforms, together with prolonged exposure increasing their potential to cause cancer (Sayyah & Mohamed, 2014). DBPs have been alledged to be the cause of bladder cancer, spontaneous abortions and birth defects (Singer, 2006). THMs, other than Chloroforms, have also been associated with negative health effects with prolonged exposure; they include dibromochloromethane, bromoform and bromodichloromethane, just to mention a few (Bitton, 2011). A controversial study published by Freese & Nozaic (2004) suggested that exposure to low doses of THMs, associated to water disinfection, is unlikely to cause negative health problems. However, the potential still exists that excessive chlorine residual in water may carry potential negative health implications (Hrudey, 2009).

Chlorine resistance mechanisms

Not all microorganisms display similar disinfection potential when exposed to chlorine (Cherich, 2011; Giddey *et al.*, 2015). Structural differences in the membrane and cell walls of microorganisms, combined with various defence mechanisms acting against oxidative damage, are considered to induce variable disinfection efficacy (Russell, 2003; Giddey *et al.*, 2015). *E. coli* for example is Gram-negative and displays less resistance to chlorine disinfection compared to Gram-positive microorganisms, due to structural variation regarding peptidoglycan layers (Russell, 2003; Van Haute *et al.*, 2013). However, there are other survival adaptions and genetic modifications that microorganisms can undergo to ensure survival. These adaptions have gained increasing attention, as they ultimately reduce the efficacy of chemical disinfectants like chlorine (Dukan & Toutati, 1996; Chapman, 2003). Literature suggests a variety of reasons responsible for this increased resistance observed (Chapman, 2003).

Primarily external stresses are suggested to enable the survival and replication of more resistant microbial strains (Dukan & Toutati, 1996; Chapman, 2003). The prolonged use of chlorine has led to microorganisms, specifically *E. coli,* to develop defence mechanisms against disinfection treatments. A study done showed *E. coli* that had survived repeated exposure to chlorine, ultimately displayed greater resistance to chlorine disinfection, compared to the original *E. coli* strain (Demple, 1996). It was therefore suggested that this natural increase in resistance was due to either phenotypic adaption, genetic alteration or genetic acquisition (Demple, 1996; Chapman, 2003). Genetic based mutations are suggested to be induced by advantageous chlorine-induced mutations, acquisition of new genetic information by horizontal gene transfer, the expression of previous silent genes, growth in a biofilm and other phenotypic alterations leading to resistant phenotypes (Chen & Stewart, 1996; Chapman, 2003). Furthermore, specific cell resistance can be due to target alteration, inactivation of inhibitors, and reduction in target access

via exclusion or efflux pumps, aided by the removal of chlorine from the targeted cells (Chapman, 2003).

The basic functioning of certain protection mechanisms, specifically those found in *E. coli*, involve multigene systems. Once triggered they aid in repair and prevention of oxidative damage incurred by oxidizing agents. The systems responsible for this protection include the *sox*RS and *oxy*R systems (Demple, 1996; Dukan & Toutati, 1996; Chapman, 2003). Once triggered, these systems induce the production of enzymes, including catalases, Superoxide dismutase and alkyl hydroperoxidases. Specifically, the oxyR radical defence system is induced by hydrogen peroxide, as well as hypochlorous acid (chlorine dissolved into water). In general the activation of these regulatory systems results in the expression of efflux pumps and the formation of enzymes, both aiding in reducing the effects of oxidative damage induced by chemical disinfectants (Chapman, 2003; Russell, 2003). Therefore these systems, once activated, enable better defence against chemical disinfectants and ultimately can reduce the effect of chlorine, which can be considered problematic.

Advantages and disadvantages

Although there are various forms of chlorine, the hypochlorite types are considered safer with better ease of use (Lewis, 2010). Sodium hypochlorite (NaOCl) is considered a safe disinfectant to transport and store, although increased costs are involved doing so (Clasen & Edmondson, 2006; Lewis, 2010). Calcium hypochlorite is, however, more stable and generally considered safer to use, although if not stored correctly it has the potential to combust or explode (Lewis, 2010). Calcium hypochlorite is known for its high oxidising potential through the action of Hypochlorous acid (HOCl). When added to water the formation of two hypochlorous acid (HOCl) molecules resulting from one calcium hypochlorite molecule proves to be advantageous, as increased germicidal action is observed under these conditions (Lewis, 2010).

Negatively, even in ambient conditions, NaOCl will only remain at the original concentration for a few months, thereafter degradation will occur, breaking down to form sodium chloride, sodium Chlorate and oxygen (Clasen & Edmondson, 2006). NaOCl degradation results in less effective disinfection, producing unexpected results if not monitored. Furthermore, temperature affects the degradation of hypochlorite substantially. An estimated rise in temperature of 10°C can speed up the degradation process by up to 3 – 4 times (Lewis, 2010, Bitton, 2011). Consequently, a low water temperature will also decrease the overall disinfection process of chlorine (Pichard, 2006). Storage conditions thus greatly influence the degree and range of degradation of sodium hypochlorite (Lewis, 2010).

Organic matter, in the form of suspended solids, have shown to significantly decrease the disinfection efficacy of chlorine, as well as promote the formation of DBPs (Sayyah & Mohamed, 2014). Thus, employing pre-treatment filtration steps before treating with chlorine treatment is strongly suggested by manufacturers (Crebelli *et al.*, 2005; Ayyildiz, 2009). Incorporating pre-

treatment steps act to maximise the disinfection potential of chlorine, due to the partial removal of organic material and suspended solids (Lewis-Ivey & Miller, 2013; Raudales *et al.*, 2014). Precautions must be taken to help ensure adequate disinfection by selecting the correct chlorine concentrations with regard to water quality (Crebelli *et al.*, 2005; Bester, 2015). In conclusion, chlorine is a well-accepted chemical disinfectant that has been used successfully for many years to control pathogenic microorganisms, regardless of the drawbacks closely associated with it (Momba, 2008).

2.12 COMBINATION TREATMENTS (CHEMICALS COMBINED WITH UV IRRADIATION)

Background

Increased human activities surrounding fresh water and overloaded sewage treatment facilities have been a major contribution to increased pollution of surface water (Obit *et al.,* 2004; DEAT, 2006).

Furthermore, over the last 20 years many studies have revealed increased microbial resistance to chemical disinfectants such as chlorine (Dukan & Toutati, 1996; Chapman, 2003). Ultimately, increased microbial resistance has led to the use of exceptionally high levels of chemical disinfectants to ensure adequate disinfection and pathogen removal (Worrall & Burt, 2009). However, simply increasing the chemical doses to achieve satisfactory disinfection is not considered economically viable or safe (Lu *et al.*, 2009). Furthermore, the formation of potentially harmful disinfection by-products (DBPs) associated with increased concentrations of chemicals has been a major concern (Wang *et al.*, 2013; Doederer *et al.*, 2014; Zhang *et al.*, 2014). Not only do increased chemical concentrations promote the formation of DBPs but also initiate the adaption and modification of microorganisms, resulting in the formation of microorganisms that display greater resistance to disinfection treatments (Hu *et al.*, 2005; Delpla *et al.*, 2009). Therefore, the development of more effective, less damaging disinfection treatments must be considered (Sharp *et al.*, 2006). One such approach involves the combination of UV light with secondary oxidants, usually chemicals such as peracetic acid or ozone and hydrogen peroxide (Rosenfeldt *et al.*, 2006; Oturan & Aaron, 2014). These combinations initiate a phenomenon which is known as an advanced oxidation process (AOPs). The process of advanced oxidation involves the production of primarily Hydroxyl Radicals (•OH), but not exclusively (Sherchan *et al.*, 2014a). Considered powerful and effective oxidisers of organic pollutants, •OH are relatively non-selective and highly reactive (Swaim *et al.*, 2008; Sherchan *et al.*, 2014b). Furthermore, they also act to reduce disinfection by-products (DBP) produced by chemical disinfectants (Lu *et al.*, 2009; Oturan & Aaron, 2014). Additionally, AOPs are beneficial as they can aid in reducing pharmaceuticals, pesticides and taste compounds that may be present in water. Research has been done primarily on combining oxidative disinfectants with UV irradiation, these include O_3/UV, H_2O_2/UV, Cl/UV and PAA/UV (Matilainen, 2010).

UV in combination with H_2O_2 has been the topic of a number of studies (Lubello *et al.*,

2002; Rosenfeldt *et al.*, 2006; Sherchan *et al.*, 2014a). When applied in combination, there is a germicidal effect induced via direct photolysis by the UV irradiation as well as an oxidative damage incurred by H_2O_2 and in addition secondary photolysis also plays a role in disinfection through the action of AOP (Pereira *et al.*, 2007). Sherchan *et al.* (2014a) reported complete inactivation of MS2 coliphage (> 7 log) when H_2O_2 (2.5 $mg.L^{-1}$) was applied in combination with UV (>100 $mJ.cm^{-2}$). It was also suggested that the complete inactivation of the MS2 coliphage was likely due to the effects of AOPs (Sherchan *et al.*, 2014a).

Koivunen & Heinonen-Tanski (2005), however, showed using a combination of H_2O_2 (3 $mg.L^{-1}$) but a lower UV dose (22 $mJ.cm^{-2}$) produced less successful reductions in the MS2 coliphage ($\pm$ 1 log). It was further suggested that AOPs contributed minimally, if any to the overall disinfection, thus, highlighting the importance of adequate UV doses to initiate the formation of •OH through AOPs (Koivunen & Heinonen-Tanski (2005). Rosenfeldt *et al*. (2006) suggested the importance of UV irradiation to initiate the additional disinfection action of AOPs, as poor optical water quality (UVT% and turbidity) did not initiate additional disinfection through AOPs when applied in combination with H_2O_2. Sherchan *et al.* (2014b) further reported •OH to be responsible for > 6 log reductions for *E. coli* vegetative cells upon exposure to the combination treatment (H_2O_2/UV). However, AOPs proved less effective in reducing *Bacillus* spores (*B. thuringiensis*), producing minimal reductions (Sherchan *et al.*, 2014b).

When considering PAA, Koivunen & Heinonen-Tanski (2005) reported on the combination of PAA (3 $mg.L^{-1}$) and UV (10 and 14 $mJ.cm^{-2}$), which was able to achieve 5.56 and 5.97 log reductions for *E.coli*, respectively. Furthermore, it was reported by Koivunen & Heinonen-Tanski (2005) that the potential exists that AOPs may contribute to the reduction of enteric bacteria when applying PAA/UV combination treatments, even at low concentrations of PAA and low UV doses. Lubello *et al.* (2002) found better reductions of Faecal Coliforms (FC) using the combination of PAA/UV to that of H_2O_2/UV, due to the increased effects of AOPs observed for PAA/UV treatments, when using low chemical concentrations for both PAA and H_2O_2. Furthermore, little differences were observed regarding FC log reductions when using 2 or 4 $mg.L^{-1}$ PAA. However, Lubello *et al.* (2002) reported to achieve similar log reductions using H_2O_2, concentrations in excess of 20 $mg.L^{-1}$ would be required.

Concerning the combination of UV and chlorine (hypochlorite types), Montemayor *et al.* (2008) suggested that combination treatments (Cl/UV) produced nearly complete removal of *E. coli* from reclaimed water. They also suggested that combination treatments provided maximum reductions achievable when considering the individual chlorine and UV treatments and thus were able to ensure public health and safety (Montemayor *et al.*, 2008).

Mode of action

There are various types of reactions that are responsible for the formation of AOPs, based on chemical, photochemical, electrochemical and sonochemical reactions (Oturan & Aaron, 2014). However, the combination of UV irradiation with powerful oxidants such as O_3 and H_2O_2 are known to produce AOPs through photochemical reactions (Comninellis *et al.*, 2008; Matilainen & Sillanpaa, 2010). Photochemical AOP are considered simpler, more effective and inexpensive than the alternative, chemically induced AOPs (Oturan & Aaron, 2014). These photochemical processes are able to reduce organic pollution present in water/wastewater by three suggested reactions, (1) photodecomposition (UV irradiation alone); (2) oxidation induced directly by the chemical disinfectants (PAA, O_3, H_2O_2) and (3) oxidation by photocatalysis (formation of •OH) (Oturan & Aaron, 2014). Considering reactions (1) and (2) which have already been discussed, reaction (3) regarding the formation of additional •OH radicals due to combination treatments will be focused on.

When considering AOPs are initiated by the presence of strong oxidants, such as H_2O_2, understanding the formation of •OH will be of relevance as they are strongly associated to AOPs (Rosenfeldt *et al.*, 2006). H_2O_2 readily undergoes photolysis when exposed to UV light (wavelengths 200 – 300 nm) which induces the homolytic scission of the single bonds (O-O) of H_2O_2, forming •OH (secondary oxidants) (Lubello *et al.*, 2002; Rosenfeldt *et al.*, 2006; Oturan & Aaron, 2014). The formation of OH radicals can be due to a variety of competitive reactions involving the decomposition of H_2O_2 when exposed to UV irradiation as described by Oturan & Aaron (2014):

$$H_2O_2 + UV \rightarrow 2\ \bullet OH \qquad (1)$$

$$\bullet OH + H_2O_2 \rightarrow H_2O_2 + HO_2\bullet \qquad (2)$$

$$HO_2\bullet + H_2O_2 \rightarrow \bullet OH + H_2O + O_2 \qquad (3)$$

$$\bullet OH + HO_2^- \rightarrow HO_2\bullet + OH^- \qquad (4)$$

$$2HO_2\bullet \rightarrow H_2O_2 + O_2 \qquad (5)$$

$$\bullet OH + HO_2\bullet \rightarrow H_2O + O_2 \qquad (6)$$

$$2\bullet OH \rightarrow H_2O_2 \qquad (7)$$

Equations 1 – 7 describe the formation as well as termination of •OH. The rate of their production is greatly dependent on a variety of factors. As established, UV light is predominately produced by LP and MP Mercury vapour lamps that emit different wavelengths (Gayán *et al.*, 2014). Photolysis

requires UV light (200 – 300 nm) to initiate the production of secondary oxidants (•OH radicles), thus LP and MP UV irradiation will be effective (Canonica *et al.*, 2008; Oturan & Aaron, 2014). However the difference in the wavelengths can affect chemical bonds differently and ultimately influence the type of degradation products formed (Rosenfeldt *et al.*, 2006; Gayán *et al.*, 2014). Furthermore the physico-chemical properties of the water will also influence the rate of production of secondary •OH, as UV irradiation is highly dependent on optical water quality parameters (UVT%, turbidity) (Rosenfeldt *et al.*, 2006; Oturan & Aaron, 2014).

•OH radicals produced through secondary photolysis (AOP) induce a germicidal action in a similar way to that of chemical oxidants (PAA, H_2O_2) (Raffellini *et al.*, 2011). Primarily •OH target cellular components of microbial cells, including the cell membrane, lipids and proteins. These cellular disruptions often lead to damaged genetic material (DNA, RNA), as well as compromised cellular function, ultimately inhibiting transcription, translation and cellular replication (Raffellini *et al.*, 2011; Linley *et al.*, 2012a; Vargas *et al.*, 2013). However, similar to primarily oxidation reactions, a variety of factors will influence the efficacy of the •OH in inducing oxidative damage on microorganisms (Oturan & Aaron, 2014), including the type of microorganisms present in the water treated and the individual degree of resistance to oxidative damage that the different microorganisms might display to oxidative damage (Sherchan *et al.*, 2014b).

As suggested by Montemayor *et al.*, 2008, the shortcomings of individual treatments was counteracted by their application in combination. Thus, in the case of (Cl/UV), chlorine proved to be more effective in the reduction of non-spore forming bacteria (*E. coli*) and faecal enterococci, however, less effective in reducing bacteriophages, viruses, pathogenic protozoa (*Cryptosporidium* spp.) and (oo)cysts, which were better reduced by low doses of UV irradiation. Thus, combination treatments are suggested to provide maximum protection against microorganisms ensuring better human safety (Montemayor *et al.*, 2008).

Conclusion

When considering the different studies done on secondary photolysis treatments (H_2O_2/UV, PAA/UV, Cl/UV) inducing the action of AOPs, potential exists in applying these treatments to achieve effective microbial reductions in polluted water. Furthermore, these combination treatments often induce a more effective disinfection that the stand-alone UV or chemical treatments. As Equations 1 – 7 describe the formation and termination of •OH for H_2O_2 specifically, similar reactions can be expected for other oxidants such as PAA (with is comprised of H_2O_2). However, as chlorine can be present in various forms, specific research regarding the actual chemical reactions involved during secondary photolysis is quite limited. Nevertheless, research has suggested that the combination of chlorine, together with UV irradiation can prove to be successful in reducing microorganisms that may be present in water (Montemayor *et al.*, 2008). As combination treatments possess the potential to reduce organic matter present in water, assessing the effect different chemical treatments, when applied in combination with both LP and

MP-UV irradiation would thus prove valuable. However, analysing the various results from different studies when determining suitable UV and chemicals doses could prove challenging, as variations in physiochemical and microbial water quality would therefore needed to be considered. Thus, characterisation of water, prior to treatment, would be recommendable when optimising AOPs (Matilainen & Sillanpaa, 2010).

2.13 CONCLUDING REMARKS

Water can be considered the one essential resource vital to maintain life on earth. Worryingly it is becoming more scares, with remaining fresh water sources undeniably becoming highly polluted. Multiple studies have shown South African rivers to have high levels of microbial pollution of pathogenic nature (Paulse et al., 2009; Britz et al., 2013; Gemmell & Schmidt, 2013; Lamprecht et al., 2014). When considering the reliance the agricultural sector has on fresh water, especially for irrigation purposes, microbial contamination presents a serious problem (Paulse *et al*., 2009; Ijabadeniyi & Buys, 2012). Food borne disease outbreaks have been linked to the consumption of faecally contaminated fresh produce. Many disease outbreaks have been reportedly caused due to consumption of *Salmonella* spp. and *Escherichia coli (E. coli)* (Warriner *et al.*, 2009; Benjamin *et al.*, 2013). Untreated river water, contaminated with faecal waste, acts as a vector responsible for the transfer of pathogens to crops, ultimately responsible for causing human illness in communities and even countries as a whole (Teklehaimanot *et al.*, 2014). (Pachepsky *et al.*, 2011; Ijabadeniyi & Buys, 2012).

The treatment of water intended for the irrigation of fresh and minimally processed produce is of vital importance, as to reduce the occurrence of human illness. Physical (sand filtration, biochar filtration), chemical (peracetic acid, chlorine, hydrogen peroxide and ozone) and photochemical (UV light irradiation) treatments could potentially, when applied to contaminated irrigation water, reduce the occurrence of potentially harmful pathogenic microorganisms. Different chemical disinfectants vary in their mode of disinfection pathways that results in microbial inactivation. chlorine has proven to be a well-accepted and capable disinfectant. More recentlyp peracetic acid and hydrogen peroxide have also proven successful in microbial reductions. However, different chemical disinfectants each have their own unique set of advantages and limitations that are often dependent on varying environmental and commercial influences.

Selecting an appropriate treatment can prove to be challenging, as environmental variations in water pH, temperature and the amount of suspended organic and inorganic matter greatly influence disinfection performance. Disinfection efficacy is further influenced by the chemical disinfection concentration used and reaction time given. Filtration methods have widely been implemented in water treatment, improving water quality significantly by reducing the levels of organic and inorganic particles present in water. Sand filtration has proven its success, especially when used in combination with other treatment methods (chemical and/or photochemical). However, most filtration methods, when used as a sole water treatment method, are unable to

adequately reduce microbial levels in water for irrigation purposes and are therefore often used as a pre-treatment method. Alternative filtration methods, such as biochar filtration, have shown potential at improving water quality, but little is known on their ability to reduce microbial numbers in water.

Pre-treatment filtration is advantageous when considering UV irradiation. A reduction in light scattering particles allows for more effective microbial reduction when implementing UV irradiation in water treatment. UV irradiation is not a novel concept; however, its full potential has not yet fully been utilised on a commercial scale in South Africa. Because microorganisms have the ability to repair damage inflicted by UV light waves and UV dose is dependent on water quality investigation, the potential of microbial repair with regards to river water is vital. Furthermore, documenting UV treatments efficacy, with regards to varying water quality will prove to be insightful, as this information will highlight the degree of feasibility of UV irradiation on a commercial scale in South Africa.

All the above-mentioned treatments have shown respectable water treatment results, however, the formation of disinfection by products (DBPs) and increased microbial resistance often influence germicidal action of the different treatments. Thus, the combination of different chemicals together with UV irradiation holds additional disinfection advantage. Combination treatments are gaining acceptance, as reductions in contact times and dose requirements are possible without compromising germicidal action as advanced oxidation processes (AOPs) are initiated.

Combination treatments also act to minimize the negatives induced by using high doses of UV and chemical disinfectants (when used as sole disinfection treatments). Although there is literature available on the disinfection efficacy of wastewater when using combination treatments, the effectiveness thereof when river water is used for irrigation is limited. Thus, investigating the potential for combination treatments to reduce the occurrence of microbial resistant microorganisms and improving disinfection efficacy hold value. Furthermore, considering alternative water treatment methods, such as biochar filtration, can initiate a whole new approach on water treatment and reduce environmental impacts greatly. However, effective control measures for commercial application can only be suggested when all the above-mentioned treatments, together with varying factors, are extensively investigated on larger scales.

Conclusively, the lack of overview that followed standardised practices, incorporating various water treatment methods that is based on a larger scale application, has resulted in great variability in results found in literature. Thus, this thesis focused on the variations in efficacy of individual water treatment methods, as well as combination treatments, as to make accurate and reliable recommendations regarding river water disinfection in South Africa.

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Chapter 3

EVALUATION OF THE DISINFECTION EFFICACY OF PERACETIC ACID, SODIUM HYPOCHLORITE, HYDROGEN PEROXIDE INDIVIDUALLY AND IN COMBINATION WITH LOW-PRESSURE UV IRRADIATION AT LABORATORY-SCALE

ABSTRACT

Determining the efficacy of ultraviolet (UV) irradiation combined with peracetic acid (PAA), chlorine (Cl) and hydrogen peroxide (H_2O_2) (PAA+UV; Cl+UV; H_2O_2+UV), required evaluating the stand-alone efficacy of each treatment first. Environmental *Escherichia coli* strains were analysed in this study, as they are considered to be indicator organisms for faecal contamination in river water. In Study 1 specific environmental *E. coli* strains, that had previously displayed a degree of resistance to UV or chemical treatments, were investigated. The environmental strains, F11.2 and MJ 58, were exposed to a Cl concentration of 6 $mg.L^{-1}$ and H_2O_2 concentration of 2.5 $mg.L^{-1}$. However, F11.2 was only exposed to PAA concentrations of 4 $mg.L^{-1}$ as F11.2 was most sensitive to PAA. Disinfection times of 15 and 25 min were allowed. Thereafter, chemically treated samples were exposed to a Low-pressure (LP) UV dose of 13 $mJ.cm^{-2}$, in an attempt to determine the effects of combination treatments and advanced oxidation processes (AOPs). A 25 min contact time produced, in most instances, significantly better ($p<0.05$) log reductions compared to the 15 min contact times for chemical treatments. The most effective reduction of F11.2 was recorded by the (PAA+UV) combination treatment with > 6 log reduction. H_2O_2 produced a > 3 log reduction for the combined treatment (H_2O_2+UV), whereas chlorine was least effective of the three chemicals for both *E. coli* strains.

In Study 2 only a 25 min contact time was investigated for river water disinfection, whilst keeping the chemical and UV doses the same as in the first study. Clear differences were seen when comparing the stand-alone treatment efficacy to that of the combination treatments. When evaluating the disinfection efficacy of treatments applied to river water, chlorine was the most effective disinfectant recording $\geq$ 4 log reduction for Faecal Coliforms (FC), regardless of the varying microbial and physico-chemical water quality associated with the river water. UV irradiation treatments were greatly influenced by poor river water quality parameters, more so than chemical treatments. Upon completion of the second study chlorine, in the form of sodium hypochlorite (NaOCl) proved most effective, successfully reducing the microbial levels recorded in river water. Stand-alone H_2O_2 treatments were least effective in reducing microbial levels present in the river water. Chloride could therefore be considered as a viable treatment option for disinfecting river water, prior to irrigation. Negatively, however, chlorine disinfection has been associated with residual chlorine levels in water, the formation of disinfection by products (DBPs) and increased microbial resistance, post-disinfection which can reduce its overall acceptability. However, combination treatment generally displayed improved disinfection potential, in most instances, with regards to a longer (25 min) contact time.

INTRODUCTION

Currently South Africa, as many other countries, is faced with the problem of water scarcity (De Bon *et al*., 2010; Hanjra & Qureshi, 2010). Worldwide the increased demand for fresh water is not only limited to the ever expanding agricultural sector, but is also the result of increased human consumption due to ever-growing populations (Wallace & Gregory, 2002; Rijsberman, 2006; Chaturvedi *et al.*, 2013). Furthermore, changes in rainfall patterns due to global warming, as well as increased pollution of the already limited fresh water sources, has led to a global water crisis (Gosling & Arnell, 2016).

Over the last 10 years multiple disease outbreaks linked to fresh produce have increased rapidly, placing focus on the microbial quality of irrigation water as a possible contamination source (Benjamin *et al.*, 2013). Many published studies have found river water to contain exceedingly high levels of microbial contamination, which, if used directly for agricultural irrigation, would pose a risk to human health (Islam *et al.*, 2005; Lynch *et al.*, 2009; Gelting *et al.*, 2011; Castro-Rosas *et al.*, 2012; Lee *et al.,* 2014). In order to ensure microbially safe irrigation water in South Africa, the Department of Water Affairs (DWAF, 1996) has established a guideline suggesting that no more than 1 000 Faecal Coliforms (FC) per 100 mL (1 000 cfu.100 mL^{-1}) should be present in water intended for irrigation purposes. According to previous studies, this guideline is often exceeded in many South African rivers (Germs *et al.*, 2004; Gemmell & Schmidt, 2013; Bester, 2015; Olivier, 2015). Britz *et al.* (2013) suggested that when evaluating a disinfection treatment, a 3 – 4 log reduction in FC should indicate adequate disinfection, thus resulting in irrigation water of acceptable microbial quality. Currently, various disinfection treatments are available, all with the aim to reduce microbial levels in water prior to irrigation.

Chemicals, such as chlorine, have proven to be successful in reducing a large variety of microorganisms present in water (Veschetti *et al.*, 2003; Mezzanotte *et al.*, 2007 Comninellis, 2008). However, as of recent years, alternative chemical disinfectants have been brought under investigation, as some countries prohibit the use of chlorine as a disinfectant altogether (Gehr *et al.*, 2003; Freese and Genthe, 2006). Peracetic acid (PAA) and hydrogen peroxide (H_2O_2) have been considered viable alternatives to chlorine disinfection for water disinfection (Freese & Nozaic, 2004; Koivunen & Heinonen-Tanski, 2005a). Multiple studies have shown PAA possess convincing disinfection potential. Gehr *et al.* (2002) reported that 2 – 6 $mg.L^{-1}$ PAA was able to reduce FC adequately, complying with the 1 000 colony forming units (cfu).100 mL^{-1} guideline (DWAF, 1996). Similarly, Morris (1993) reported that a concentration of 5 $mg.L^{-1}$ PAA for 20 min was sufficient in reducing total and FC by 4 – 5 log. H_2O_2 has also been used to evaluate its potential as a contending disinfectant, with multiple studies done over the past few years (Koivunen & Heinonen-Tanski, 2005a). Vargas *et al*. (2013) reported a 4 log reduction of coliforms using a H_2O_2 concentration of 10 $mg.L^{-1}$, for 10 min.

Concerns highlighted when using chemical disinfectants include the formation of harmful DBPs (disinfection by-products) during chlorine disinfection, increased microbial resistance to

chemical disinfectants and the inability to target a broad range of microorganisms that could be present in river water (Freese & Nozaic, 2004; Sayyah & Mohamed, 2014; Al-Juboori *et al.*, 2015).

Ultraviolet (UV) light has successfully been implemented in the water industry, inducing adequate reductions in microbial levels (Gayán *et al.*, 2014). Primarily UV irradiation affects the genetic material (DNA, RNA) of a large range of microorganisms. Disruption of cellular components and proteins have also been reported after UV light exposure (Premi *et al.*, 2015). UV wavelengths of 200 – 280 nm have been suggested to be the most germicidal wavelengths, as optimal absorption of wavelengths (photons) by genetic material occurs in this range (Koutchma, 2009). During irrigation treatment UV light is produced by either low-pressure (LP) or medium-pressure (MP) mercury vapour lamps, differentiated on their ability to produce different wavelengths (Sakai *et al.*, 2007; Gayán *et al.*, 2014). As UV irradiation is considered a non-thermal process that doesn't require chemical intervention, there are no residual products released into the environment which could result in the formation of harmful disinfection by-products (DBPs), which are often associated with chemical disinfectants (Gayán *et al.*, 2014). However, potential drawbacks have been identified that could affect the efficacy of UV irradiation negatively.

Disinfection efficacy can be influenced by the ever changing microbial composition and water quality within a water system. Researchers have reported that microorganisms possess DNA-repair mechanisms that could reverse damage induced by UV irradiation, decreasing the lethality of UV irradiation (Sinha & Häder, 2002; Guo *et al.*, 2011; Kneuttinger *et al.*, 2014). Furthermore, poor water quality with regards to ultraviolet transmission (UVT%), turbidity and chemical oxygen demand (COD) are often associated with decreased UV irradiation efficacy as less UV light is able to penetrate a sample, thus shielding microorganisms from the UV rays. Guo *et al.* (2009) reported just over 3 log and 2 log reductions for *E. coli* and total coliforms respectively, during LP-UV disinfection (13 $mJ.cm^{-2}$). Koivunen & Heinonen-Tanski (2005) supported these findings by reporting that *E. coli* were successfully reduced by 1.44 log at a UV dose of 14 $mJ.cm^{-2}$.

As of recent years, the combination of UV irradiation and chemical disinfectants has gained increasing attention in the water industry (Koivunen & Heinonen-Tanski, 2005a; Hadjok *et al.*, 2008; Montemayor *et al.*, 2008). These combination treatments ultimately minimise the shortcoming of individual disinfection treatments by potentially reducing the amount of residual chemicals entering the environment, reducing costs and increasing the variety of microorganisms targeted (Montemayor *et al.*, 2008; Oller *et al.*, 2011).

Advanced oxidation processes (AOPs) are considered to be the leading cause for the benefits incurred by employing combination treatments. AOPs are expected to enhance the disinfection potential of the two individual treatments to a greater extent than simply their summed disinfection capabilities (Wang & Xu, 2012; Sherchan *et al.*, 2014). The combination of chemicals (PAA or H_2O_2), together with UV light, initiates the formation of additional free hydroxyl radicals (•OH). These •OH molecules are expected to have a high oxidation potential for organic matter and therefore account for the additional disinfection properties initiated by AOPs (Rosenfeldt *et al.*,

2006; Sherchan *et al.*, 2014). Furthermore, the enhanced formation of •OH molecules is expected when chemical disinfectants are added before UV light exposure, as UV light acts directly on the chemical disinfectants to produce additional •OH molecules (Caretti & Lubello, 2003; Sherchan *et al.* 2014). Koivunen & Heinonen-Tanski (2005a) reported that for a UV dose of 14 $mJ.cm^{-2}$ and PAA concentration of 3 $mg.L^{-1}$, 5.97 log reductions were achieved for *E. coli*. This was greater than the sum of the two stand-alone treatments of UV (achieving a 1.44 log reduction) and PAA (resulting in a 2.81 log reduction). Furthermore, Montemayor *et al.* (2008) also reported increased potential when using chlorine and UV irradiation in combination with each other.

The main aim of this study was thus to evaluate, at laboratory scale, the disinfection efficacy of chemicals (PAA, Cl and H_2O_2) in combination with LP-UV irradiation in reducing microbial levels in water. In order to establish a suitable disinfection time for the chemical disinfectants under investigation, combination treatments were first conducted on reference *E. coli* strains in simple saline solution (SSS). Thereafter, the efficiency of the optimised combination treatments were tested on river water. The effect that water quality parameters, such as UVT%, turbidity, COD, total and Volatile Suspended Solids (TSS and VSS), pH, alkalinity and conductivity had on the disinfection efficacy, were also considered.

MATERIALS AND METHODS

Research study design

High concentrations of chemical disinfectants are often used in combination with short contact times to treat fresh produce before distribution to consumers, ensuring human safety upon consumption (Saby *et al.*, 2002; Gehr *et al.*, 2003; Parish *et al.*, 2003; Koide *et al.*, 2009; Durak *et al.*, 2012). However, these methods are often associated with the formation of undesirable disinfection by-products (DBPs) and can result in increased microbial resistance over time (Freese & Nozaic, 2004; Sayyah & Mohamed, 2014; Al-Juboori *et al.*, 2015). Study 1 aimed to evaluate the effect of longer contact times (15 – 25 min) combined with lower doses of chemicals and UV irradiation on reducing environmental *E. coli* strains. Caretti & Lubello (2003) reported increased disinfection for *E. coli* when chemicals were added before UV light exposure, in contrast to if chemicals were added after UV irradiation. The combination of UV irradiation with chemical disinfectants can induce additional germicidal effects through the action of advanced oxidation processes (AOPs), specifically when exposed to UV post-chemical treatment. AOPs can prove advantageous, as they act directly on the reduction of DBPs. Furthermore, AOPs have the ability to target a larger variety of microorganisms than simply summing the effects of the stand-alone treatments (Montemayor *et al.*, 2008; Üstün *et al.*, 2011; Wang & Xu, 2012; Sherchan *et al.*, 2014). A UV dose of 13 $mJ.cm^{-2}$ was used in this study as Olivier (2015) reported > 3 log reduction for environmental *E. coli* strains at a LP UV dose of 10 – 13 $mJ.cm^{-2}$. Furthermore, Koivunen &

Heinonen-Tanski (2005a) reported that low concentrations of chemicals in combination with LP-UV, a dose of 14 mJ.cm^{-2} was adequate to reduce *E. coli* by > 4 log.

E. coli strain selection was based on research done by Bester (2015) and Olivier (2015). Strains that had showed increased resistance to PAA, Cl, H_2O_2 and UV irradiation were thus investigated. Contact times of 15 and 25 min were allowed for chemical treatments. Olivier (2015) reported increased resistance to LP-UV irradiation at lower UV doses for *E. coli* strain F11.2. Therefore, F11.2 was used throughout Study 1 to evaluate the efficacy of UV irradiation (13 mJ.cm^{-2}) as a stand-alone treatment and in combination (PAA+UV; Cl+UV and H_2O_2+UV). Furthermore, increased resistance to Cl and H_2O_2 treatments was observed by *E. coli* strain MJ58 (Bester, 2015; Olivier, 2015). Giddey *et al.* (2015) also reported increased resistance to environmental strain MJ58 to H_2O_2 treatments. However, PAA treatments proved less effective against F11.2 than MJ58 (Bester, 2015). Thus, in this study the disinfection efficacy of PAA and UV was only tested on *E. coli* strain F11.2, as it had displayed increased resistance to both UV and PAA disinfection treatments previously (Olivier, 2015).

Study 1: Efficacy of UV irradiation in combination with chemical disinfectants on reducing *E. coli* strains

The sensitivity of two environmental *E. coli* strains, (F11.2 and MJ58), when exposed to PAA, Cl and H_2O_2 in combination with UV irradiation were investigated (Fig. 1). The treatments were evaluated as stand-alone treatments, as well as in combination (PAA+UV; Cl+UV and H_2O_2+UV). The chemical concentrations and UV doses used were at concentrations lower than those recommended for commercial application. According to previous research, environmental strains F11.2 and MJ58 displayed the most resistance to either specific chemicals or UV irradiation treatments (Bester, 2015; Oliver 2015). In this study PAA was used at 4 mg.L^{-1}, while Cl and H_2O_2 were used at 6 mg.L^{-1} and at 2.5 mg.L^{-1}, respectively, for both F11.2 and MJ58. Throughout this study chemical disinfectants were always dosed prior to UV irradiation, allowing either 15 or 25 min contact intervals. Samples subjected to chemical disinfectants (PAA, Cl and H_2O_2) were exposed to LP-UV irradiation at a dose of 13 mJ.cm^{-2}, using a collimated beam device. Using standard plate count methods, *E. coli* were enumerated on VRBA (Biolab, South Africa) before and after treatments. All tests were conducted in 0.85% (m.v^{-1}) SSS.

Study 2: Influence of water quality on disinfection treatments

Unfiltered river water was sampled from the Plankenburg River in Stellenbosch, kept cool during transport and exposed to either one of the following chemicals before UV irradiation: PAA (4 mg.L^{-1}), Cl (NaOCl) (6 mg.L^{-1}) or H_2O_2 (2.5 mg.L^{-1}). A contact time of 25 min was allowed for chemical disinfectants prior to UV light exposure. UV was dosed at 13 mJ.cm^{-2} using a LP collimated beam device. The microbial content of the river water, before and after disinfection treatments, was determined by enumerating total and Faecal Coliforms, as well as the aerobic

plate count (ACC). All enumeration was done using standard plate count techniques according to standard methods. Treatments were performed in triplicate and the physico-chemical properties of the river water was established prior to disinfection treatments.

GENERAL MATERIALS AND METHODS

E. coli inoculum

Two environmental *E. coli* strains were used to investigate the efficacy of chemical and UV disinfection methods. The selection of the two isolates was based on studies completed by Bester (2015) and Olivier (2015), who concluded that environmental strains F11.2 and MJ58 displayed greater resistance to specific stand-alone chemical and UV irradiation disinfection treatments. MJ58 showed increased resistance to Cl and H_2O_2 treatments and F11.2 showed increased resistance to PAA and UV irradiation treatments (Bester, 2015; Giddey *et al.*, 2015; Olivier, 2015).

The *E. coli* isolates were stored at – 80°C in 40% glycerol (v/v^{-1}) solution (Merck, South Africa). In order to revive these *E. coli* cultures, 100 µL of the defrosted bacterial suspension was inoculated in 5 mL nutrient broth (NB) (Biolab, South Africa). After incubation for 24 h at 37°C, a loop full of the mixture was streaked out on Levine's Eosin Methylene-Blue agar (L-EMB) (Oxoid, South Africa). Thereafter, plates were incubated for 20 h at 37°C. Pure *E. coli* colonies had a shiny metallic green appearance on L-EMB agar (Merck, 2005). Before disinfection experiments could be completed, pure *E. coli* colonies were transferred from L-EMB agar plates to sterile Nutrient Broth. The inoculated Nutrient Broth was then incubated for 24 h at 36°C, where after a specific volume was transferred to sterile SSS to obtain an approximate cell density equal to a 0.5 McFarland standard (BioMerieux, South Africa).

E. coli enumeration (Study 1)

Microbial analysis throughout this study was done using standard pour plate methods (Fig. 1). In all instances serial dilutions (10^{-1} – 10^{-6}) were prepared before and after all the specific disinfection treatments. All dilutions and plating was done in compliance with the South African National Standards (SANS) method 6886-1 (SANS, 1999). Violet Red Bile Agar (VRBA, Biolab, Merck) was used as the growth medium for *E. coli* throughout this study. All plating was done in duplicate, thereafter the poured plates were incubated for a period of 18 – 24 h at 36°C. The *E. coli* colonies were identified as red colonies surrounded by a red halo (Merck, 2007). Colonies were counted following standard guidelines (SANS 4832, 2007). The results were recorded as colony forming units per 100 mL (cfu.100 mL^{-1}).

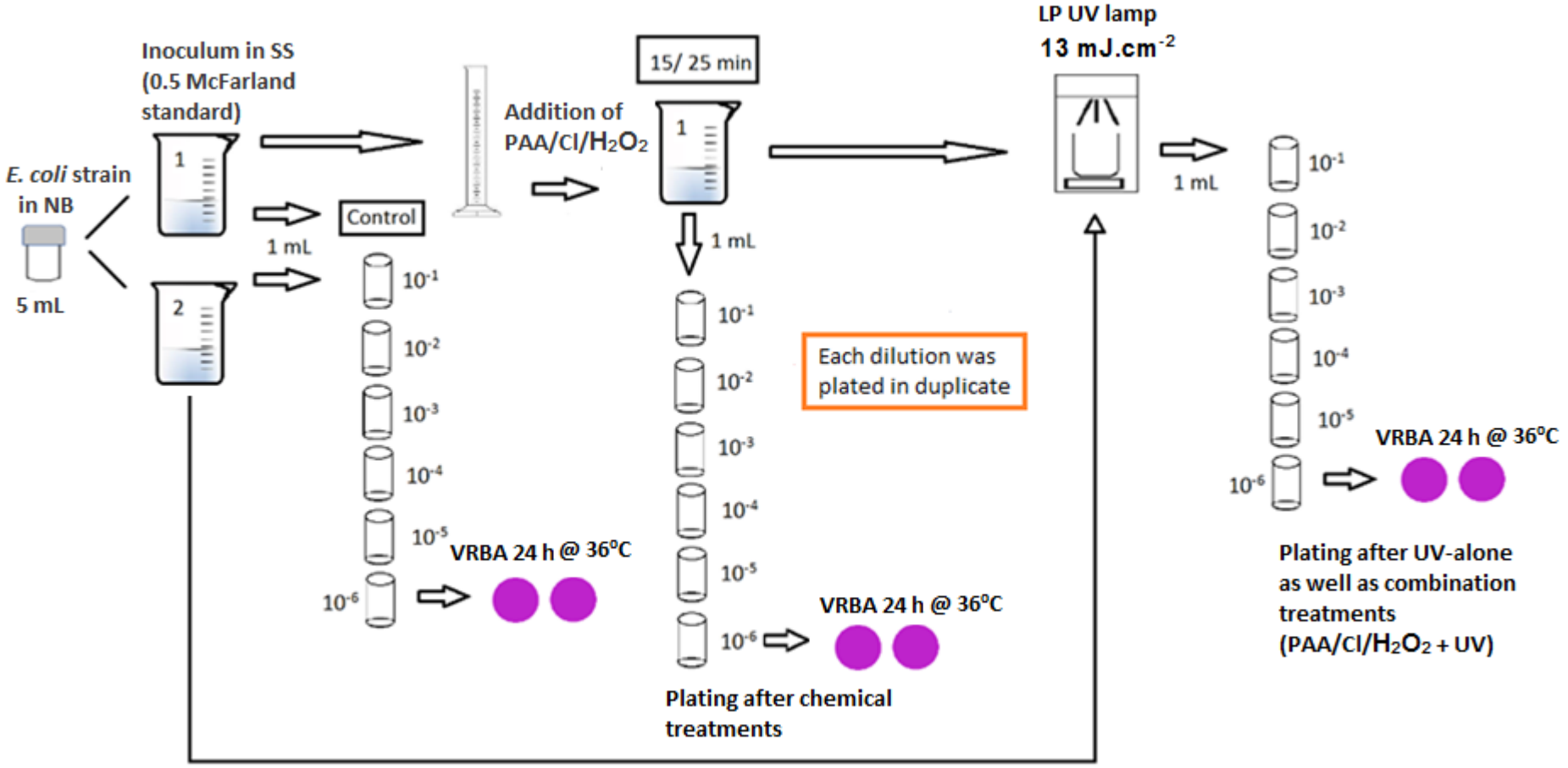

Figure 1 Experimental design used in Study 1 to determine the log reduction of environmental *E. coli* strains following the application stand-alone treatments and (UV; PAA, Cl; H_2O_2) combination treatments (PAA+UV;Cl+UV;H_2O_2+UV).

River water sampling (Study 2)

The river water used in Study 2 was obtained from the Plankenburg River in Stellenbosch, South Africa (33°56'15.4''S, 18°50'53.0''E). Sampling was done according to standard methods (SANS 5667- 6, 2006) in sterile 2 L bottles. Water was introduced into the bottles by submerging the bottle under the water and only then were the lids opened. When filled, lids were replaced whilst the bottles were still submerged. River water samples were transported in a cooler box and analysed within two hours after sampling.

Microbial enumeration (Study 2)

As in Study 1, microbial enumeration in Study 2 also involved standard pour plate techniques to quantify the number of microorganisms present before and after disinfection treatments. The total aerobic population was quantified using Plate Count Agar (PCA), (Merck, South Africa) and poured plates were inversely incubated for 48 h at 30±0.5°C, in accordance to South African National Standards (SANS) method 4833 (SANS, 2007b). This enumerated group was referred to as ACC (aerobic colony counts) and microbial growth was identified as glowing white colonies within the agar (SANS, 2007b). The total and Faecal Coliforms (TC and FC) were tested for using Violet Red Bile Agar (VRBA) (Biolab, South Africa) in accordance to the SANS method 4832 (SANS, 2007a). TC plates were inversely incubated at 36±0.5°C for 18 – 24 h. Faecal Coliforms (FC) were incubated at 44±0.5°C (Schraft & Watterworth, 2005). The coliforms were identified as red colonies surrounded by a red band of precipitate, according to Merck (2005). Experimentation was completed as shown in Figure 2.

LP-UV disinfection (Study 1 and 2)

All laboratory-scale studies that involved UV disinfection as a stand-alone treatment, or as part of a combined treatment (PAA+UV; Cl+UV and H_2O_2+UV), were carried out using a bench-top collimated beam device (Diagram 1) (Berson, The Netherlands). The disinfection was brought about by exposing the sample (either *E. coli* suspensions or river water) to an Amalgam Low-Pressure (LP) mercury vapour lamp (UV-Technik, Germany). The LP mercury lamp produced a power output of 40 W, which had an arc length of 250 mm. These LP-UV lamp dominantly emit light at a wavelength of 253.7 nm. In order to determine the intensity of the light produced by the lamp, a ILT1400 radiometer (International Light Technologies, USA) linked to a XRL140T254 detector (International Light Technologies, USA) was used. The subsequent reading was used in an equation (Morowitz, 1950; Hallmich & Gehr, 2010) to determine the time needed to induce a specific UV dose:

$$\textit{Desired dose } (mJ.cm^{-2}) = \textit{Average intensity } (mW.cm^{-2}) \times \textit{Exposure time (sec)} \quad (1)$$

Therefore, to attain the required UV dose the contact time had to be determined using the average intensity of the LP-UV lamp (at that specific time) and the desired UV dose required (1).

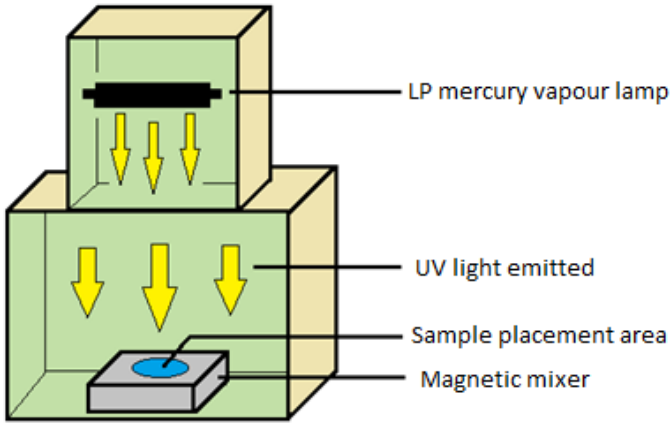

Diagram 1 Schematic representation of the bench-top collimated beam device used for laboratory-scale UV disinfection experiments.

Chemical disinfectants (Study 1 and 2)

Study 1 and 2 involved the use of three different commercial disinfectants, namely peracetic acid, sodium hypochlorite and hydrogen peroxide. A commercial peracetic acid (PAA) solution (Tsunami 100), composed of 31% acetic acid ($m.v^{-1}$), 15% peroxyacetic acid and 11% hydrogen Peroxide ($m.v^{-1}$) (Ecolab, South Africa) was used. The sodium hypochlorite (NaOCl) solution, with 15% ($m.v^{-1}$) available chlorine, was supplied by Metsi Water Solutions (South Africa). Hydrogen peroxide (H_2O_2) disinfectant used was prepared from 30% ($v.v^{-1}$) H_2O_2 (Merck, South Africa). Confirmation of the exact chemical concentrations for H_2O_2 and Cl was established prior to treatments, using the respective Spectroquant® Cell Tests (Merck, South Africa). In order to confirm the concentrations used for H_2O_2, a Spectroquant® Hydrogen Peroxide Cell Test (2.0 – 200 $mg.L^{-1}$) (Merck, South Africa) was used. To determine the exact concentrations of chlorine used the Spectroquant^R Chlorine Cell Test was used to determine the free chlorine available, as Chlorine is expected to deteriorate over time of storage. MQuant™ Peracetic Acid Test strips (Merck, South Africa) were used to indicate true concentrations of PAA in solution. Sodium Thiosulfate ($Na_2S_2O_3$) (Merck, South Africa) stock solution (1%) ($m.v^{-1}$) was used to the quench activity of PAA and Cl in order to obtain exact contact times for the Cl and PAA experiments. This was done by adding 1 mL of the Sodium Thiosulfate stock solution to 8 mL of SSS after the treatment time, which was then used for the 10^{-1} dilution according to the method of Mazzola *et al*. (2006).

Dilutions used were freshly prepared on each day of experimental analysis for PAA treatments and 4 $mg.L^{-1}$ solution was prepared and added to a sterile 500 mL glass beaker that contained 125 mL sample (*E. coli* suspended in SSS for Study 1 and river water for Study 2).

Following the same procedure a 6 mg.L^{-1} Cl solution and a 2.5 mg.L^{-1} H_2O_2 solution was prepared when required. The specific quantity of 125 mL sample was decided on to maintain similar properties with reference to future potential upscaling to pilot-scale (Chapter 4). The 125 mL sample produced a depth of approximately 22 mm in a 500 mL glass beaker. The distance from the UV lamp through water was estimated at approximately 22 mm in the pilot-scale to be tested in Chapter 4. In an attempt to simulate pilot-plant conditions a 125 mL sample was therefore used through Study 1 and 2. The exact disinfection protocol followed is displayed in Figures 1 and 2.

Statistical analysis

Upon completion of the trials the data obtained was analysed using Statistica 13.0 (Statsoft, 2014). One way, two way and mixed model repeated measures ANOVA were done where required.

River water disinfection (Study 2)

In Study 2 river water with varying microbial and physico-chemical quality was tested, instead of SSS inoculated with *E. coli*. The experimental procedure stayed the same as displayed in Figure 1, however, instead of 125 mL SSS solution 125 mL unfiltered river water was used (Fig. 2). Following aseptic procedures, a dilution series was prepared (10^{-1} – 10^{-6}) that served as a control at time = 0, prior to treatments. Post-disinfection the microorganisms surviving the different treatments were enumerated by preparing dilution series (10^{-1} – 10^{-6}), plated in duplicate using VRBA as well as PCA (Fig. 2). Only one contact time (25 min) was used for all three chemicals tested.

Physico-chemical parameters of River Water (Study 2)

pH, Electrical Conductivity, Turbidity and Ultraviolet Transmission Percentage (UVT%)

In order to measure the pH and temperature of the untreated river water, a WTW 320 pH meter (WTW, Germany) was used. The electrical conductivity (EC) of the water was measured using a HI 8733 conductivity meter (Hanna Instruments, USA) and the units expressed in mS.m^{-1}. Water turbidity was measured using a turbidity meter (Orion AQ3010 Turbidity Meter, Thermo Scientific, USA). The units used to express the turbidity are measured in Nephelometric Turbidity Units (NTU), and it is an expression of the effectiveness of light to pass through the specific sample. The UVT% of the untreated river water was measured by a hand-held, Sense™ Ultraviolet Transmittance Monitor (Berson, Germany) and the instrument was calibrated using deionised water before measurements.

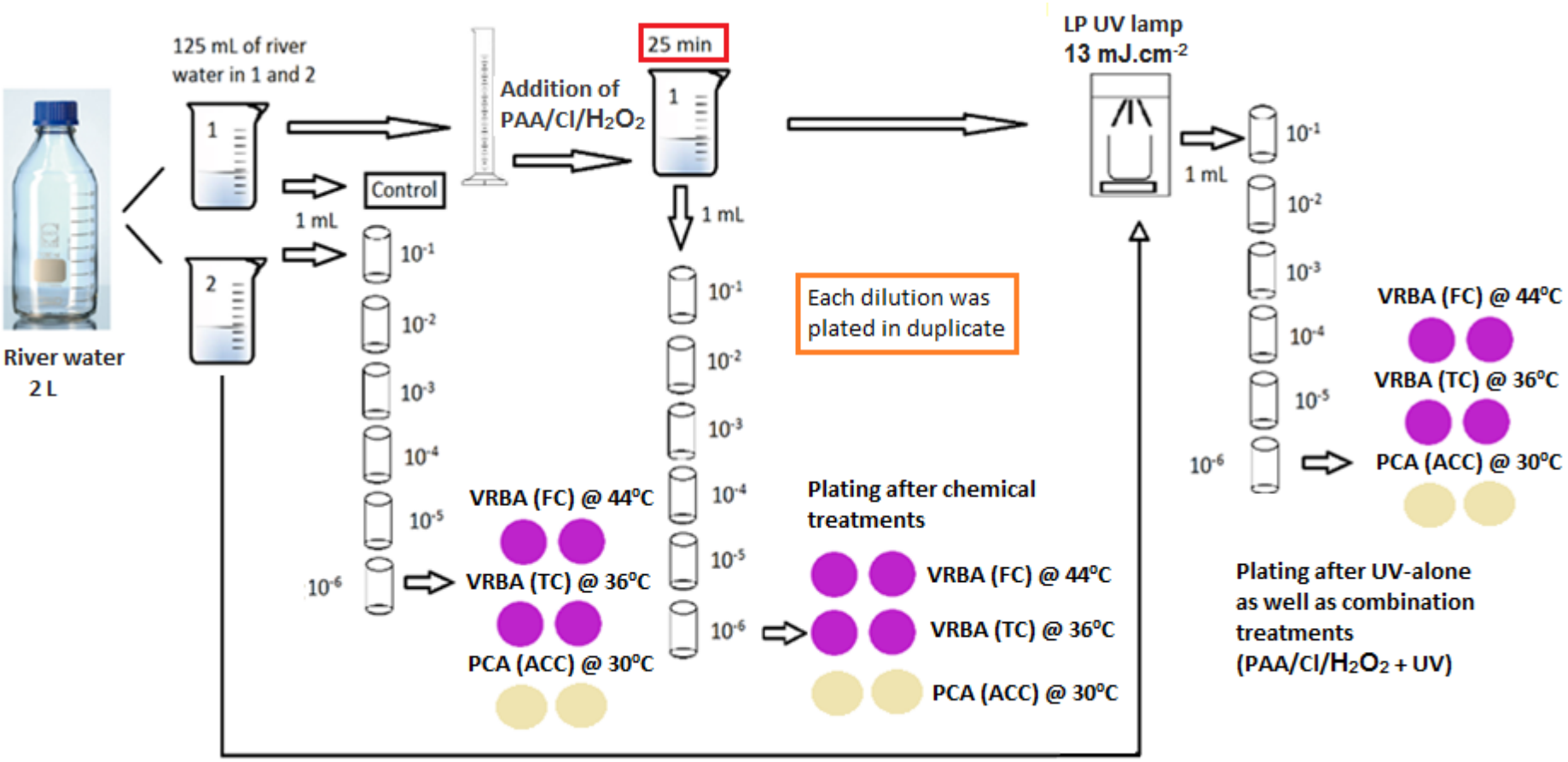

Figure 2 Experimental design used in Study 2 to determine the log reductions achieved in river water following the application of stand-alone treatments (UV; PAA;Cl;H_2O_2) and combination treatments (PAA+UV; Cl+UV; H_2O_2+UV)

TSS, COD and alkalinity

The Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), alkalinity and Chemical Oxygen Demand (COD) were measured according to the Standard Methods (APHA, 2005). The COD of the sample was measured using a Spectroquat® Nova 60 measured according to the Standard Methods (APHA, 2005). The COD range measured in was 10 – 150 $mg.L^{-1}$ and the specific COD Solutions A and B were used for this range (Merck Millipore, South Africa).

As certain physico-chemical parameters are suggested to be indicators of water quality, guidelines have been established regarding acceptable irrigation water quality for fresh or minimally processed produce (Table 1).

Table 1 Guidelines established for physico-chemical and microbial quality of water intended to be used for irrigational purposes of fresh produce (DWAF, 1996)

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

RESULTS AND DISCUSSION

Study 1: Efficacy of combination treatments on reducing E. coli strains in SSS

The effect of PAA and LP-UV (strain F11.2)

The effectiveness of PAA (4 $mg.L^{-1}$ for 15 and 25 min) as well as UV irradiation at a dose of 13 $mJ.cm^{-2}$ on *E. coli* strain F11.2 is shown in Fig. 3.

The results (Fig. 3) show a clear difference in log reductions for the different treatments. The stand-alone UV treatment recorded a 3.40 log reduction for *E. coli* strain F11.2. A 3.25 log reduction was recorded for the stand-alone PAA treatment (15/PAA) after a 15 min contact time (Fig. 3), which was not significantly more effective than the stand-alone UV treatment. This was in line with the results reported by Caretti & Lubello (2003), who found that after a 25 min contact time PAA (4 $mg.L^{-1}$) produced a 3.37 log reduction for *E. coli*. In this study, however, after a 25 min contact time, significantly better ($p<0.05$) log reductions were achieved for the stand-alone PAA treatment (25/PAA), with no growth recorded.

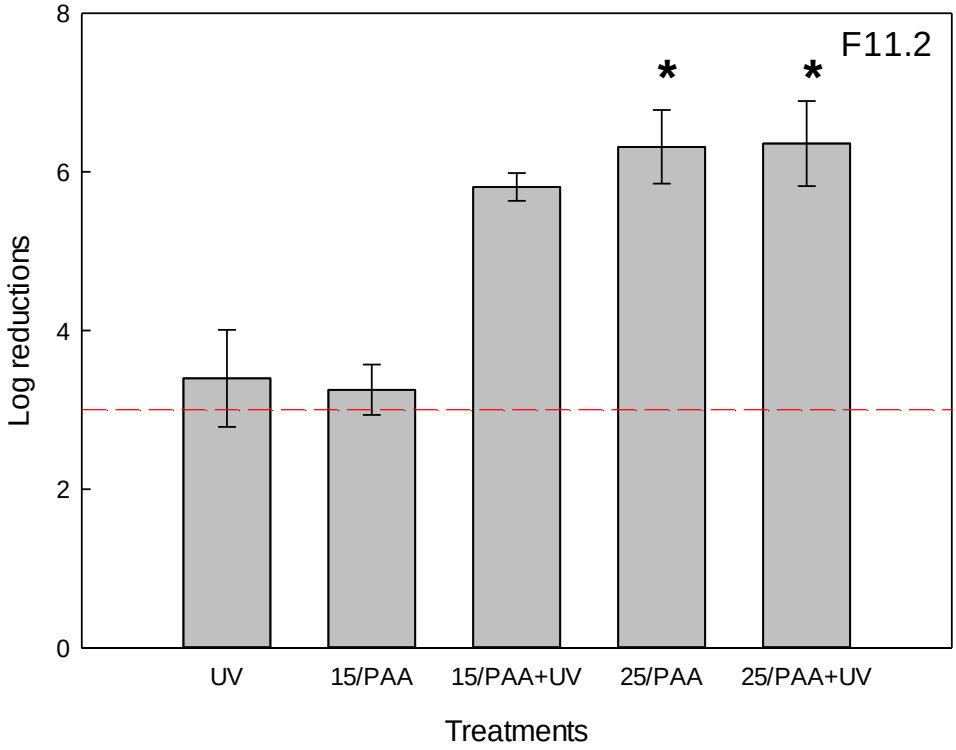

Figure 3 Disinfection efficacy observed after 15 and 25 min for PAA (4 mg.L^{-1}), UV (13 mJ.cm^{-2}) and combination (4 mg.L^{-1}+13 mJ.cm^{-2}) on environmental *E. coli* strain F11.2 in SSS. Error bars represent standard deviation calculated at a 95% confidence level.

* - No growth detected at lowest dilution (10^{0})

Thus, a 25 min contact time for PAA produced significantly better ($p<0.05$) log reductions for strain F11.2 than a 15 min contact time. The combination treatments, (15/PAA+UV) and (25/PAA+UV), produced similar disinfection, with no statistical difference ($p>0.05$) recorded between the two treatments. Over 5 log reductions were thus reported for (25/PAA), (15/PAA+UV) and (25/PAA+UV) treatments, with the combination treatments successfully eliminating *E. coli* strain F11.2 (Fig. 3) for nearly all the contact times tested.

Britz *et al.* (2013) suggested that at least, for most instances, a 3 log reduction in *E. coli* numbers is required to ensure river water of acceptable quality (< 1 000 cfu. 100 mL^{-1}). As this study was undertaken to ultimately contribute to the adequate disinfection of river water, a 3 log reduction was considered to give a good indication of the potential efficacy of the different treatments tested. The target 3 log reduction, indicated by the dotted red line (Fig. 3), was met by the (25/PAA), (15/PAA+UV) and (25/PAA+UV) treatments.

It is, therefore, clear that the stand-alone UV (13 mJ.cm^{-2}) and stand-alone (15/PAA) (4mg.L^{-1}) treatments, could be considered the lesser effective disinfection treatments (Fig. 3). Significantly better ($p<0.05$) disinfection was observed for both combination treatments (15/PAA+UV and 25/PAA+UV) as well as (25/PAA). Researchers also found that increasing the

contact time for chemicals was nearly always met with an increased disinfection, even when maintaining the same chemical concentration (Kits, 2004; Koivunen & Heinonen-Tanski, 2005a).

The effect of chlorine and LP-UV (strains F11.2 and MJ58)

As displayed in Figure 4, the disinfection efficacy of Cl (6 mg.L^{-1}) and LP-UV irradiation treatments, for both environmental *E. coli* strains MJ58 and F11.2, was investigated. The UV dose was kept constant throughout the treatments (13 mJ.cm^{-2}).

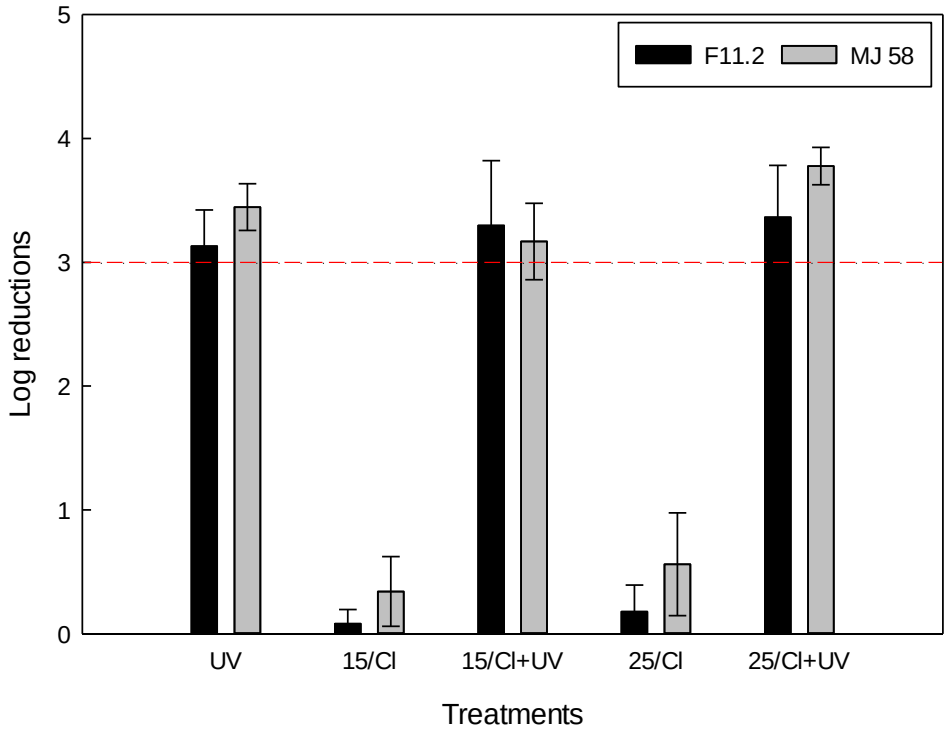

Figure 4 Log reductions observed after 15 and 25 min for Cl (6 mg.L^{-1}), UV (13 mJ.cm^{-2}) and a combination thereof on environmental *E. coli* strains F11.2 and MJ58 in SSS. Error bars represent standard deviation calculated at 95% confidence level.

For both *E. coli* strains F11.2 and MJ58, significantly better ($p<0.05$) disinfection for stand-alone UV treatments was observed (> 3 log reduction), compared to the stand-alone Cl treatments (15/Cl and 25/Cl). Stand-alone Cl treatments showed poor log reductions (< 1 log) in saline for both F11.2 and MJ58. Significant difference in log reductions were not found between F11.2 and MJ58 at either 15 or 25 min contact times for stand-alone Cl treatments. Neither were significant differences observed in log reductions for F11.2 and MJ58 when contact time was increased from 15 to 25 min (Fig. 4). According to Mezzanotte *et al.* (2007) contact time had little effect on the disinfection efficacy of Cl when dosing with low concentrations.

Combination treatments (15/Cl+UV and 25/Cl+UV) also did not produce significantly better ($p>0.05$) log reductions compared to the stand-alone UV treatments, therefore indicating that UV irradiation was the main contributor to the disinfection observed in the combination treatments.

Montemayor *et al.* (2008) reported a 3 log reduction in *E. coli* for combination treatments (Cl+UV) furthermore suggesting that UV irradiation was more effective than Cl regarding the combination treatments. Thus combination treatments (Cl+UV) as well as stand-alone UV treatments were overall more effective than the stand-alone Cl treatments at both 15 and 25 min contact times. When considering the 3 log reduction target (indicated by the dotted red line) all treatments that included UV irradiation (UV, 15/Cl+UV; 25/Cl+UV) were able to meet this requirement for both F11.2 and MJ58.

The effect of H2O2 and LP-UV (strains F11.2 and MJ58)

Lastly, the effect of H_2O_2 in combination with UV irradiation was investigated for both *E. coli* strains F11.2 and MJ58. The disinfection potential of H_2O_2 at a concentration of 2.5 $mg.L^{-1}$ was combined with the UV irradiation (13 $mJ.cm^{-2}$) and the results are presented in Fig. 5.

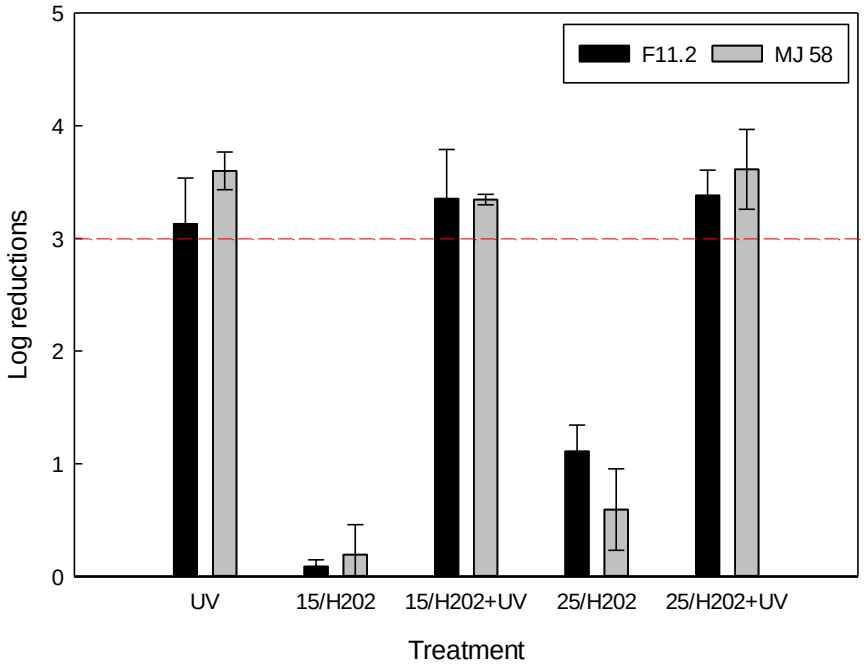

Figure 5 Log reductions observed after 15 and 25 min for H_2O_2 (2.5 $mg.L^{-1}$), UV (13 $mJ.cm^{-2}$) and in combination on *E. coli* strains F11.2 and MJ58 in SSS. Error bars represent standard deviation calculated at 95% confidence level.

From the results (Fig. 5), it was clear that stand-alone UV irradiation was significantly more ($p<0.05$) effective than the chemical disinfectant (H_2O_2), similar observation for stand-alone UV irradiation were shown for the PAA disinfection Trial (Fig. 3). The stand-alone disinfection efficacy of H_2O_2 at both contact times (15 and 25 min) for *E. coli* strains F11.2 and MJ58 was unable to reach the 3 log target reduction (indicated by the dotted red line). However, there was a

significantly better ($p<0.05$) log reduction value recorded for strain F11.2 after 25 min ($25/H_2O_2$) compared to 15 min ($15/H_2O_2$) contact times. For strain MJ58 there was no significant difference ($p>0.05$) in disinfection efficacy recorded between the two contact times ($15/H_2O_2$ vs $25/H_2O_2$). Similar log reductions were recorded by Linley *et al.* (2012), who observed a 0.25 log decrease for environmental *E. coli* after allowing 15 min contact time with H_2O_2 (2.5 $mg.L^{-1}$). Furthermore, Linley *et al*. (2012) showed that up to 30 $mg.L^{-1}$ H_2O_2 produced only 1 log reduction for environmental *E. coli*. Olivier (2015) reported just over a log reduction for F11.2 at a H_2O_2 concentration of 2.5 $mg.L^{-1}$, similar to the reductions observed in this study after the ($25/H_2O_2$) treatment (Fig. 5).

Significant differences ($p<0.05$) were observed between the stand-alone H_2O_2 treatments ($15/H_2O_2$ and $25/H_2O_2$) when compared to the treatments involving UV irradiation (UV, $15/H_2O_2$+UV; $25/H_2O_2$+UV). Thus, all treatments involving UV irradiation were able to meet the target 3 log reduction for both strains (F11.2 and MJ58) (Fig. 5). Furthermore the combination treatments ($15/H_2O_2$+UV and $25/H_2O_2$+UV) did not produce significantly better ($p>0.05$) disinfection compared to the stand-alone UV treatment, thus suggesting UV disinfection to be the dominant contributor to combination treatments efficacy.

Overall, the stand-alone UV treatment (13 $mJ.cm^{-2}$) was able to meet the 3 log target reduction in all instances (Figs. 3 – 5). Cl (6 $mg.L^{-1}$) and H_2O_2 (2.5 $mg.L^{-1}$) stand-alone treatments (Fig. 4 and 5) proved to be significantly less ($p<0.05$) effective in reducing *E. coli* strains F11.2 and MJ58 than UV irradiation treatments at both time intervals (15 and 25 min). PAA, however, did not follow the same trends as Cl and H_2O_2, as the UV treatment was not significantly ($p>0.05$) more effective than the stand-alone PAA treatment after 15 min (15/PAA). Furthermore, allowing 25 min contact time for PAA (25/PAA), a significantly better ($p<0.05$) reduction was observed for F11.2 than the stand-alone UV treatment (Fig. 3). Koivunen & Heinonen-Tanski (2005b) reported that PAA (3 $mg.L^{-1}$) in combination with UV (14 $mJ.cm^{-2}$) were able to produce an *E. coli* log reduction of 5.97 ± 0.34 log. Their results were thus similar to results presented in Fig. 3, reporting a > 5 log reduction for *E. coli* strain F11.2. According to Mezzanotte *et al.* (2007) contact time carried minimal significance regarding Cl disinfection efficacy but had a definite influence regarding PAA disinfection at low concentrations. Their findings are consistent with the results presented in Figures 3 and 4, as there was a significantly better ($p<0.05$) disinfection observed for PAA after 25 min to that of 15 min.

It could be concluded that the stand-alone PAA treatments (4 $mg.L^{-1}$) (15/PAA and 25/PAA) were able to meet the target 3 log reduction, whereas Cl and H_2O_2 treatments were unable to achieve more than 1 log reduction for either F11.2 or MJ58. Therefore, PAA was the more effective chemical disinfectant when compared to Cl and H_2O_2 for strain F11.2 (Fig. 3 – 5). H_2O_2 treatments (Fig. 5) resulted in increased log reductions for 25 min contact times compared to reductions after only 15 min, showing a similar trend as was observed for PAA (Fig.3). Increased contact times (25 min) would thus be considered more effective for PAA and H_2O_2 treatments.

All stand-alone UV treatments and combinations treatments for both Cl and H_2O_2 recorded just over a 3 log reduction for both strains (F11.2 and MJ58), thus satisfying the target 3 log reduction (Fig.4 and 5). When considering the poor contributions made by the stand-alone Cl and H_2O_2 treatments it was clear that UV irradiation was the fundamental contributor to the disinfection reported for the combination treatments (Fig. 4 and 5).

Lastly, not a great degree of variation was recorded between the stand-alone UV treatments and the combination thereof with Cl and H_2O_2 (Fig. 4 and 5), as both were able to meet the 3 log target reduction. Research has shown that combination treatments can be considered more reliable, as potential shortcomings of the stand-alone treatments can be counteracted when applied in combination with one another (Sharp *et al.*, 2006; Pereira *et al.*, 2007). Also, as the combination treatments efficacy is due to the sum of the stand-alone treatments, a broader range of microorganisms can be targeted, as different microorganisms have shown to vary in resistance to different chemical and photochemical treatments (Bester, 2015; Giddey *et al*., 2015; Olivier, 2015). Montemayor *et al.* (2008) also concluded that the combination of UV irradiation together with low doses of Cl would be recommended to sufficiently reduce a broad range of microorganisms and leave an acceptable residual for enhanced safety for irrigation water. Furthermore, these findings were critical, as current disinfection methods involve the application of high concentrations of chemical disinfectants in order to produce successful disinfection. Durak *et al.* (2012) reported that using high concentration of Cl only was able to minimal increase in the disinfection efficacy observed for Cl disinfection, even when using Cl concentrations of up to 200 $mg.L^{-1}$.

In conclusion, potential exists in applying combination treatments (Fig. 3 – 5) to successfully reduce *E. coli* even when applied in low concentrations for chemicals (PAA, Cl and H_2O_2) and UV irradiation (13 $mJ.cm^{-2}$). The effect of varying water quality on the combination treatment efficacy is, however, not yet known.

Study 2: Influence of river water on the efficacy of chemical and UV treatments

River water quality

The water quality of the river water used for each treatment was evaluated by determining a variety of physico-chemical parameters (Table 2). These physico-chemical properties were compared to guidelines set by the Department of Water Affairs (DWA) regarding irrigation water quality. The initial microbial loads present in the river water on each of the specific sampling days were also recorded (Table 3). The results recorded in Table 2 provide a better understanding of the water quality and thus the disinfection potential of the different treatments investigated (Fig. 6 – 8). Upon evaluation of the water quality presented in Table 2, there was clear variation observed between the physico-chemical properties of the water used in the different Trials 1 – 3.

Table 2 Physico-chemical properties of river water sampled from the Plankenburg River prior to disinfection treatments

Parameter	Trial 1	Trial 2	Trial 3
UVT %	68.00	63.30	11.30
Turbidity (NTU)	6.70	4.28	151.00
Conductivity ($mS.m^{-1}$)	42.00	33.30	51.50
pH	6.60	6.74	6.35
Alkalinity ($mg.L^{-1}$ $CaCO_3$)	75.00	50.00	40.00
COD ($mg.L^{-1}$)	23.00	19.80	55.80
TSS ($mg.L^{-1}$)	7.00	7.00	20.60
VSS ($mg.L^{-1}$)	3.00	5.00	16.00

The results (Table 2) show that, with regard to the ultraviolet transmission (UVT%), water sampled in Trials 1 and 2 had similar UVT% values, while the water in Trial 3 had a much lower UVT% (11.30%). As UVT% is often used as an indicator of UV irradiation efficacy, it was expected that the water quality in Trial 3 would influence the efficacy of UV irradiation more negatively than in Trials 1 and 2 (Salgot *et al.*, 2002; Koutchma, 2009).

Another important optical characteristic measured was the water turbidity. The water used in Trial 3 had an exceptionally high value (151.00 NTU) when compared to the results recorded for Trials 1 and 2 (Table 2). Freese & Nozaic (2004) stated that high turbidity levels could have a negative effect on UV disinfection. In addition to the lowest UVT% and highest turbidity, Trial 3 river water also had the highest TSS (20.60 $mg.L^{-1}$) value of the three Trials. The river water investigated for Trials 1 and 2 each recorded TSS values of 7.00 $mg.L^{-1}$ (Table 2), TSS is considered a measure of the suspended organic and inorganic matter present in water (Bilotta & Brazier, 2008). The TSS guideline for irrigation water is, however, < 50 $mg.L^{-1}$, placing all three trials within the acceptable range (DWAF, 1996). According to Koutchma (2009), adverse water quality parameters, as measured in Trial 3, could be responsible for reducing the amount of photons penetrating the water and therefore compromising the efficacy of UV irradiation.

The conductivity measured in Trial 2 was able to meet the guideline of 40 $mS.m^{-1}$ (DWAF, 1996), however, it was exceeded for Trials 1 and 3, recording 42.00 and 51.50 $mS.m^{-1}$ respectively. EC gives an indication on the amount of dissolved inorganic salts that may be present in water and is often associated with an increased COD value (DWAF, 1996). In this study, the water used in Trial 3 recorded the highest EC also recorded the highest COD value (55.80 $mg.L^{-1}$) when compared to Trials 1 and 2 (Table 2). According to Wagner *et al.* (2002) a COD value reported in the range of 20 – 120 $mg.L^{-1}$ would be considered an intermediate wastewater, thus classifying the water of Trial 3 as something between secondary effluent and

untreated sewage water. Therefore, the least favourable water was sampled for Trial 3, with lower COD values measured for the water of Trials 1 and 2 (reporting COD values of 23.00 and 19.80 $mg.L^{-1}$) respectively (Table 2).

The water collected for Trial 2 was the only sample that had a conductivity value falling within the guideline limits. It also had the lowest turbidity and COD values for all three trials. It was concluded that the water used in Trial 2 was of better quality, when compared to Trials 1 and 3 (Table 3). Furthermore, guidelines (DWAF, 1996) suggested that the optimal pH for irrigation water is pH 6.5 - 8.4, thus the water for Trials 1 and 2 (Table 2) were able to comply with this guideline. A pH value of 6.35 was however measured for the water tested in Trial 3, which was slightly more acidic than the water in Trials 1 and 2. As the water sampled for Trial 3 recorded a high turbidity, the lowest UVT% and highest COD values, amongst others, it displayed the poorest water quality from a physico-chemical perspective, in comparison to the water sampled in Trials 1 and 2. Thus, decreased disinfection efficacy was expected for Trial 3.

When evaluating Trials 1 – 3 (Table 3), it became clear that the river water not only had varying physico-chemical properties, but also considerable microbial variations for the different days of sampling. The initial microbial numbers presented in Table 3, recorded in cfu.100 mL^{-1}, revealed extremely high levels of microbial contamination for the enumerated microbial groups (ACC, TC and FC) (Trials 1 – 3). When considering the DWAF (1996) guideline regarding FC levels, allowing only 1 000 cfu.100 mL^{-1}, the microbial levels recorded for all trials were above the acceptable levels (Table 3). Irrigating with the river water (Trials 1 – 3) would pose a huge risk to human safety without any intervention prior to irrigation, increased possibility of potentially pathogenic microorganisms being transferred from crops to humans. Park *et al.* (2012) suggested that high levels of microbial contamination detected in river water, and ultimately in irrigation water, could be the main cause of pathogens transferred to fresh produce before human consumption.

When comparing the initial microbial levels of Trials 1 – 3 (Table 3), it was clear that the water sampled in Trial 3 had the highest number of ACC (2 390 000 cfu.100 mL^{-1}), TC (320 000 cfu.100 mL^{-1}) and FC (60 900 cfu.100 mL^{-1}) averages, far above the recommended guideline (DWAF, 1996). High levels of microbial contamination have also been reported by other researches regarding the water quality of the Plankenburg River (Bester, 2015; Giddey, 2015; Olivier, 2015). Paulse *et al.* (2009) reported FC levels as high as 3.5×10^{6} present in the water from the Plankenburg River.

The water sampled in Trial 3, which reported the poorest physico-chemical properties (Table 2), also recorded the least favourable microbial quality (Table 3). Water used in Trial 2, which reported the most acceptable physico-chemical quality, also had the lowest microbial counts of the water sampled for the three trials.

Table 3 Microbial counts for unfiltered river water (Trials 1 – 3) before disinfection experiments, recorded in (cfu. 100 mL^{-1})

	UV	PAA	Cl	H_2O_2	Average
Trial 1					
ACC	1.56×10^6	4.9×10^5	1.14×10^6	9.9×10^5	1.45×10^6
TC	1.46×10^5	1.41×10^5	3.71×10^4	1.35×10^5	1.15×10^5
FC	2.24×10^4	1.47×10^4	1.8×10^4	9.0×10^4	3.6×10^4
Trial 2					
ACC	9.7×10^5	8.4×10^5	5.0×10^5	1.01×10^6	8.3×10^5
TC	1.51×10^5	8.2×10^4	6.4×10^4	8.5×10^4	9.55×10^4
FC	6.0×10^4	2.9×10^4	1.14×10^4	4.2×10^4	3.56×10^4
Trial 3					
ACC	3.7×10^6	1.11×10^6	2.1×10^6	2.64×10^6	2.39×10^6
TC	3.3×10^5	2.7×10^5	2.4×10^5	4.4×10^5	3.2×10^5
FC	9.1×10^4	7×10^4	3.48×10^4	4.8×10^4	6.09×10^4

ACC – Aerobic colony count; TC – Total coliforms; FC – Faecal Coliforms

River water disinfection

The disinfection results for Trial 1 (Figure 6), clearly show variations between the different treatments investigated. When considering the stand-alone treatments, UV disinfection appeared to be the most effective treatment (for TC and FC) when compared to that of PAA, Cl and H_2O_2 (Fig. 6). The stand-alone UV treatment (13 $mJ.cm^{-2}$) recorded log reductions of 5.16 and 4.35 log for TC and FC respectively, although only 1.66 log reduction was recorded for ACC (Fig. 6). Furthermore, stand-alone UV irradiation was adequate to reduce TC and FC levels completely (no growth was detected at the lowest dilution), however, significantly less ($p<0.05$) effective reductions were recorded for aerobic colonies (ACC).

With the use of the collimated beam, LP-UV wavelengths are predominantly emitted at the specific wavelength of 253 nm, which has proven effective for *E. coli* disinfection, which are Gram-negative microorganisms associated with the coliform groups (Zimmer & Slawson, 2002; Hijnen *et al.*, 2006; Ijpelaar *et al.*, 2010). Beauchamp & Lacroix, (2012) however, suggested increased UV resistance displayed by Gram-positive microorganisms when compared to Gram-negative bacteria. Furthermore, aerobic populations included a greater variety of microorganisms (including Gram-positive) which could potentially display greater resistance to UV light disinfection. For this reason, significantly poorer ($p<0.05$) log reductions were nearly always reported for ACC numbers when compared to the TC and FC groups in Trial 1 (Fig. 6). Predictably

similar trends were observed for the combination treatments (PAA+UV; Cl+UV and H_2O_2+UV) with regard to the variations between ACC and the coliform groups.

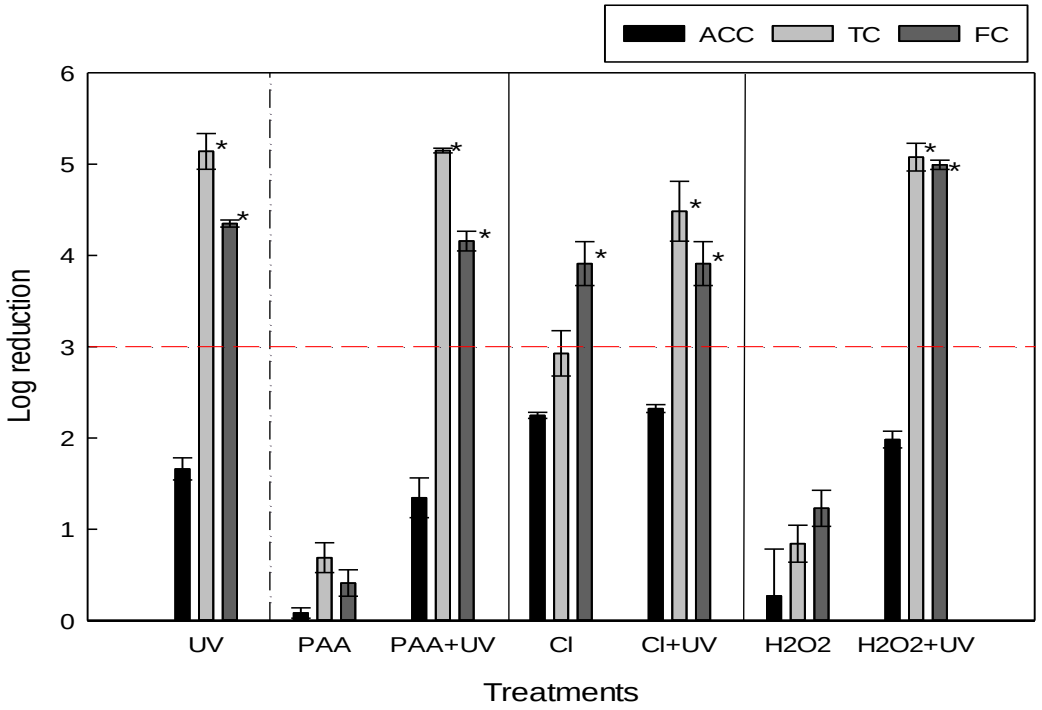

Figure 6 Log reductions observed for Trial 1 after river water was exposed to PAA (4 $mg.L^{-1}$), Cl (6 $mg.L^{-1}$) and H_2O_2 (2.5 $mg.L^{-1}$) for 25 min, alone and in combination with UV (13 $mJ.cm^{-2}$). Error bars were calculated from a standard deviation at a 95% confidence level. * - No growth detected at lowest dilution (10^0)

When evaluating the log reductions achieved for the stand-alone PAA and H_2O_2 treatments in Trial 1 (Fig. 6), it was clear that there was little overall contribution made by these chemicals at the low concentrations tested. PAA was unable to produce even 1 log reduction for all enumerated groups (ACC, TC and FC). H_2O_2 treatments, however, reported significantly better ($p<0.05$) FC disinfection compared to PAA, reaching a 1 log reduction (1.23 log) for FC. Significantly greater ($p<0.05$) microbial reductions were reported for the stand-alone Cl treatment (6 $mg.L^{-1}$) for ACC, TC and FC when compared to that achieved by PAA and H_2O_2. Chlorine disinfection was also the only stand-alone chemical treatment able to reach the target 3 log reduction for FC (Fig. 6), indicated by the dotted red line. When considering the FC levels of the river water (Table 3) chlorine, thus, effectively induced up to a 4 log reduction in FC, as no microbial growth was detected after the treatment.

The combination treatments (PAA+UV and H_2O_2+UV) were able to produce adequate log reductions, reaching the target 3 log reduction for TC and FC. It was, however, clear that UV irradiation was the main contributor to the overall disinfection efficacy reported for combination treatments (PAA+UV) and (H_2O_2+UV), as poor stand-alone disinfection efficacy was achieved for the individual chemicals.

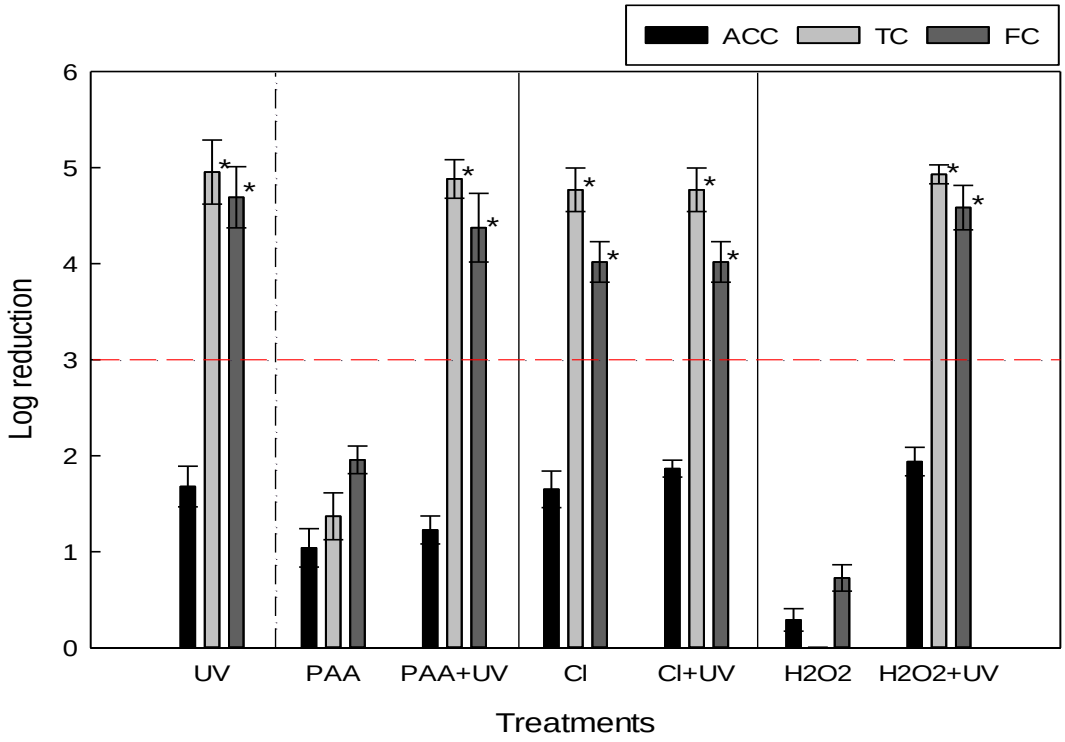

Figure 7 Log reductions observed for Trial 2 after river water was exposed to PAA (4 mg.L^{-1}), Cl (6 mg.L^{-1}) and H_2O_2 (2.5 mg.L^{-1}) for 25 min, alone and in combination with UV (13 mJ.cm^{-2}). Error bars were calculated from standard deviation at a 95% confidence level. * - No growth detected at lowest dilution (10^0)

The results of Trial 2 (Fig. 7) indicated similar disinfection trends as observed for Trial 1 for the stand-alone UV treatments, with FC being reduced by 4.69 log. As in Trial 1 the stand-alone UV treatment (13 mJ.cm^{-2}) was sufficient to meet the target 3 log reduction for the coliform groups.

Furthermore, combination treatments (PAA, H_2O_2 and UV) reported significantly better ($p<0.05$) log reductions for FC and TC to that of the stand-alone chemical treatments, with the exception of the stand-alone Cl treatment. For the stand-alone Cl treatment there were no significant differences ($p>0.05$) observed in log reductions of enumerated groups (ACC, TC and FC) compared to that of the combination treatment (Cl+UV). Indicating (from the results of Trial 2) stand-alone Cl treatments to be the most effective disinfectant between PAA, Cl and H_2O_2. Furthermore, in all the combination treatments no growth was detected for FC and TC at the lowest dilution (10^{-6}), therefore, satisfying the target 3 log reduction requirement (Fig. 7).

The physico-chemical parameters (specifically optical characteristics, UVT% and turbidity) measured for Trial 2 were also similar to Trial 1, which could have contributed to the similar UV efficacy observed between the two trials (Figs. 6 and 7). Improved Cl and PAA disinfections were, however, reported for Trial 2 compared to that achieved for Trial 1. This could be related to variation in the microbial quality (species and strains), as well as slightly lower turbidity, conductivity, alkalinity and COD values of the river water sampled in Trial 2.

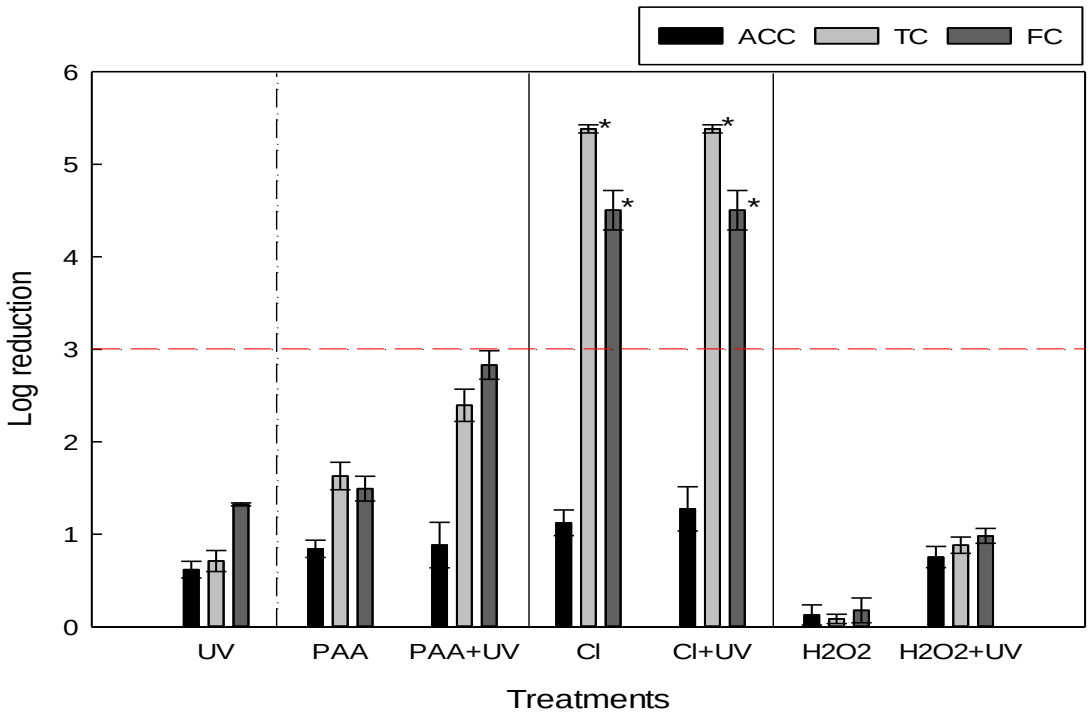

Figure 8 Log reductions observed for Trial 3 after river water was exposed to PAA (4 $mg.L^{-1}$), Cl (6 $mg.L^{-1}$) and H_2O_2 (2.5 $mg.L^{-1}$) for 25 min, alone and in combination with UV (13 $mJ.cm^{-2}$). Error bars are calculated from standard deviation at a 95% confidence level. * - No growth detected at lowest dilution (10^0)

The results presented in Fig. 8 indicated that the stand-alone UV treatments investigated in Trial 3, resulted in significantly lower ($p<0.05$) log reductions in ACC, TC and FC when compared to Trials 1 and 2. UV irradiation resulted in log reductions of only 0.62 log (ACC), 0.71 log (TC) and 1.32 log (FC). Koutchma (2009) reported that poor UVT% would influence the overall UV disinfection efficacy negatively. A poor UVT% is often associated with increased organic and inorganic particles present in liquid, which can shield microorganisms from the damaging UV light waves (Koutchma, 2009). Trial 3 had the poorest UVT% of all three trials, measuring a value as low was 11.30% (Table 2).

The stand-alone Cl treatment was very effective in reducing TC and FC numbers, with no microbial growth detected at the lowest dilution. Dissimilar results were seen for the ACC groups, as in most instances the microbial populations enumerated for the ACC groups displayed increased resistance to the disinfection treatments. When comparing the log reductions observed in Trial 2, with regards to the stand-alone Cl treatments, there were not significant differences ($p>0.05$) observed, regardless of the poorer water quality measured in Trial 3. Similar to the results recorded in Trials 1 and 2, the stand-alone PAA and H_2O_2 treatments were both unable to reach the target 3 log reduction for the coliform groups (Figs. 6 and 7).

H_2O_2 treatments produced poor log reductions, achieving no more than 1 log reduction in FC (Fig.8). The poor contribution made by UV irradiation to the overall disinfection observed in

Trial 3, could potentially be related to the poor optical characteristics (UVT% and turbidity) recorded for the river water on the specific day of sampling (Table 2).

It was clear that there were factors influencing the efficacy of UV disinfection for all the stand-alone UV treatments (Trials 1 – 3), as similar log reductions were not recorded for all 3 trials (Figs. 5 – 7). Trials 1 and 2 had comparable water quality (Table 2), which could have contributed to similar log reductions being observed in the two trials (Figs. 6 and 7). In both Trials 1 and 2, a 4 – 5 log reduction for both TC and FC were observed, thus meeting the target 3 log reduction. However, during Trial 3 (Fig. 8) significantly poorer ($p<0.05$) log reductions (ACC, TC and FC) was observed with regards to the UV treatments. Considering the water quality parameters presented in Table 3, the water of Trial 3 recorded a very low UVT% (11.30), high turbidity (151.00 NTU), as well as the least favourable TSS value of the three trials (Table 2). Therefore, variation in disinfection achieved by UV irradiation could be accounted to unfavourable water quality. In UV irradiation a UV dose of 13 $mJ.cm^{-2}$ was sufficient to inactivate TC and FC (Trials 1 – 2). However, UV efficacy was compromised in Trial 3 (Fig. 8) in comparison to Trials 1 and 2; this could be directly linked to poor optical water quality parameters. Due to the unpredictable and ever changing nature of the microbial and physico-chemical parameters associated with river water, variation in disinfection efficacy for similar treatments has been reported (Koutchma, 2009; Britz *et al.*, 2013; Olivier, 2015).

Considering the stand-alone chemical treatments, Cl at a concentration of 6 $mg.L^{-1}$ for all trials (Figs. 6 – 8) was the most effective disinfectant compared to PAA and H_2O_2, regardless of the water quality (Table 3). Furthermore, the stand-alone Cl treatments were able to meet the target 3 log reduction for FC in all three trials. Poorest Cl log reductions were however reported for water sampled in Trial 1, which also measured the highest alkalinity of the three trials. Watts & Linden (2007) suggested an increased alkalinity value were linked to an increased chlorine demand when treating water. Therefore, suggesting a possible explanation for the less effective disinfection when using chlorine for Trial 1. PAA (4 $mg.L^{-1}$) was not capable of reaching the target 3 log reduction for FC and was significantly less ($p<0.05$) effective than Cl in the three trials (Figs. 6 – 8). Veschetti *et al.* (2003) also found that PAA was less effective than Cl in reducing more resistant environmental *E. coli*. Mezzanotte *et al.* (2007) also reported that Cl treatments (6 $mg.L^{-1}$/25 min) resulted in a 4 log reduction for both TC and FC. Similar results were observed in this study (Figs. 6 – 8).

H_2O_2 treatments produced the poorest log reductions (Figs. 6 – 8) and was significantly less ($p<0.05$) effective than Cl, especially for the TC and FC. Koivunen & Heinonen-Tanski (2005a) also reported poor disinfection efficacy when using H_2O_2 (3 $mg.L^{-1}$), further suggesting PAA to be more effective than H_2O_2 in reducing a large variety of microorganisms. According to Wagner *et al.* (2002) PAA may penetrate microbial cells more easily, therefore improving its disinfection properties compared to that of H_2O_2. Some microorganisms may also be protected against H_2O_2 by the activity of catalase enzymes, which are less effective against PAA disinfection

(Chapman, 2003). H_2O_2 disinfection was highly variable between the three trials, which made it difficult to identify trends between log reductions expected for ACC, TC and FC groups during each of the three trials.

Significantly better ($p<0.05$) log reductions were reported for PAA disinfection in Trials 2 and 3 compared to Trial 1 for ACC, TC and FC. According to Gehr *et al.* (2003) high COD levels (>100 $mg.L^{-1}$) resulted in poor microbial reductions when using PAA as a disinfectant. However, as the COD of Trial 1 was nearly half that recorded for Trial 3 (where PAA disinfection produced better log reductions), it is unlikely that the COD content was the reason for the less effective log reductions observed in Trial 1. Furthermore, differences in alkalinity were reported for Trials 1 and 2, with the water in Trial 1 recording a higher alkalinity (75 $mg.L^{-1}$ $CaCO_3$) when compared to Trial 2 (50 $mg.L^{-1}$ $CaCO_3$). A higher alkalinity value could result in water with a greater buffering potential (resistance to changes in pH), often chemical disinfectants depend on changes in pH to induce a germicidal effect, specifically in the case of PAA disinfection (Kitis, 2004; Newman, 2004). Thus, significantly poorer ($p<0.05$) disinfection was observed for PAA treatments (Trial 1), when compared to Trial 2, could be linked to the higher alkalinity reported for the water in Trial 1 (Table 2).

Differences in initial microbial numbers (Table 3) also suggested a possible reason for variation in disinfection (between different trials but for the same treatments). As the river water was sampled on three different days (for the three trials), the water was expected to be of a heterogeneous nature, implying variations in quantities, species and strains of microorganisms. The day to day variations in microorganisms present in the river water, in addition to the variations brought about by the differences observed in physico-chemical parameters, could thus explain the significant differences ($p<0.05$) observed between the same treatments but on different days.

Other factors could also contribute to variations in disinfection efficacy and these include the current growth phase of the microorganisms and the degree of environmental stress they were exposed to before the specific disinfection treatments (Berney *et al.*, 2006; Gayán *et al.*, 2014). Furthermore, other factors such a microorganisms exposure to sunlight prior to disinfection, the presence of chemical pollution in the water and general resistance built up over time due to previous disinfection treatments, all decrease disinfection efficacy (van der Veen & Abee, 2011).

Combination treatments (PAA+UV, Cl+UV, H_2O_2+UV) investigated in Trials 1 and 2 resulted in no microbial growth for TC and FC. However, in Trial 3 the (PAA+UV) treatment was only able to completely reduce the FC group. (Cl+UV) was sufficient to reduce both the TC and FC till a point where no growth was detected (Trials 1 – 3). However, when poor UV disinfection was reported, linked to unfavourable physico-chemical parameters (Trial 3), compromised disinfection was generally the result thereof regarding combination treatments.

Stand-alone Cl treatments seemed to be the most effective chemical disinfectant treatments, followed by stand-alone PAA, which showed a significantly better ($p<0.05$) disinfection efficacy compared to that of H_2O_2 (Trials 1 – 3). Similar trends were followed for the combination

treatments. Lubello *et al.* (2002) also concluded that the combination of (PAA+UV) had a greater disinfection effect than that of (H_2O_2+UV).

Nevertheless, although stand-alone chlorine disinfection proved to be the most effective, residual chorine levels post-disinfection had to be considered (Table 4). A guideline of $\leq$ 0.25 mg.L^{-1} (DWA, 2013b) has been suggested for wastewater intended to be used for irrigation purposes, which in the case of this study was exceeded by all three trials (Table 4) for the stand-alone Cl residual levels. Trial 3 recorded the lowest residual (1.35 mg.L^{-1}) for the post-treated water, which was still nearly 5 times that of the guideline (DWA, 2013b). Thus, chlorine 6 mg.L^{-1} proved more effective in this study. However residual levels would need to be considered as high levels would promote the formation of DBPs (Diehl *et al.*, 2000; Al-Juboori *et al.*, 2015).

Table 4 Residual stand-alone chlorine levels recorded in river water, post-treatment, when chlorine was dosed at 6 mg.L^{-1}, for Trials 1 – 3

TRIAL	**Residual Cl (mg.L^{-1})**
Trial 1	1.54 mg.L^{-1}
Trial 2	1.62 mg.L^{-1}
Trial 3	1.35 mg.L^{-1}

Exceptionally high microbial levels were recorded for the river water sampled from the Plankenburg River in Stellenbosch (Table 3). The sampling site is situated directly downstream from the informal settlement, Kyamandi, as well as an industrial area, flowing past agricultural and residential areas further upstream (Britz *et al.*, 2013). All of these factors contribute to the contamination of the river water (Paulse *et al.*, 2009). The informal settlement, Kyamandi, situated directly upstream from the sampling site, is suggested to be a main contributor of microbial pollution in the river in the form of untreated sewage (Britz *et al.*, 2013). The efficacy of the different treatments could therefore be influenced by various contaminants introduced by the different upstream developments, effecting the characteristics of the river water on the days of sampling.

Greater disinfection resistance has been reported for aerobic colonies (Figs. 1 – 3) when compared to the TC and FC. The large variety of microorganisms associated with ACC therefore do not all display similar sensitivity to the disinfection treatments under evaluation (Figs. 6 – 8). Generally coliforms, like *E. coli*, which are Gram-negative, are less sensitive to UV irradiation as well as chemical oxidants, whereas Gram-positive bacteria often display greater resistance to UV and chemical treatments (Beauchamp & Lacroix, 2012; Gayán *et al.*, 2014). However, considering LP-UV irradiation was only investigated in this study, future research investigating MP-UV irradiation could be insightful. MP-UV systems emit UV light at a larger range than LP-UV, and

should thus be considered if targeting a larger variety of microorganisms, such as the ACC groups (Ijpelaar *et al.*, 2010; Gayán *et al.*, 2014).

CONCLUSIONS

According to the results obtained in Study 1, there was variability in the disinfection efficacy seen between the different treatments. The environmental *E. coli* strains tested showed differences in resistance to the different treatments. Overall it was evident that the *E. coli* strain F11.2 was most sensitive to the stand-alone effects of PAA treatment, with little disinfection contributed by Cl and H_2O_2 for environmental strain F11.2. Microorganisms are equipped with various types of defence mechanisms that respond differently to different oxidative chemical stress, therefore differences will be observed between different disinfection treatments. Increased chemical contact time (t =25 min) resulted in increased disinfection for PAA, Cl and H_2O_2 treatments. Longer contact times are associated with sufficient time for the chemicals to penetrate the bacterial cells and cause oxidative damage. Disinfection potential was further enhanced by combination treatments (PAA+UV, Cl+UV and H_2O_2+UV). This study showed the benefits of combining chemical and UV treatments to produce up to 3 log reductions.

The results reported in Study 2 show the dominant effect of varying water quality has on the disinfection capabilities of the different treatments. The water for the final trial completed (Trial 3) measured a poor UVT% and subsequently a poor UV disinfection was observed throughout the trial. UV irradiation therefore was not consistent and proved to be unreliable with poor water quality. A strong correlation was observed between physico-chemical water parameters and disinfection potential of different treatments. However, for all three trials Cl (6 $mg.L^{-1}$) produced the best disinfection and therefore is considered the most reliable treatment. The stand-alone Cl treatment was able to reach the 3 log target reduction for FC. The stand-alone effect of PAA and H_2O_2 resulted in ineffective disinfection (not able to achieve the target 3 log reduction required). H_2O_2 was the least effective disinfectant, recording a maximum of just over 1 log reduction for FC. The combination treatment of (PAA+UV) was the only treatment that seemed to show significant synergy benefits, greater than the sum of the two stand-alone treatments.

When effective conditions prevailed for UV disinfection (good UVT%) all the combination treatments were sufficient in reaching the target 3 log reduction, although UV irradiation alone could be considered the main contributor thereof. Using higher chemical concentrations would increase the costs, while longer contact times would require larger storage capacity at up-scaled disinfection. Combination treatments thus hold the potential to meet both these conditions, granted adequate chemical and UV doses are used to treat with to induce the additional germicidal effects of AOPs. Nevertheless, using excessive chemical concentration must be approached with caution as increased costs as well as the formation of DBP will be greatly increased. Thus, dosing with, specifically, chlorine 6 $mg.L^{-1}$ proved effective, but recorded higher than allowed chlorine residual

levels (DWA, 2013b), highlighting the importance of maintaining low enough chemical concentrations whilst still allowing for adequate disinfection.

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Chapter 4

PILOT-SCALE INVESTIGATION OF MEDIUM-PRESSURE UV IRRADIATION IN COMBINATION WITH CHEMICAL DISINFECTANTS WHILST CONSIDERING THE IMPACT OF PHOTO-REPAIR

ABSTRACT

Making use of medium pressure (MP) ultraviolet (UV) irradiation as a stand-alone treatment, as well as in combination with peracetic acid (PAA), chlorine (Cl) and hydrogen peroxide (H_2O_2), as combination treatments, was investigated for river water at pilot-scale. The Faecal Coliform (FC) levels present in the water were, in some instances, successfully reduced by over 3 log for UV treatments. However, significantly poorer ($p<0.05$) disinfection was reported for all the chemical treatments. UV irradiation was directly affected by poor optical water characteristics measured for the river water, as a poor ultraviolet transmission (UVT%) was often associated with less than acceptable reductions in microbial counts. Chlorine disinfection proved to be the most effective of the chemical treatments investigated, but regardless thereof, was only able to reduce FC by 1.58 log at best, which was inadequate considering the > 6.0 log initial FC levels. Chlorine residual levels never exceeded 0.50 $mg.L^{-1}$, therefore minimising the potential formation of disinfection by-products (DBPs). In most instances no significant differences ($p>0.05$) were observed between stand-alone UV treatments and combination treatments, thus suggestive of insignificant contributions made by advanced oxidation processes (AOPs).

Further studies reported up to 13.72% and 15.86% photo-recovery for TC and FC respectively, after UV irradiated river water was subjected to visible light at 3.5 kLux intensity for 3 h. The importance of UV irradiation, as demonstrated in the first study, would thus be compromised when considering a 15.86% recovery rate for FC post-UV irradiation. From the studies completed it was concluded that chemical disinfectants, as investigated, showed poor viability at low concentrations, with significantly better ($p<0.05$) disinfection reported for MP-UV irradiation. Nevertheless, UV treatments at a dose range of 25 – 30 $mJ.cm^{-2}$ proved ineffective for some trials, specifically when poor physico-chemical and microbial properties were reported for the river water. The investigation of more effective filtration processes, combined with increased chemical and UV doses should be investigated to further optimise UV disinfection and ultimately combination treatments with chemical disinfectants.

INTRODUCTION

As the demand for fresh water is increasing yearly, many countries like South Africa are faced with the ever-growing problem of water scarcity. Furthermore, many South African rivers have been reported to be extremely polluted with regards to microbiological levels (Paulse *et al.*, 2009; Britz *et al.*, 2013; Olivier 2015). Alarmingly, regardless of the high levels of faecal indicator microorganisms reported by multiple studies, river water is often used for irrigation purposes,

posing an immense health risk to humans (Castro-Rosas *et al.*, 2012; Britz *et al.*, 2013; Lee *et al.*, 2014). Thus, to ensure microbially safe irrigation water, the Department of Water Affairs (DWAF, 1996) has established a guideline suggesting no more than 1 000 Faecal Coliforms per 100 mL (1 000 cfu.100 mL^{-1}) to be present in irrigation water (DWAF, 1996).

Chemical disinfectants and ultraviolet (UV) light disinfection methods are well-accepted treatments used to reduce microbial contamination in water (Gehr *et al.*, 2003; Koivunen & Heinonen-Tanski, 2005; Ijpelaar *et al.*, 2010). Nevertheless, the survival of pathogens post-disinfection is often still a reality when using the available disinfection treatments. Furthermore, disinfection treatments are often influenced by the varying physico-chemical and microbial quality of river water (Lu *et al.*, 2009; Ijpelaar *et al.*, 2010; Olivier, 2015). Research has shown that the shortcomings associated with individual disinfection treatments can greatly be reduced, by applying chemical disinfectants in combination with UV irradiation (Caretti & Lubello, 2003; Zhang *et al.*, 2014).

UV irradiation has proven to be an effective, non-thermal disinfection treatment, successfully reducing microorganisms associated with polluted water (Koutchma, 2009; Gayán *et al.*, 2014). Compared to the chemical alternatives, UV irradiation does not promote the formation of harmful DBPs (Lu *et al.*, 2009; Matilainen & Sillanpaa, 2010). Predominantly UV irradiation acts directly on the genetic material of microorganisms, as photons (UV light) are easily absorbed by DNA and RNA. These genetic disruptions are most prevalent at UV wavelengths of 210 – 280 nm, suggested to be the most germicidal wavelength range produced by UV irradiation (Koutchma, 2009; Gayán *et al.*, 2014).

Low and medium-pressure (LP and MP) mercury vapour lamps are generally used, differentiated by their ability to produce different UV wavelengths (Sakai *et al.*, 2007). Advantageously, limited formation of DBPs are reported when using UV irradiation. Negatively, no residual disinfection action post-treatment is offered (Mezzanotte *et al.*, 2007). As no residual disinfection is offered when using UV irradiation, the potential of microorganisms to increase post-disinfection exists (Hu *et al.*, 2005; Guo *et al.*, 2011). The possibility of microorganisms increasing in numbers post-treatment is of major concern and is a result of their ability to ‘repair’ damage induced by UV irradiation. Two main repair mechanisms have been identified, namely light-mediated repair (photo-reactivation) and also repair in the absence of light (dark-repair) (Gayán *et al.*, 2014). Light-mediated repair (photo-repair) is considered to be of greater concern than dark-repair, which is less likely to occur (Guo *et al.*, 2011; Gayán *et al.*, 2014). Photo-repair is primarily carried out through the action of cyclobutane pyrimidine dimers (CPD) lyases, also known as photolyases, which act directly against the action of damage inducing CPD photoproducts. Photolyases make use of visible light (wavelengths of 350 – 500 nm) in order to reverse the damage induced specifically by CPD photoproducts (Rastogi *et al.*, 2010; Gayán *et al.*, 2014). Dark-repair, as the name suggests, is not dependent on the availability of visible light to repair UV

induced damage, but rather is associated with more accurate repair than photo-repair (Douki, 2013; Premi *et al.*, 2015).

As microorganisms have the ability to repair damage induced by UV irradiation, great potential exists that sublethal UV doses will lead to inadequate microbial reductions. However MP-UV treatments are considered less likely to promote microbial regrowth than the LP alternatives (Hu *et al.*, 2005; Guo *et al.*, 2009). MP-UV wavelengths are more likely to be emitted over a broader wavelength range than that of LP wavelengths (Oguma *et al.*, 2002; Sakai *et al.*, 2007), enabling MP-UV irradiation to target a larger variety of microorganisms, assuming that microorganisms have different wavelengths at which they are more sensitive to UV light (Eischeid & Linden, 2007). Furthermore, MP-UV irradiation has the potential to target other cellular components within microbial cells, ultimately leading to compromised cellular functioning (Quek & Hu, 2013; Gayán *et al.*, 2014). Investigating MP-UV irradiation, as well as the potential of photo-repair is thus important when making recommendations on effective dose requirements with regards to river water disinfection.

Combination treatments have been gaining attention due to their success in targeting a large variety of microorganisms (Zhang *et al.*, 2014). These treatments primarily make use of chemical disinfectants in combination with UV irradiation and are considered more effective than the sum of the stand-alone treatments used (Koivunen & Heinonen-Tanski, 2005). Additional disinfection can be ascribed to the formation of reactive hydroxyl radicals (•OH), formed by advanced oxidation processes (AOP) (Comninellis *et al.*, 2008; Wang *et al.*, 2011). These •OH are considered effective and powerful oxidisers of organic matter, which are relatively non-selective, targeting a large variety of organic matter (Swaim *et al.*, 2008). Applying chemical treatments in combination with MP-UV irradiation aims at decreasing the potential shortcomings of individual treatments, ultimately producing more consistent and reliable disinfection (Sharp *et al.*, 2006). Combination treatments not only provide a potential to decrease the chemical concentrations used, but also at reducing costs and ensuring better disinfection than the individual treatments (Lu *et al.*, 2009).

The aim of the current study was thus to investigate the potential of MP-UV irradiation in combination with different chemical disinfectants on reducing the microbial numbers present in river water intended for irrigation. A series of trials were completed at a pilot-scale disinfection treatment facility, evaluating the potential of chemical disinfectants (PAA, Cl and H_2O_2), UV disinfection, as well as combination treatments of chemical and UV disinfection (PAA+UV), (Cl+UV) and (H_2O_2+UV). The influence of varying water quality on the disinfection efficacy of the different treatments was also considered. This was followed by experiments evaluating the potential of photo-repair on MP-UV irradiated river water samples, as to gain a better understanding of the true disinfection efficacy present UV irradiation at pilot-scale.

MATERIALS AND METHODS

Pilot-plant description

Pilot-scale disinfection experiments were completed at a custom built pilot-scale disinfection treatment facility situated next to the Plankenburg River in Stellenbosch, South Africa (33°56'15.4''S,18°50'53.0''E). The pilot-scale disinfection treatment facility (Figure 1) was specifically designed to test the efficacy of different chemical and MP-UV light treatments on large water volumes. Water was pumped from the river through a standard sand filter and directed to one of three holding tanks each, with a holding capacity of 2 500 L (Fig. 1). UV disinfection was performed by making use of an in-line UV system (Berson, The NetherlandsExperiments were performed during the months of January – March (summer and early autumn). In terms of rainfall these months are dry, as the Western Cape is a winter rainfall region in South Africa. Thus, optimisation experiments were strategically completed in the dryer months, as sub-optimal water quality would be expected when compared to the other seasons. According to Britz *et al.* (2013) the highest microbial levels were reported for the Plankenburg river for the months of January, February and March. These findings were in agreement with Paulse *et al.* (2009), reporting highest TC and *E.coli* in the summer months for river water sampled in the Plankenburg River.

Water sampling

Sterile 2 L bottles were used to collect water samples at the sampling points indicated in Figure 1. A control sample was taken at point 1 (after the sand filter) before any treatments were applied to the water, to evaluate the initial microbial and physico-chemical quality of the river water on the specific day. A second sample was collected at point 2 (Fig. 1), after UV exposure at a specific UV dose. Up until this point no chemical treatment had been applied. Thereafter the relevant chemical disinfectant was added to the 2 500 L holding tank, allowing a 25 min contact time. A sample was collected at point 3, representing the river water exposed to chemical disinfectants for 25 min. Directly thereafter the chemically treated water was exposed to the MP-UV irradiation at the desired dose and a sample was collected at point 4, representing the combination treatment for chemical and UV irradiation. All water samples were transported in cooler boxes and analysed within two hours after sampling took place.

Chemical disinfectants

The peracetic acid (PAA) solution used in disinfection studies was a commercial preparation (Tsunami 100, Ecolab, South Africa) comprising of 31% acetic acid, 15% peroxyacetic acid and 11% hydrogen peroxide. In all Cl disinfection treatments a commercial sodium hypochlorite solution was used (XY 12 – hypochlorite, Ecolab, South Africa). Lastly, a 30% ($v.v^{-1}$) hydrogen peroxide (H_2O_2) stock solution was used (Merck, South Africa).

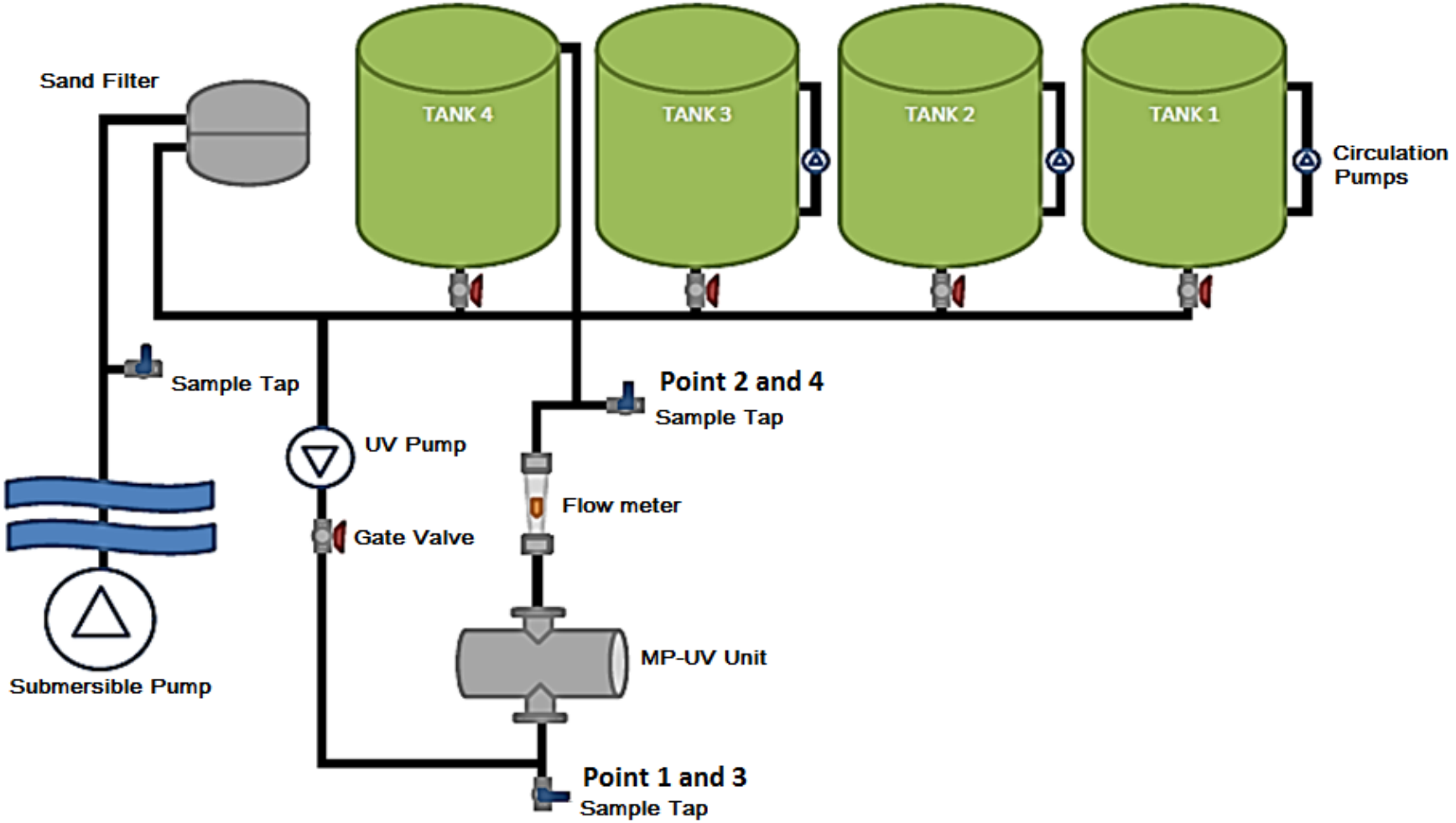

Figure 1 Diagrammatic representation of the pilot-scale disinfection system installed alongside the Plankenburg River, Stellenbosch. Indicating the MP-UV disinfection system installation.

Pilot-scale MP-UV disinfection

UV disinfection was done using a Berson InLine 40+ UV disinfection system (Berson, The Netherlands). The UV irradiation system produced medium pressure (MP) UV light waves, ranging between 220 – 580 nm. The UV light was emitted by a B810H MP-UV mercury lamp placed perpendicular to the flow of water in the piping network. The desired UV dose required had to be calculated with regards to the UVT% of the water on the specific day. Thereafter the desired UV dose was achieved by altering the flow rate of the water through the piping network. As the UV system was not capable of automatically adjusting the flow rate of the water, this was done manually by adjusting a regulatory valve.

Microbiological analysis

Before and after all disinfection treatments and photo-repair experiments the microbial quality of the river water was determined. All microbial enumeration was done in triplicate after the dilution series were prepared (10^{-1} – 10^{-6}) in duplicate.

Aerobic Colony Counts (ACC), Total Coliforms (TC) and Faecal Coliforms (FC)

ACC were determined according to the South African National Standards (SANS) methods 4833 (SANS, 2007b). Samples were serially diluted (10^{-1} – 10^{-6}) and plated in duplicate, using Plate Count Agar (PCA) (Merck, South Africa). The poured plates were incubated at 30±0.5°C for 48 h (SANS, 2007b). Total Coliforms (TC) were enumerated according to the (SANS) methods 4832 (SANS, 2007a). Violet Red Bile Agar (VRBA) (Merck, South Africa) was used for the preparation of duplicate pour plates, incubated at 36±0.5°C for 24 h. Faecal Coliforms (FC) were also enumerated using VRBA (Merck, South Africa) but incubated at 44±0.5°C for 24 h (Schraft & Watterworth, 2005).

Water quality analysis

The physico-chemical parameters of the sand-filtered river water prior to any treatments was determined according to Standard Methods (APHA, 2005). The parameters measured included the chemical oxygen demand (COD), Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), pH, UV transmission percentage (UVT%), alkalinity and electrical conductivity (EC). The COD was measured using a Spectroquat® Nova 60. Guidelines regarding irrigation water intended to be used for fresh or minimally processed crops have been established for certain water parameters (Table 1) and were used to evaluate the river water quality throughout the study.

The turbidity was measured using of a portable Orion AQ3010 Turbidity Meter (Thermo Science, USA). The instrument calibration was confirmed using solutions of known turbidity as stipulated by the manufacturer (Thermo Science, USA). Analysis of the water was then completed in

duplicate. The UVT% was measured using a hand held Sense™ T UV-Transmittance Sensor (Berson, The Netherlands) used in compliance with the instructions provided by the manufacturer. Prior to any measurements the instrument was calibrated using deionised water. A portable HI 8733 conductivity meter (Hanna Instruments, USA) was used to measure EC (measured in $mS.m^{-1}$), indicating the presence of dissolved salts present in the river water.

Table 1 Guidelines for physico-chemical and microbial parameters for irrigation water (DWAF, 1996)

Water quality parameter	**Guideline limit**
Faecal Coliforms (FC)	1 000 $cfu.100\ mL^{-1}$
pH	6.5 – 8.4
Conductivity	40.00 $mS.m^{-1}$
Total suspended solids (TSS)	50.00 $mg.L^{-1}$

Photo-repair following MP-UV irradiation

The phenomena of photo-repair (photo-reactivation) post-UV irradiation at pilot-scale was also investigated. The possibility of microbial repair was evaluated, using a closed container equipped with two 10 W fluorescent light bulbs (STR-GX3006A, C10W, Eurolux, South Africa) emitting light at 3.5 kLux intensities (Fig. 2). The light intensity of the lamps was confirmed using a portable Jaz spectrometer (Ocean optics, USA). Samples that had been exposed to UV irradiation were placed under the fluorescent light in sterile 500 mL glass beakers while being subjected to minimal agitation induced by magnetic stirrer and bar. The reactivation of the microorganisms was recorded as log-reactivation and the percentage regrowth was calculated using the equation of Lindenauer & Darby (1994):

$$Percentage\ photo-repair\ (\%) = \frac{(N_p - N)}{(N_0 - N)} \times 100\%$$

With, N_p = the number of microbial cells in reactivated sample ($cfu.mL^{-1}$), N = cell microbial number immediately after UV irradiation in $cfu.mL^{-1}$ and N_0 = the number of microbial cells before UV irradiation ($cfu.mL^{-1}$).

Statistical analysis

The statistical analysis was performed using Statistica 13.0 software (StatSoft, USA). The data was analysed using two-way and mixed model ANOVA. Fisher's least significance difference (LSD) post

hoc analyses were performed using a 5% significance level ($p<0.05$) as guideline for significant results.

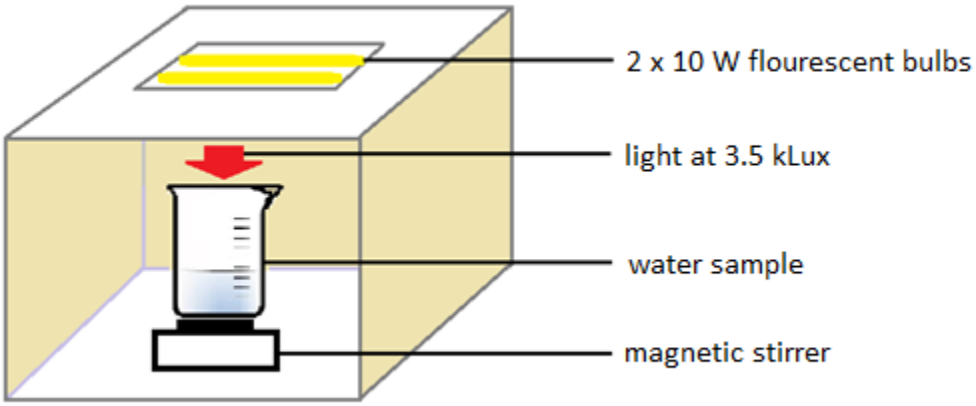

Figure 2 MP-UV irradiated water samples exposed to fluorescent light at an intensity of 3.5 kLux in a closed container.

RESEARCH STUDY DESIGN

Study 1: Influence of water quality on MP-UV irradiation and chemical treatments, and a combination thereof

MP-UV irradiation in combination with PAA

Filtered river water was exposed to a UV dose that ranged from 25 – 30 mJ.cm^{-2}. It was difficult to establish a single UV dose (even for the same trials), as varying water quality made maintaining a specific UV dose near impossible at pilot-scale. The 2 500 L holding tanks were filled, where after samples were taken at sampling points 1 and 2, representing the control and water exposed to UV irradiation respectively (Fig. 1). Thereafter the 2 500 L holding tank was dosed with PAA (4 mg.L^{-1}) and allowed a contact time of 25 min, before subjecting the chemically treated water to MP-UV light (25 – 30 mJ.cm^{-2}). Additional samples were collected at points 3 and 4, representing the PAA treatment and (PAA+UV) combination treatments respectively (Fig. 1). The log-inactivation were determined for ACC, TC and FC for the PAA, UV and the combination treatments (PAA+UV). Furthermore, results for the microbial and water quality were analysed and compared to the guidelines established for irrigation water set by the DWAF (1996). Three trials were completed for each treatment on alternative days. All trials were completed in the dryer (summer) months as it was expected that the river water quality would be at its worst.

MP-UV irradiation in combination with Cl

The disinfection efficacy of sodium hypochlorite (3 $mg.L^{-1}$) was also investigated. A Cl contact time of 25 min was allowed, followed by UV irradiation. The UV dose was maintained in the range of 25 – 30 $mJ.cm^{-2}$ (for all trials completed). Similar sampling protocols were followed as described for PAA treatment. Results for the microbial and physico-chemical water quality were analysed and compared to the guidelines (DWAF, 1996).

MP-UV irradiation in combination with H2O2

Additionally, the potential of H_2O_2 (2.5 $mg.L^{-1}$) as a viable chemical disinfectant used for river water was evaluated as a stand-alone treatment as well as in combination with MP-UV irradiation at pilot-scale. As for Cl and PAA treatments, a contact time of 25 min was allowed for H_2O_2 treatments before UV irradiation was performed. A UV dosing rage of 25 – 30 $mJ.cm^{-2}$ was maintained for all trials. The same sampling protocol was followed as described for PAA and Cl treatments.

Study 2: The potential of photo-repair following MP-UV irradiation

The possibility of microbial photo-repair (photo-reactivation) after UV irradiation was investigated at pilot-scale. The river water was irradiated at a UV dose of 25 – 30 $mJ.cm^{-2}$, where after the water was transferred to sterile glass beakers and subjected to artificial light at an intensity of 3.5 kLux. Samples were placed in the reactivation chamber (Fig. 2) for a period of 3 h. Throughout the 3 h reactivation period, constant agitation was induced by a magnetic stirrer. ACC, TC and FC were enumerated before UV irradiation as well as directly after and again after 3 h exposure to fluorescent light. The regrowth of the microorganisms was reported as log-reactivation.

RESULTS AND DISCUSSION

Study 1: Pilot-scale MP-UV irradiation and chemical disinfection

Study 1 evaluated the efficacy of PAA (4 $mg.L^{-1}$), Cl (3 $mg.L^{-1}$) and H_2O_2 (2.5 $mg.L^{-1}$), as well as UV irradiation as stand-alone and combination treatments (PAA+UV, Cl+UV and H_2O_2+UV) at pilot-scale. River water was sampled on three alternative days for each chemical disinfectant investigated as Trials 1^{PAA} – 3^{PAA}, Trials 1^{Cl} – 3^{Cl} and Trials 1^{H202} – 3^{H2O2}. The aim of this study was to determine the most effective disinfection treatments, whilst monitoring the effects of varying microbial and physico-chemical water quality. The efficacy of the different chemical treatments was determined by allowing a contact time of 25 min. The stand-alone and combination effects of UV irradiation were determined at a UV dose range of 25 – 30 $mJ.cm^{-2}$. Results were reported as log reductions and compared to guidelines set by the Department of Water Affairs (DWAF, 1996).

PAA and MP-UV treatments

River water quality

The river water quality parameters were first determined before disinfection treatments were completed for each of the pilot-scale trials. The microbial and physico-chemical parameters were determined (Trials 1^{PAA} – 3^{PAA}) as presented in Table 2.

Table 2 Physico-chemical and microbial quality characteristics of sand-filtered river water prior to PAA and MP-UV disinfection, Trials 1^{PAA} – 3^{PAA}

Quality parameter	Trial 1^{PAA}	Trial 2^{PAA}	Trial 3^{PAA}
UVT%	27.40	29.05	35.20
COD (mg.L^{-1})	160.50	137.70	103.50
Turbidity (NTU)	39.50	23.30	21.35
TSS (mg.L^{-1})	28.00	23.00	22.00
VSS (mg.L^{-1})	24.00	19.00	17.00
pH	7.46	6.96	6.95
Alkalinity (mg $CaCO_3$.L^{-1})	125.00	100.00	150.00
Conductivity (mS.m^{-1})	56.50	67.40	40.60
Aerobic colony count (ACC) (log cfu.100 mL^{-1})	8.04	7.90	7.58
Total coliforms (TC) (log cfu.100 mL^{-1})	6.89	7.14	7.29
Faecal Coliforms (FC) (log cfu.100 mL^{-1})	6.42	6.82	6.92

Evaluation of the microbial counts (Table 2) showed extremely high levels of bacterial contamination for ACC, TC and FC for each of the respective trials. The ACC microbial groups measured the highest levels of microbial contamination, reporting up to 8.04 log cfu.100 mL^{-1}. The FC always represented the lowest levels of microorganisms (for enumerated groups), although average counts of 6.42 – 6.92 log were still recorded. The river water sampled (Trials 1^{PAA} – 3^{PAA}) was unable to comply with the guideline set for irrigation water regarding FC contamination levels (DWAF, 1996) (Table 1). The high microbial, specifically faecal contamination present in the Plankenburg River (Table 2), was similar to that reported in literature by other researchers (Huisamen, 2012; Bester 2015; Olivier, 2015). It was found that up to 3.92 log reductions in FC would be required to produce irrigation water of acceptable microbial quality in Trials 1^{PAA} – 3^{PAA} (i.e. to achieve the < 1 000 cfu.100 mL^{-1} FC guideline suggested by the DWAF (1996) (Table 2). Britz *et al.* (2013) recommended that a 3 – 4 log reduction in FC would likely produce microbially acceptable irrigation water. These findings were based on

previous studies completed regarding river water quality in the Western Cape and consideration to guidelines established by the DWAF (1996) (Britz *et al.*, 2013). The highest levels of expected faecal contamination was reported for Trial 3 (Table 2), recording 6.92 log for FC, although similar FC values were recorded for the water sampled for all three trials. These findings (Table 2) further highlight the importance of implementing effective disinfection treatments in order to ensure safe agricultural use of river water.

The physico-chemical parameters (Table 2) showed clear variations between the different trials (Trials 1^{PAA} – 3^{PAA}). The poorest UVT% value was recorded for the water sampled in Trial 1^{PAA} (27.40%) with Trials 2^{PAA} and 3^{PAA} reporting 29.05% and 35.20% respectively (Table 2). As lower UVT% often coincide with higher turbidity values (Gayán *et al.*, 2014), the water sampled in Trial 1^{PAA} which measured the lowest UVT%, also recorded the highest turbidity value of the three trials (Table 2), indicating the possibility of compromised ultraviolet transmission for Trial 1^{PAA}. Trial 3^{PAA} recorded the lowest turbidity value (21.35 NTU), which correlated to the highest UVT%, indicating improved ultraviolet transmission capabilities of the water sampled for Trial 3^{PAA}. Poor UVT% and turbidity are associated with increased concentrations of organic and inorganic substances that can possibly interfere with UV irradiation, by scattering or absorbing UV light (Abdul-halim & Davey, 2016). Trial 1^{PAA} also measured the highest COD value (160.5 $mg.L^{-1}$) recorded for the river water sampled of 3 trials (Table 2). Lubello *et al.* (2002) reported a COD of 75 $mg.L^{-1}$ for pilot-scale water disinfection treatments. Thus higher COD values for this study (Table 2) could potentially indicate compromised water quality. The highest TSS and VSS values were also measured for the water sampled in Trial 1^{PAA}, correlating with the poorer UVT%, turbidity and COD values recorded (Table 2).

The TSS and pH values were within the guidelines established by the DWAF (1996) (Table 1). However the conductivity for Trials 1^{PAA} – 3^{PAA} exceeded the guideline of 40 $mS.m^{-1}$. The water sampled in Trial 3^{PAA} recorded the highest alkalinity (150 mg. $CaCO_3$.L-1) of the three trials. Higher alkalinity is related to an increased buffer capacity displayed by water, thus potentially decreasing the efficacy of certain chemical oxidants that induce germicidal action through pH alteration (Kitis, 2004; Newman, 2004). In conclusion, the water sampled in Trial 1^{PAA} was of poorer quality when compared to that measured for Trials 2^{PAA} and 3^{PAA}.

Microbial reductions

The log reductions presented in Figure 3 showed clear variation between the different treatments for Trials 1^{PAA} – 3^{PAA}. Stand-alone PAA treatments showed the lowest disinfection efficacy (Trials 1^{PAA} – 3^{PAA}) for the different disinfection treatments investigated, recording less than 1 log reduction for ACC, TC and FC (Fig. 2). Log reductions in Trial 1^{PAA} and Trial 2^{PAA} were similar for ACC, TC and FC, while Trial 3^{PAA} reported lowest log reductions for all enumerated groups.

UV irradiation was significantly more ($p<0.05$) effective than the stand-alone PAA treatments (Trials 1^{PAA} – 3^{PAA}) for all enumerated groups. Trial 3^{PAA} reported lower log reductions in ACC than for Trials 1^{PAA} and 2^{PAA}. TC were always reduced by > 3 log for all three trials, however FC were reduced by > 3 log for Trials 2^{PAA} and 3^{PAA}, with slightly lower reductions reported for Trial 1^{PAA}.

Similarly, the combination treatments (PAA+UV) recorded significantly improved ($p<0.05$) disinfection compared to the stand-alone PAA treatments for Trials 1^{PAA} – 3^{PAA} (Fig. 2). Minimal variation was observed between the stand-alone UV treatments and the combination treatments (PAA+UV)

(Fig. 2). Regarding the log reductions for Trials 1^{PAA} and 2^{PAA} for the stand-alone UV treatments and combination treatments (PAA+UV), minimal variations were reported between the two treatments. Therefore, UV irradiation could be considered almost solely responsible for the disinfection reported for the (PAA+UV) combination treatments (Trials 1^{PAA} – 3^{PAA}).

When considering the physico-chemical parameters (Table 2), the poor optical characteristics measured for the water in Trial 1^{PAA} were thus related to a poorer UV disinfection reported (Fig. 2). Gayán *et al.* (2014) suggested higher UVT% values were accompanied by improved disinfection, as UV light would more easily penetrate a body of water. Poorest disinfection was observed for the stand-alone PAA treatment in Trial 3^{PAA} for ACC and FC, although when considering the alkalinity of the water measured in Trial 3^{PAA} (Table 2) increased buffer capacity was to be expected, thus decreasing the efficacy of PAA (Kitis, 2004) when compared to Trials 1^{PAA} and 2^{PAA}.

MP-UV irradiation and Cl disinfection

River water quality

Microbial levels presented in Table 3 revealed unacceptable water quality when considering the DWAF (1996) guideline (1 000 cfu. $100mL^{-1}$) as exceptionally high levels of FC levels were measured. The water sampled for Trial 1^{Cl} reported up to 6.29 log for FC, meaning a 3.29 log reduction would be necessary to produce water of acceptable irrigation quality (DWAF, 1996). In comparison to the microbial levels recorded in Table 2 (PAA disinfection), similar FC levels were recorded for Trials 1^{Cl} and 3^{Cl} (6.29 and 6.59 log) (Table 3), all trials measured unacceptable FC level in the river water sampled from the Plankenburg River (DWAF, 1996).

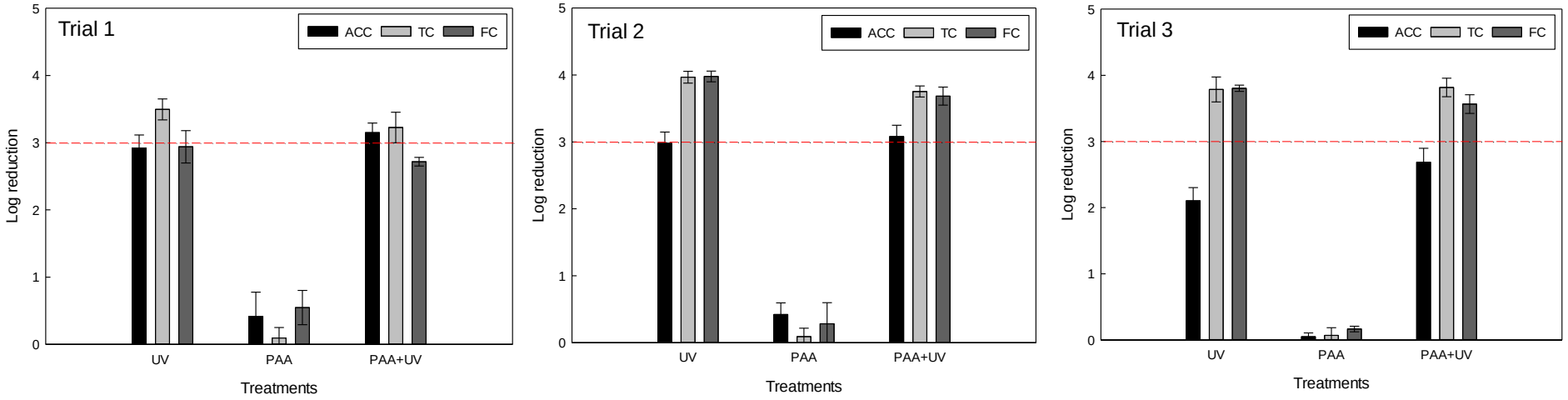

Figure 3 Log reductions reported for filtered river water exposed PAA (4 mg.L^{-1}) and a UV dose of 25 – 30 mJ.cm^{-2} and a combination thereof, represented by Trials 1^{PAA} – 3^{PAA}.

Table 3 Physico-chemical and microbial quality characteristics of sand-filtered river water prior to Cl and MP-UV disinfection, Trials 1^{Cl} – 3^{Cl}

Quality parameter	Trial 1^{Cl}	Trial 2^{Cl}	Trial 3^{Cl}
UVT%	24.10	39.50	33.90
COD ($mg.L^{-1}$)	170.10	99.00	146.70
Turbidity (NTU)	38.60	11.54	15.95
TSS ($mg.L^{-1}$)	28.00	19.00	21.00
VSS ($mg.L^{-1}$)	23.00	14.00	17.00
pH	7.46	6.69	6.85
Alkalinity (mg $CaCO_3.L^{-1}$)	150.00	75.00	100.00
Conductivity ($mS.m^{-1}$)	46.50	46.00	41.30
Aerobic colony count (ACC) (log cfu.100 mL^{-1})	6.83	5.89	7.97
Total coliforms (TC) (log cfu.100 mL^{-1})	6.63	5.14	7.10
Faecal Coliforms (FC) (log cfu.100 mL^{-1})	6.29	4.89	6.59

Variations in the physico-chemical water parameters between the different trials were clear (Table 3). The UVT% value was poorest for the water sampled in Trial 1^{Cl}, recording a value of 24.1% (Table 3). Better UVT% values were recorded for Trials 2^{Cl} and 3^{Cl}, however still indicative of a poor ultraviolet transmission. Trial 1^{Cl} measured the highest turbidity (38.6 NTU), which was more than double that for the water sampled in Trials 2^{Cl} and 3^{Cl} (Table 3). Furthermore, the water sampled in Trial 1^{Cl} had the highest COD (170.1 $mg.L^{-1}$), TSS as well as VSS values of the three trials (Table 3). Thus, the water used in Trial 1^{Cl} had the least favourable physico-chemical properties of the three trials. Trial 2^{Cl} however reported the lowest COD, TSS as well as VSS values, together with only a marginally better UVT% and turbidity when compared to Trials 1^{Cl} and 3^{Cl} (Table 3).

A higher COD value, can be linked to increased concentrations of organic matter present in water, which can ultimately increases the chlorine demand (Van Haute *et al.*, 2013). The higher alkalinity values recoded for Trial 1^{Cl} could also influence chemical disinfection as an increased chlorine demand is associated with an increased alkalinity (Watts & Linden, 2007), thus highlighting the poorer water quality of Trial 1.

The pH of all three trials (Table 3) fell within the guideline of 6.5 – 8.4, established by the DWAF (1996). However, the EC guideline of 40 $mS.m^{-1}$ was not met for any of the trials (Table 3), with Trial 1^{Cl} recording the highest value (46.5 $mS.m^{-1}$).

Microbial reductions

Variation in terms of log reductions were observed for the different disinfection treatments (Figure 4). Stand-alone Cl treatments (Trials1^{Cl} – 3^{Cl}) were unable to meet the target 3 log reduction (indicated by the red dotted line) for FC (Fig. 4). The microbial populations enumerated for Trial 2^{Cl}

displayed the least resistance to Cl treatments, reporting significantly better ($p<0.05$) disinfection for ACC and TC to that of Trials 1^{Cl} and 3^{Cl} (Fig. 4). Nevertheless, neither of the enumerated microbial groups reported more than a 2 log microbial reduction for stand-alone Cl treatments. Considering the physico-chemical parameters of Trial 2^{Cl} (Table 3) improved disinfection was expected, as the lowest COD as well as pH was recorded for the water used in this trial (Fig. 4). COD is indicative of organic matter present in water, thus a lower COD is often met with a decreased Cl demand, associated with increased disinfection efficacy (Van Haute *et al.*, 2013). Furthermore, the lower pH was favoured for Cl treatments, as more effective disinfection molecules are formed at a lower pH (Newman, 2004; Lang *et al*., 2008).

Stand-alone UV treatments produced significantly better ($p<0.05$) log reductions for all enumerated microbial groups when compared to the stand-alone Cl treatments (Fig. 4). UV treatments for Trial 2^{Cl} was able to meet the 3 log target reduction, reporting significantly better ($p<0.05$) log reductions for FC than Trials 1^{Cl} and 3^{Cl}. Trial 1^{Cl} recorded the poorest UV disinfection of the 3 trials, as significantly poorer ($p<0.05$) log reductions were observed for FC than for Trials 2^{Cl} and 3^{Cl}. When evaluating the optical water parameters (Table 3), the lowest UVT% and highest turbidity was reported for the water in Trial 1^{Cl}, which subsequently also reported the poorest UV disinfection of the three trials (Fig. 4). Alternatively, the water investigated in Trial 2^{Cl} possessed the better UVT% and turbidity, consequently reporting the best UV disinfection of the three trials (Fig. 4), thus correlating the influence UVT% and turbidity had on UV irradiation efficacy, even for poor UVT%, as measured for Trials 1^{Cl} – 3^{Cl} (Table 3). However Cl disinfection produced greater reductions in FC than stand-alone PAA treatments with similar water quality measured for both PAA trials (Table 2) and Cl trials (Table 3).

Lastly, combination treatments produced no significant differences in log reductions for FC when compared to the stand-alone UV treatments ($p=0.26$, $p=0.91$ and $p=0.20$ for Trials 1^{Cl} – 3^{Cl} respectively), emphasising the reliance on MP-UV irradiation. Trial 2^{Cl} reported the highest ACC, TC and FC microbial reduction (2.75 log, 4.19 log and 4.21 log, respectively) for the combination treatments (Fig. 4)

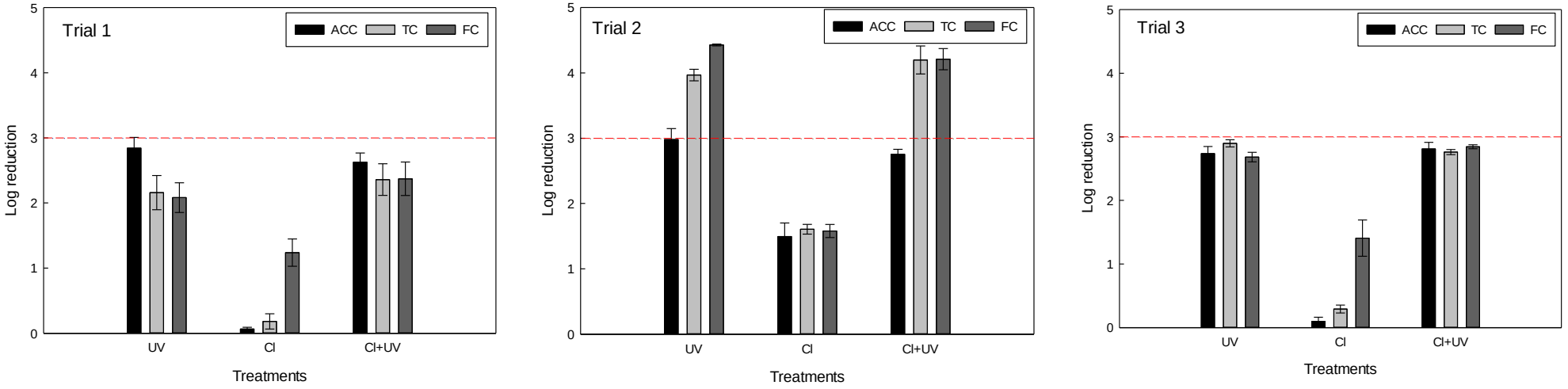

Figure 4 Log reductions reported for sand-filtered river water exposed Cl (3 mg.L^{-1}) and a UV dose of 25 – 30 mJ.cm^{-2} and a combination thereof, represented by Trials 1^{Cl} – 3^{Cl}.

H_2O_2 and MP-UV disinfection

River water quality

Similarly to PAA and Cl disinfection trials (Tables 2 and 3), high levels of microbial contamination was observed for the water sampled in Trials 1^{H2O2} – 3^{H2O2} (Table 4), with FC levels far exceeding the guideline established by the DWAF (1996). As FC levels exceeded 7 log, a > 4 log reduction would thus be required to sufficiently reduce the microbial levels of the water sampled from the Plankenburg River, to fulfil the recommended guideline of < 1 000 cfu.100 mL^{-1} (DWAF, 1996).

Table 4 Physico-chemical and microbial quality characteristics of sand-filtered river water prior to H_2O_2 and MP-UV disinfection, Trials 1^{H2O2} – 3^{H2O2}

Quality parameter	Trial 1^{H2O2}	Trial 2^{H2O2}	Trial 3^{H2O2}
UVT%	54.30	33.50	38.00
COD ($mg.L^{-1}$)	30.60	48.60	51.10
Turbidity (NTU)	9.29	15.53	15.22
TSS ($mg.L^{-1}$)	18.00	21.00	22.00
VSS ($mg.L^{-1}$)	13.00	16.00	16.00
pH	7.00	6.87	6.92
Alkalinity (mg $CaCO_3.L^{-1}$)	150.00	125.00	125.00
Conductivity ($mS.m^{-1}$)	44.10	46.50	42.30
Aerobic colony count (ACC) (log cfu.100 mL^{-1})	6.73	6.33	7.10
Total coliforms (TC) (log cfu.100 mL^{-1})	6.60	5.70	6.29
Faecal Coliforms (FC) (log cfu.100 mL^{-1})	6.30	5.30	6.45

Nearly identical physico-chemical parameters were recorded for the water sampled in Trials 2^{H2O2} and 3^{H2O2} (Figure 4), however dissimilar microbial parameters were measured as Trial 3^{H2O2} reported a 1.15 log higher level of FC than for Trial 2^{H2O2}. The water sampled in Trial 1^{H2O2} was able to measure better turbidity, COD, TSS and VSS values compared to that measured of Trials 2^{H2O2} and 3^{H2O2}. Since these parameters reflect on the absorbent quality of the water, it was expected that the UV transmission (UVT%) would be lowest for Trial 1^{H2O2}, which was the case. Surprisingly, the highest alkalinity value was recorded for the water used in Trial 1^{H2O2}, potentially indicating an improved buffer potential of the river water for this trial (Kitis, 2004; Newman, 2004).

The pH and TSS measured for the water used in Trials 1^{H2O2} – 3^{H2O2} were able to meet the guidelines established by the DWAF (1996) (Table 1),. However, this was not the case for the conductivity. All three trials exceeded the conductivity guideline of 40 $mS.m^{-1}$, with Trial 2^{H2O2} recording the highest value (46.5 $mS.m^{-1}$).

When evaluating the physico-chemical properties of the water used for PAA disinfection trials (Table 2) and Cl disinfection trials (Table 3), similarities were obvious regarding poor UVT%

linked to increased turbidity and TSS values and often an increased COD value as well for the river water sampled from the Plankenburg River for the summer months.

Microbial reductions

The stand-alone H_2O_2 treatments for Trials 1^{H2O2} – 3^{H2O2} (Fig. 5) recorded significantly less ($p<0.05$) effective disinfection compared to the stand-alone UV treatments for all enumerated groups (ACC, TC and FC). Stand-alone H_2O_2 treatments were unable to achieve even 1 log reduction for any of the trials (Fig. 5), overall proving to be a very ineffective disinfection treatment. Furthermore, the poorest reduction in FC was observed for Trial 1^{H2O2}, as significantly better ($p<0.05$) reduction were measured for Trials 2^{H2O2} and 3^{H2O2}. Considering the highest alkalinity value was measured for Trial 1^{H2O2} (Table 4) an increased buffer capacity could thus be responsible for the poorer FC reductions, as chemical oxidant often rely on changes in pH to intensify their disinfection potential (Kitis, 2004; Newman, 2004).

Trial 1^{H2O2} recorded significantly better ($p<0.05$) log reductions for stand-alone UV treatments, consequently also measuring the better UVT% of the three trials (Table 4). The poorer UVT% recorded for water used in Trials 2^{H2O2} and 3^{H2O2} had obvious implications on microbial reductions, as less than 2 log reductions were measured for all enumerated groups. Furthermore, only Trial 1^{H2O2} was able to meet the target 3 log reduction for FC (indicated by the dotted red line), measuring 3.02 log reduction. However, significantly better ($p< 0.05$) log reductions for TC and FC were observed when compared to ACC, regardless of the broad range of wavelengths produced by MP-UV irradiation, suggested to affect a larger range of microorganisms than LP-UV systems (Hu *et al.*, 2005; Eischeid & Linden, 2007; Ijpelaar *et al.*, 2010).

Lastly, all combination treatments were able to produce statistically better ($p<0.05$) microbial reductions than the stand-alone H_2O_2 treatments (Fig. 5). However, minimal variations in log reductions were observed between the combination treatments and the stand-alone UV treatments. This was expected as the stand-alone H_2O_2 treatments contributed minimally to the overall disinfection recoded for combination treatments. Thus concluding that microbial reductions were highly dependent on MP-UV irradiation when applied in combination with H_2O_2. Similar trends were reported for the previous studies done on PAA and Cl (Figs. 3 and 4), both of which reported significantly better ($p<0.05$) reductions for combination treatments than for the stand-alone chemical treatments. Therefore unfavourable physico-chemical parameters are ultimately responsible for poor microbial inactivation, especially when considering the poor contributions made by the chemical treatments.

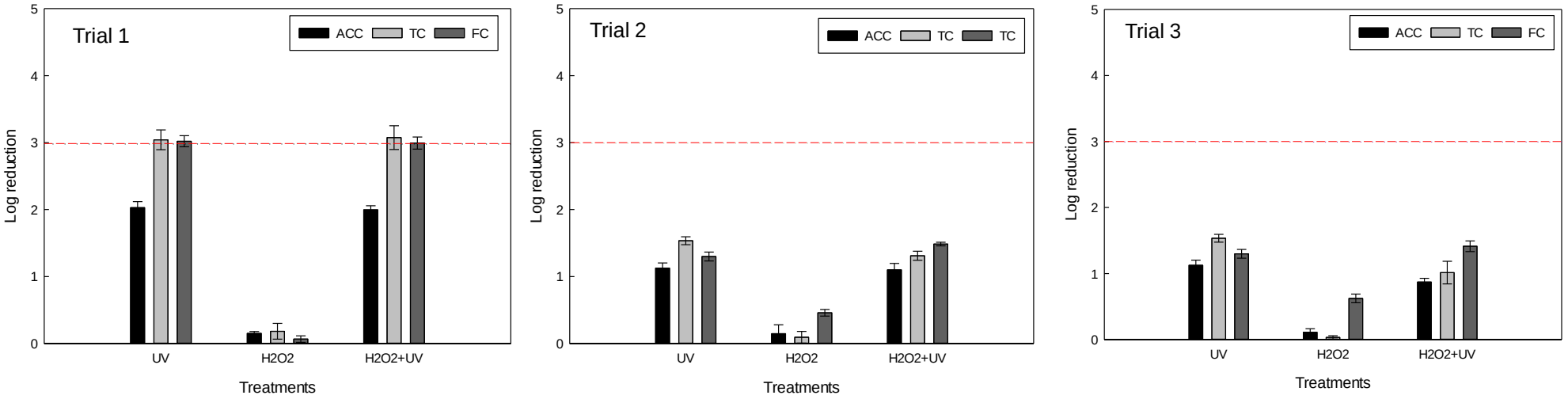

Figure 5 Log reductions reported for filtered river water exposed H_2O_2 (2.5 mg.L^{-1}) and a UV dose of 25 – 30 mJ.cm^{-2} and a combination thereof, represented by Trials 1^{H2O2} – 3^{H2O2}.

From the results obtained, as presented in Figs. 3 – 5, it could be concluded that MP-UV irradiation at pilot-scale was generally more effective than the stand-alone PAA, Cl and H_2O_2 treatments investigated. The ACC, TC and FC log reductions obtained, following PAA disinfection (Trial 1^{PAA}), were comparable to that of Trial 2^{PAA} (Fig. 3). However, poorer reductions were observed for the third trial (Trial 3^{PAA}). Nevertheless, none of the PAA trials (Fig. 3) resulted in much more than 0.5 log reductions for any of the enumerated groups. In contrast to these results a greater overall decrease in microbial numbers was observed after chlorine disinfection (Fig. 4). Comparable disinfection was observed for ACC, TC and FC for Trials 1^{Cl} and 3^{Cl} (Fig. 4), however significantly better ($p<0.05$) reductions for ACC and TC were observed for Trial 2 (Fig. 4). Chlorine was able to reduce FC by about 1.5 log (Trials 1^{Cl} – 3^{Cl}), significantly better ($p<0.05$) than observed for PAA (Fig. 3). Lastly, significantly poorer ($p<0.05$) reductions in FC were observed after H_2O_2 disinfection for Trials 1^{H2O2} – 3^{H2O2} (Fig. 5) than for chlorine disinfection. H_2O_2 treatments were only able to achieve 0.06, 0.46 and 0.62 log reduction in FC for Trials 1^{H2O2} – 3^{H2O2} respectively. Variations in microbial resistance observed within the same set of trials (for the same treatments), strongly highlighting the influence of microbial and physico-chemical parameters on disinfection efficacy.

However, regardless of the fact that dissimilar microorganisms would react differently to PAA and H_2O_2 treatments, minimal differences were observed between the two treatments, as both displayed poor disinfection efficacy for the enumerated microbial groups. Considering that H_2O_2 forms part of the commercial PAA solution used in this study, it can be postulated as to why similar sensitivity was observed for the separate PAA and H_2O_2 treatments (Figs. 3 and 5 respectively).

This study investigated the potential of chlorine at half the concentration (3 $mg.L^{-1}$) used in the previous Chapter 3. Half the dose proved to significantly reduce the occurrence of residual chlorine post-treatment, as < 0.50 $mg.L^{-1}$ residual was measured for all Trials. When considering the proposed guideline of < 0.25 $mg.L^{-1}$ (DWA, 2013b) greater potential exists that using chlorine at a concentration of 3 $mg.L^{-1}$ will be more acceptable than chlorine at a concentration of 6 $mg.L^{-1}$. Half the dose also would reduce the potential formation of disinfection by-products (DBP), as lower chlorine doses have been reported to reduce the formation of DBPs (Crebelli *et al.*, 2005; Richardson *et al.*, 2007). In conclusion, chlorine treatments were most effective than the PAA and H_2O_2 treatments against the microorganisms present in the river water, with lower efficacy reported for PAA and H_2O_2 treatments. Nevertheless, none of the stand-alone chemical disinfectants tested were able to reduce initial microbial numbers to acceptable levels, as suggested by the DWAF (1996).

Alternatively, MP-UV irradiation was adequate to reduce the FC loads present in the river water to acceptable levels, as suggested by the DWAF (1996), granted the ultraviolet transmission of the water was of an acceptable standard. Even though the target 3 log reduction was met for many of the trials, insufficient FC inactivation was still however reported in many instances, due to

extremely high levels of initial FC contamination in the river water (Tables 2 – 4). When considering the stand-alone UV treatments, and reductions achieved for TC and FC, the most effective microbial reductions were observed for Trial 2^{Cl} (Fig. 4), with similar reductions reported in Trials 2^{PAA} and 3^{PAA} (Fig. 3). Although similarities in log reduction were observed, which was expected as the UV dose was kept constant for all trials (25 – 30 $mJ.cm^{-2}$), in some instances there were significant differences ($p<0.05$) observed between the log reductions. These differences in disinfection efficacy reported, strongly suggest additional factors that could impact the efficacy of UV irradiation. Limitations regarding UV disinfection at pilot-scale, together with factors such as varying physico-chemical and microbial properties of the river water, could thus be important when considering the efficacy of MP-UV irradiation. The Berson InLine 40+ UV disinfection apparatus (Berson, The Netherlands) used at pilot-scale produced effectively the same UV dose for each trial, as the system altered the intensity of the UV light according to the UVT% of the river water measured for each respective trial. Therefore, the UV dose range was able to be kept constant throughout the trials, thus delivering the same level of germicidal efficacy each time. Olivier (2015) reported that maintaining a single UV dose using a Berson InLine 40+ UV disinfection apparatus at pilot-scale (similar to that used for this study), would be challenging, indicating the possibility that exactly the same UV dose would unlikely be achieved for each trial. Thus reporting a UV disinfection range, instead of a single UV dose, might be considered a more accurate description when reporting on the UV dose used for the trials in this study.

River water can host a large diversity of microorganisms. So, the probability that microorganisms displaying different levels of resistance exists. UV disinfection induces a germicidal effect by primarily targeting the genetic material of microorganisms, thus a difference in microorganisms may ultimately induce variation in disinfection (Gayán *et al.*, 2014). There are various factors responsible for differences in sensitivity observed for different disinfection treatments, as biochemical and physical differences between different microbial cells may both play a role. Firstly, the ability of microorganisms to repair damage induced by UV irradiation to genetic material, will influence the disinfection efficacy of UV irradiation, as some microorganisms have complex systems that once activated are able to significantly reduce the damage induced by UV irradiation (Hallmich & Gehr, 2010; Guo *et al.*, 2011). Thus, investigating the possibility of photo-reactivation and its influence on microbial reductions in river water is of importance.

Secondly, structural differences between Gram-negative and Gram-positive microorganisms have been shown to be responsible for variation in disinfection efficacy. Improved disinfection has been associated with coliforms from the groups (TC and FC), which are considered to be comprised mostly of Gram-negative bacteria such as *E. coli* (Beauchamp & Lacroix, 2012; Gayán *et al.*, 2014). Gram-positive microorganisms that are more often associated with ACC, have proven to be less effectively reduced by UV irradiation (Koutchma, 2009; Beauchamp & Lacroix, 2012). Structural variations may thus better postulate why differences in log reductions were observed for the different enumerated microbial groups (Figs. 3 – 5).

Furthermore, as similar microorganisms are usually associated with a specific enumerated microbial group, variation in log reductions within each of the microbial groups may be due to strain differences between similar microorganisms (Giddey *et al.*, 2015). Thus, different strains may display variable resistance to disinfection treatments, even within the same microbial group (Gayán *et al.*, 2011; Giddey *et al.*, 2015). Therefore proposing why differences observed for Trial 2^{PAA} (Fig. 3) with similar water quality as Trial 1 (Fig. 3), recorded significantly better ($p<0.05$) log reductions for FC and TC. Similar trends were observed for Trials 2^{Cl} and 3^{Cl} (Fig. 4), with Trial 3^{Cl} (Fig. 4) measuring significantly poorer ($p<0.05$) disinfection for TC and FC than observed in Trial 2^{Cl}. In conclusion, stand-alone UV disinfection has the potential to be used as an effective water disinfection method, however, the potential of microorganisms to repair damage induced by UV irradiation, together with varying microbial and physico-chemical parameters associated with river water, must not be overlooked. Further research would be vital, as to establish a UV dose that could be considered more reliable if used as a sole disinfection treatment.

Lastly, combination treatments (Figs. 3 – 5) proved to significantly better ($p<0.05$) microbial reductions were observed for all enumerated microbial groups (ACC, TC and FC), when compared to the stand-alone chemical treatments (Figs. 3 – 5). However, minimal differences were observed between the stand-alone UV treatments and the combination treatments, suggesting poor contribution made by chemical disinfectants. Combination treatment efficacy was hence highly dependent on the efficacy of UV irradiation, thus suggesting variable microbial resistance, as already discussed, likely to affect the efficacy of combination treatments as well.

The potential of Advanced Oxidation Processes (AOP) being initiated whilst using low chemical and UV doses in combination, would require further research. Increased chemical concentrations would, however, be required to induce more obvious disinfection benefits of Advanced Oxidation Processes (AOPs). Alternatively, completely new approaches could be considered, as the impact of varying microbial and physico-chemical parameters will always influence chemical as well as UV disinfection. Research has suggested improving filtration methods to reduce the variation in water quality upon treatment with chemicals or UV. Alternative filtration media, such as Biochar, has been investigated due to its efficacy at improving water quality, although little has been reported on its ability to reduce microbial levels in water.

Study 2: Potential of photo-repair following MP-UV irradiation

Investigating the potential of photo-repair after irradiating river water with a UV dose of 25 – 30 $mJ.cm^{-2}$, at pilot-scale, was done by completing three separate trials. The physico-chemical parameters measured for river water sampled (Table 5), showed the UVT% to range from 33.50% to 54.30% for Trials 1 – 3. The UVT% measured (Trials 1 – 3) could generally be considered unfavourable regarding UV irradiation efficacy. The lowest COD (30.60 $mg.L^{-1}$) was measured for the water used in Trial 2, which also reported the lowest turbidity (9.29 NTU), TSS and VSS of the three Trials. Trial 1 recorded the highest COD, turbidity, TSS and VSS, thus

indicating water of a poorer quality when compared to the water sampled for Trials 2 and 3 (Table 5). These above mentioned physico-chemical parameters are all indicative of a higher degree of suspended organic matter present in the water (Van Haute *et al.*, 2013), as was expected from Trial 1 because of the poor UVT%, turbidity, TSS and COD recorded. Nevertheless, the TSS values for the water sampled in all 3 trials were able to meet the suggested guideline of $\leq$ 50 $mg.L^{-1}$ (DWAF, 1996).

The pH of the river water for Trials 1 – 3 was able to meet the guideline (Table 1), however, the conductivity recorded (Table 5) was unable to do so, as the guideline suggests the conductivity of the water not to exceed 40 $mS.m^{-1}$. Similar physico-chemical characteristics were also reported by a number of researchers regarding the river water quality of the Plankenburg River (Britz *et al.*, 2013; Bester, 2015; Olivier, 2015). In conclusion, Trial 1 and 3 appeared to measure poorer water quality, with improved water quality recorded for the water sampled in Trial 2 (which was still not of a good quality).

River water quality

Table 5 Physico-chemical and microbial properties of the sand-filtered river water sampled from the Plankenburg River for reactivation Trials 1 – 3

Quality parameter	Trial 1	Trial 2	Trial 3
UVT%	35.20	54.30	33.50
COD ($mg.L^{-1}$)	103.50	30.60	48.60
Turbidity (NTU)	21.35	9.29	15.53
TSS ($mg.L^{-1}$)	22.00	18.00	21.00
VSS ($mg.L^{-1}$)	17.00	13.00	16.00
pH	6.95	7.00	6.87
Alkalinity (mg $CaCO_3.L^{-1}$)	100.00	75.00	125.00
Conductivity ($mS.m^{-1}$)	40.60	44.10	46.50

Photo-repair Following UV irradiation

Figure 6 presented the percentage log recovery of both TC and FC after UV irradiated water was exposed to 3 h of visible light, at and intensity of 3.5 kLux. Only Coliform groups were investigated, as it is these groups which are usually listed in guidelines regarding irrigation water quality (DWAF, 1996). UV-induced damage may be repaired through the action of photolyases, described as the process of photo-repair (also known as photo-reactivation) (Guo *et al.*, 2011; Gayán *et al.*, 2014). Trial 1 (Fig. 6) reported 11.16% recovery in TC and 15.86% recovery for FC, after being exposed to 3 h of photo-reactivating light. Similarly, TC for Trial 2 recorded a log recovery of 10.91%, however, significantly less ($p<0.05$) log recovery was reported for FC than for

Trial 1, reporting 8.23% recovery (Fig. 9). A final trial (Trial 3) measured a log recovery of 13.72% for TC and 15.36% for FC.

Similar FC log recovery was reported for Trials 1 and 3 (15.86% and 15.36% respectively), however, significantly less ($p<0.05$) recovery was reported for Trial 2 (10.91%). When considering the physico-chemical parameters (Table 5), the water sampled for Trials 1 and 3 reported poor UVT% values (35.20% and 33.50% respectively). Gayán *et al.* (2014) suggested that poor UVT% would be indicative of less effective disinfection, as a decreased UV light intensity would be associated to it. Thus, an explanation is provided as to why FC for Trial 1 and 3 were able to regenerate themselves more easily (due to less damage) than the FC detected in the water of Trial 2, which had an improved UVT% (Table 5). The lowest percentage log recovery for the Coliforms was reported for the water of Trial 2 (TC 10.91% and FC 8.23%), which also reported the better UVT%, COD, turbidity, TSS and VSS (Table 5).

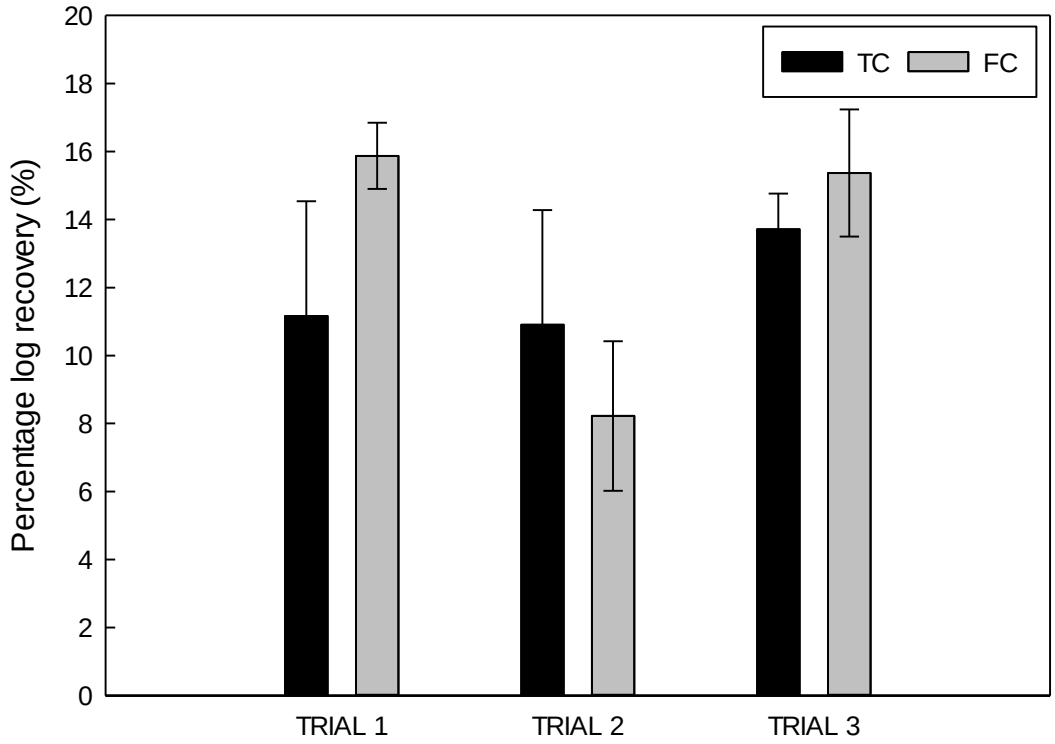

Figure 6 Log reactivation for TC and FC for Trials 1 – 3 when MP UV irradiated samples were exposed to 3.5 kLux visible light for 3 h.

Photo-repair has been investigated by a number of researchers, as findings may drastically alter the overall efficacy of UV irradiation. Similar trends have been observed by other researches, as reported in this study, regarding photo-reactivation. Guo *et al.* (2009) showed little reactivation reported at high UV doses for both LP and MP-UV systems, however, making use of lower UV doses resulted in 20.00% recovery for *E. coli*. As most research has been done on low-pressure (LP) UV irradiation water, investigating MP disinfection systems (as used in this study) could provide a more informative understanding on the repair of microorganisms. MP-UV systems are

more often used in the treatment of surface water at up-scaled water treatment facilities (Guo *et al.*, 2009; Koutchma, 2009). Pyrimidine dimer formation has been linked to a decreased repair of damaged genetic material within microorganisms (Eischeid & Linden, 2007; Poepping *et al.*, 2014). Quek & Hu (2013) reported increased photolyase damage, as higher UV doses (± 20 $mJ.cm^{-2}$) are associated with decreased repair potential of microorganisms.

According to a study completed by Olivier (2015), up to 29.07% log recovery was reported after MP-UV irradiated river water, at a UV dose of 24 $mJcm^{-2}$, when exposed to visible light for 3 h. In comparison to this study, where less than half that log recovery was reported, regardless of the fact that similar physico-chemical parameters as well as UV doses were reported between the two studies. Microorganisms displaying increased resistance to UV irradiation will thus be 'less damaged' and more likely to recover than microorganisms displaying decreased resistance to UV irradiation. Guo *et al.* (2011) suggested that when evaluating photo-repair, considering the potential of normal growth of the unaffected microorganisms, as well as, the normal growth of regenerated microorganisms, must both be considered contributing factors to the overall increase in microorganisms after UV irradiation. When considering no significant difference were observed for the non-UV irradiation control after 3 h, suggested that photo-reactivation was the dominant process responsible for the increased microbial numbers, post-UV irradiation.

According to Fig. 6 up to a 15.86% recovery for FC and 13.72% recovery for TC were observed, when both were exposed to 3.5 kLux visible light for 3 h. Research has reported 50 – 70% recovery at increased light intensity ranging between 6 – 11.5 kLux (Quek & Hu, 2008b). When considering that sunlight intensity has been reported to reach up to 100 kLux, increased reactivation potential is plausible (Lichtsteiner, 2008). In view of the proposed 3 log target reduction a 15.86% log recovery, as reported in this study (Fig. 6), would thus propose an adequate reduction of 3.50 log, if factoring in up to a 15.86% log recovery. When considering the log reductions achieved for Study 1, certain trials were just able to reach the target 3 log reduction but considering that up to 15.86% recovery could be expected after 3 h, < 3 log would therefore be a more accurate representation of the actual log reduction of UV irradiation. Furthermore, considering the importance UV irradiation has for the combination treatments for Study 1, a 15.86% recovery could would significantly alter the overall efficacy.

Overall, a variety of factors have shown to alter the photo-repair potential induced by the photolyase enzymes such as UV dose, light intensity, water and air temperature (Guo *et al.*, 2011; Quek & Hu, 2013). Furthermore, factoring in the microbial and physico-chemical variability of river water further adds to this complex phenomena of microbial repair. In order to gain a better understanding of the photo-repair potential, under more realistic conditions, less optimal conditions will need to be investigated. UV-irradiation has proved effective in reducing the initial microbial loads in river water through the studies completed. Considering the potential of microbial regeneration, following UV-irradiation as investigated in this study, even as little as 15.86% regeneration could influence the acceptability of river water for irrigation purposes. Therefore,

investigations into higher UV doses would provide better insight on the potential of microbial damage repair. As disinfection treatments have shown variability in efficacy due to ever changing microbial and physico-chemical quality of the river water, employing pre-treatment steps in an attempt to 'standardise' the water, should be considered. When considering sand filtration, as used in this study, poor UVT%, turbidity and COD values were still recorded for the filtrate (following sand filtration), thus considering more effective filtration methods should be investigated.

CONCLUSIONS

In this study, investigating the potential of MP-UV irradiation, in combination with different chemical disinfectants on river water at pilot-scale, was investigated. As observed in Study 1 the river water sampled for all trials, from a microbiological perspective, was unable to meet the guideline proposed by the DWAF (1996) for acceptable irrigation water. Similar trends were followed for most of the physico-chemical properties measured for the river water, as often poor UVT%, COD and turbidity values were recorded. Correlating the river water quality to chemical disinfection efficacy was difficult, due to chemical disinfectants being unable to achieve acceptable water quality without the addition of MP-UV irradiation (for combination treatments). Overall stand-alone chemical disinfectants (PAA, Cl and H_2O_2) proved to be significantly less ($p<0.05$) effective than MP-UV irradiation at reducing the initial microbial levels measured in the river water. Chlorine treatments (3 $mg.L^{-1}$) were the most effective of the stand-alone chemical disinfectants. Nevertheless, stand-alone UV treatments (25 – 30 $mJ.cm^{-2}$), for most trials, were significantly more ($p<0.05$) effective than chlorine treatments, sufficiently able to reduce the FC levels in river water to an acceptable point (< 1 000 cfu.100 mL^{-1}) (DWAF, 1996). The influence of poor optical water characteristics could, however, not be overlooked, as a poor UVT% and turbidity were associated with sub-optimal UV disinfection, as observed in some instances. Consequently MP-UV irradiation, as tested, was unable to be achieve reliable and effective microbial reductions, as varying microbial and water quality had a direct influence on disinfection efficacy. The poor contribution made by chemical disinfectants to the overall log reductions reported (for combination treatments), emphasised the dependence on UV irradiation when aiming to achieving adequate disinfection, which was not always effective. It was also expected that minimal production of disinfection by-products (DBPs) were prevalent at the chemical concentrations used, as <0.50 $mg.L^{-1}$ residual chlorine was available in the water following chemical treatments.

In a second study completed, the potential of microbial damage repair (photo-repair), following MP-UV irradiation was investigated at pilot-scale. River water treated with a UV dose between 25 – 30 $mJ.cm^{-2}$ reported up to 13.72% recovery and 15.86% recovery for TC and FC respectively. As considerable variation in river water quality was measured for the water sampled for the different trials, variability in the degree of photo-repair was also observed. Regardless thereof, the potential of reactivation was plausible and thus its influence on lowering the originally perceived disinfection efficacy (in log reductions) should be factored in.

It would be advisable, if exclusively using UV irradiation as a water disinfection treatment, to utilise the UV treated water directly after irradiation as to decrease the potential for photo-repair. Nevertheless, MP-UV irradiation applied as a stand-alone treatment or in combination with PAA (4 $mg.L^{-1}$), Cl (3 $mg.L^{-1}$) or H_2O_2 (2.5 $mg.L^{-1}$), was unable to reduce the FC to acceptable levels. No evidence of advanced oxidation processes (AOP) were made available upon evaluation of the combination treatments efficacy, as no significant differences ($p<0.05$) were observed between the stand-alone UV treatments and combination treatments (Figs. 3 – 5). As river water, sampled in the dry months (summer months), was used as a test medium, poor water quality was expected. Improved disinfection efficacy was, however, expected if river water was to be sampled in other months (non-summer months), as a more 'diluted' water quality could be expected as reported by other researches (Paulse *et al.*, 2009). As the UVT% seldom exceeded 40% in this Study, a theoretical improved disinfection could be expected for water displaying improved UVT%, which will often also associated with improved microbial and physico-chemical water quality. Effectively, combination treatments hold potential to minimise the effect varying microbial and water quality may have on the individual treatments and increase the certainty of effect disinfection, although the investigation of increased chemical concentrations would need to be completed.

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Chapter 5

EVALUATING THE EFFICACY OF BIOCHAR AS A VIABLE FILTER MEDIA TO IMPROVE RIVER WATER QUALITY

ABSTRACT

The possibility of using biochar as an alternative filtration method for the pre-treatment of river water was explored in this study. The efficacy of the disinfection treatments studied in Chapter 3 and 4, was clearly affected by varying microbial and physico-chemical properties of river water. Thus, the ability of biochar to improve the initial microbial and physico-chemical quality of river water was evaluated. Two different types of biochar were investigated, namely Eucalyptus and Pine. The efficacy of both Pine and Eucalyptus biochar filter columns were compared to a control sand filter column.

Significant improvements ($p<0.05$) to river water quality were observed for the filtrate collected from the Eucalyptus biochar filter columns, with significantly less effective filtration recorded for Pine biochar filter columns. Secondly, the potential of microbial washout was determined for previously 'used' Eucalyptus filter columns after 48 – 72 h of discontinued water flow. After autoclaved, distilled water was filtered through the 'used' Eucalyptus filter columns, > 3 log Faecal Coliform (FC) levels were detected in the filtrate. This was unexpected, as the same filter columns, when not previously used, recorded no microbial growth in the filtrate, even when river water with 3 – 4 log FC levels was allowed to filter through previously unused columns. Eucalyptus filter columns were able to improve the ultraviolet transmission (UVT%) of the river water from 52.40% to 91.50%, as well as reduce the chemical oxygen demand (COD) from 120.60 $mg.L^{-1}$ to 16.20 $mg.L^{-1}$.

Additionally no microbial growth was detected in filtrates for any of the enumerated groups (ACC, TC and FC) when using Eucalyptus biochar filter columns. Unfavourable change in water pH was recorded for the biochar filtrate, as water recorded a pH change from a near neutral to a more basic pH ($\pm$ pH 8 – 9) post-filtration for both Pine and Eucalyptus biochar filter columns. Overall the different biochar filter media investigated demonstrated variability in efficacy. Pine biochar filter columns showed little difference from a standard sand filter column filtration regarding improvement to the microbial quality of river water. Eucalyptus biochar showed a high probability, when incorporated as a filtration media, at being effective in significantly improving the microbial as well as physico-chemical properties of water, even when subjected to extreme water quality.

INTRODUCTION

South African water sources have shown an alarming increase in pollution levels over the years (Paulse *et al.*, 2009). River water is often contaminated with high levels of microbial pollution, pharmaceuticals and detergents (Gemmell & Schmidt, 2012; Yao *et al.*, 2012). In terms of the microbial pollution, high levels of faecal contamination are often associated with many South

African rivers, often as a direct result of poor waste management and improper wastewater treatment (Schaefer, 2008; Britz *et al.,* 2013). Attempts to control microbial pollution has led to the use of, often costly, chemical disinfectants prior to irrigation (Worrall & Burt, 2009). Disinfection efficacy is, however, not always guaranteed due to the ever changing water quality, especially that of river water (Britz *et al.*, 2013, Olivier, 2015).

Irrigating crops with microbially unsafe water can have major implications regarding human health, as well as economic losses (DWAF, 1996). Alternatives to chemicals disinfectants, such as UV irradiation, have been shown to produce effective microbial reductions with regards to river water, even when highly polluted water is treated (Hijnen *et al.*, 2006; Koutchma, 2009; Gayán *et al.*, 2014). UV irradiation, however, is not risk free. Photo-reactivation and decreased disinfection efficacy, associated with poor and varying water quality, often present challenges when implementing UV irradiation (Rastogi *et al.*, 2010; Guo *et al.*, 2011; Gayán *et al.*, 2014). Employing pre-treatment filtration steps may be considered a viable option to 'standardise' the river water. If a more uniform water quality is achieved post-filtration, increased reliability can be expected for the chemical and UV treatments used to disinfect water. Biochar filtration, when used as a filtration step, could produce a more uniform and consistent water quality, further optimising chemical and UV disinfection.

Sand filtration is a well-accepted pre-treatment filtration method, but only allows minimal improvements to water quality post-filtration (Haig *et al.*, 2011; Corral *et al.*, 2014). Recently biochar has been evaluated as an alternative filtration media for application in wastewater treatment (Ahmad *et al.*, 2014; Mohan *et al.*, 2014). Due to unique characteristics associated with biochar, it is gaining acceptance as a low-cost alternative sorbent, capable of removing high levels of organic and inorganic pollution from water (Alam *et al.*, 2009; Yao *et al.*, 2012; Ahmad *et al.*, 2014; Baltrenaite, 2016). Biochar has proven effective in water remediation by removing inorganic and organic compounds such as dyes and phenolic compounds. The highly porous charcoal-like nature of biochar allows for increased absorption capabilities, as well as increased surface area when compared to alternative filtration media such as standard sand filters (Alam *et al.*, 2009; Hunt *et al.*, 2010; Mohan *et al.*, 2014).

Biochar is primarily produced through a thermal process, referred to as pyrolysis, which aims to convert discarded agricultural waste to an environmentally beneficial product (Chen *et al.*, 2008; Spokas & Reicosky, 2009). Alam *et al.* (2009) showed that the adsorption capacity of different biochar samples increased with an increase in pyrolysis temperature, thus highlighting variability in absorption capabilities between different types of biochar.

Research has suggested that using virgin (unused) biochar as a filtration media could result in changes in pH and nutrient levels, as increases in inorganic content have been reported for water that has undergone biochar filtration (McClellan *et al.*, 2007). Guidelines established for irrigation water, suggesting an acceptable pH range of 6.5 – 8.4, must be kept in mind when evaluating biochar as a filter media (DWAF, 1996). Undesirable changes in pH are also likely to

influence the efficacy of chemical treatments such as chlorine (Newman, 2004; Lewis, 2010). Evaluating the potential changes in pH is thus essential when considering biochar as a filtration method. Limited research has been published on the potential of biochar to effectively reduce microbial numbers post-filtration (Dempster *et al.*, 2012; Ahmad *et al.*, 2014). Hunt *et al.* (2010) recommended biochar to be a prospective wastewater treatment, due to specific surface functional groups promoting the removal of contaminants from water (even those of microbial origin).

The objectives of this study were to investigate the potential of biochar to be used as a viable filtration method to improve the physico-chemical properties of river water and also reduce microbial numbers. In order to do so two different biochar types, namely Pine and Eucalyptus biochar, were compared to standard sand filters. Thereafter the impact of discontinued water flow over a period of time, on the filtration capabilities of used biochar filters to reducing microbial levels, was also determined.

MATERIALS AND METHODS

Research study design

The potential of biochar to be used as an effective filtration media was investigated by completing a series of experiments at laboratory-scale. Efficacy testing was based on the biochar filters' capacity to improve the physico-chemical properties and reduce microbial numbers present in river water. Pine and Eucalyptus biochar types were investigated. Constructing sterile filter columns, containing biochar and river sand, untreated river water samples (collected from the Plankenburg River in Stellenbosch) were allowed to filter through the different filter columns. Microbial and physico-chemical analyses were completed before and after filtration in order to determine differences in filtration capacities between the biochar types. Results were compared to guidelines established by the Department of Water Affairs (DWAF) for acceptable irrigation water quality.

Study 1: Evaluating the potential of biochar to be used as a viable filter media for improving river water quality

The filtration efficacy of different biochar types was investigated. Two different biochar types were tested, namely Eucalyptus and Pine biochar. River water was sampled from the Plankenburg River, followed by determining the initial microbial and physico-chemical quality of the water under laboratory conditions. River water (500 mL) was filtered through specially designed filter columns (Figure 1). Thereafter microbial enumeration was done on the filtrate collected, so as to determine possible changes in aerobic colony counts (ACC), as well as in Total and Faecal Coliforms (TC and FC). Possible changes in the physico-chemical properties of the river water after filtration was also investigated and compared to standards established by the DWAF (1996). Control filters containing only sand were used to determine the contributions of the sand to the overall filtration efficacy, as all the filter columns contained sand as part of the filter design.

Study 2: Evaluating potential microbial washout from used biochar filters

In order to gain insight into the potential long term use of biochar filters, the ability of used biochar filters in maintaining their filtration capabilities was investigated. The used biochar filters (which had been used to filter 5 L of river water in Study 1) were sealed with autoclaved aluminium foil to prevent airborne microbial contamination, and stored for 48 – 72 h at room temperature (24±2°C). Thereafter 500 mL of sterile distilled water was allowed to filter through each of the filter columns and the filtrate was collected. Potential microbial growth within the used filter columns was investigated by analysing the filtrate collected for microbial washout. The filtrate was tested for ACC, TC and FC.

GENERAL MATERIALS AND METHODS

Construction of sterile biochar filters

Filtration columns were constructed using glass columns with open ends. A sterile metal mesh was fixed over one of the open ends. Sterile glass wool was then placed at the bottom of the column (kept in place by the mesh). A layer of prepared river sand (100 g) formed the next layer. After the addition of a layer of biochar (300 g), another layer of sand (200 g) was added. The two layers of sand were intended to keep the biochar in place (Fig. 1).

Standard sieved river sand used in the construction of the filter columns, with particle sizes of 0.5 – 1.0 mm, was used throughout. Before the river sand was incorporated into the filters it was acid-washed with a hydrochloric acid solution (0.1 N), prepared by adding 10 mL of concentrated hydrochloric acid (HCl) to 1 L distilled water. The acid washing process aimed to remove any volatiles and heavy metals that could be associated with the sand. These could potentially affect the pH as well as other physico-chemical properties of the filtered water. Using distilled water, the excess acid was removed until a neutral pH was achieved using MColorpHast – pH indicator strips (Merck, SA). The acid washed sand was then autoclaved for 20 min at 121°C and dried at 100°C.

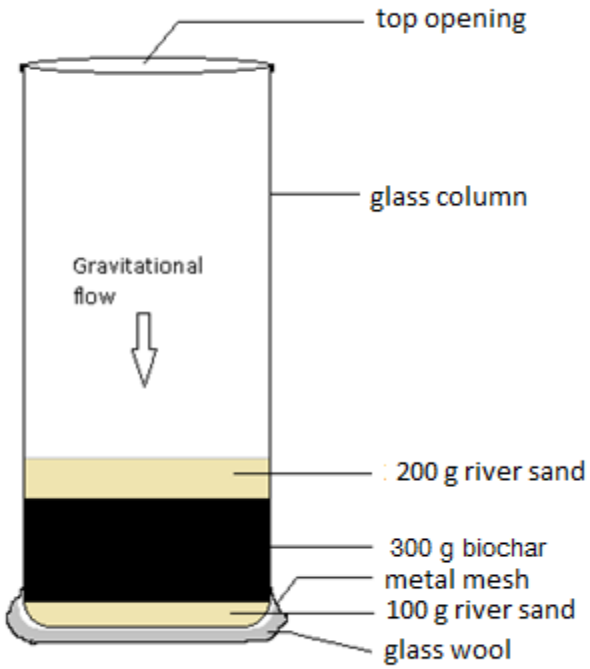

Figure 1 Biochar filter layout used in Studies 1 and 2, highlighting fundamental components and their relevant proportions.

The glass wool, metal mesh, glass columns and cable-ties were sterilised in an autoclave for 20 min at 121°C to ensure the aseptic construction of filter columns. The filtrate was collected in 1 L autoclaved glass bottles (Fig. 2).

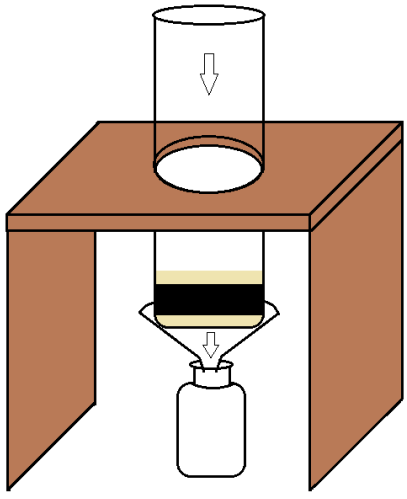

Figure 2 Set up of complete biochar filter column and filtrate collection process in 1 L glass bottle below the biochar filter column.

River water sampling and site location (Study 1)

The river water used was sampled from the Plankenburg River in Stellenbosch (33°56'15.4''S, 18°50'53.0''E). Sampling was done according standard methods (SANS 5667-6, 2006). The river water was collected in sterile 5 L glass bottles. Samples were kept and transported in cooler boxes until further analysis done within 4 h of sampling.

Statistical analysis

The statistical analysis was done using Statistica 13.0 software (StatSoft, USA). The data was analysed using one-way, two-way and mixed model ANOVA. Fisher least significance difference (LSD) post hoc analyses were performed using a 5% significance level ($p<0.05$) as guideline for significant results.

The filtration process

The experimental procedure followed to determine the effect of Biochar on river water quality is summarised in Fig. 3. An identical protocol was used in both the sand and biochar filter columns (Fig. 3). A dilution series was prepared (10^{-1} – 10^{-6}) and analysed from the unfiltered river water, that served as pre-treatment control to determine the microbiological quality of the water before filtration.

Autoclaved distilled water (500 mL) was added to the columns before experimentation took place. This served the dual purpose of washing away any impurities from the biochar and allowed the biochar to absorb moisture. 'Moistening' the biochar also allowed the individual particles to move more closely to one another, minimising air pocket size. Results from preliminary trials suggested that 100 g of biochar had the potential to retain about 300 mL of a liquid, at any given time. After 'moistening', 500 mL of river water was allowed to filter through the biochar columns, before the filtrate was collected for further testing.

River water filtrate collected from both biochar and sand-only filter columns was again used for microbial analysis. The dilution series were prepared in triplicate.

Microbiological analysis (Study 1 and 2)

Before and after all filtration experiments, the microbial quality of the river water was determined using standard plate count methods. For Study 2 microbial enumeration was completed on the filtrate only, as autoclaved, distilled water was used to filter through the columns. All microbial enumeration was done in duplicate after dilution series were prepared (10^{0} – 10^{-6}) in triplicate.

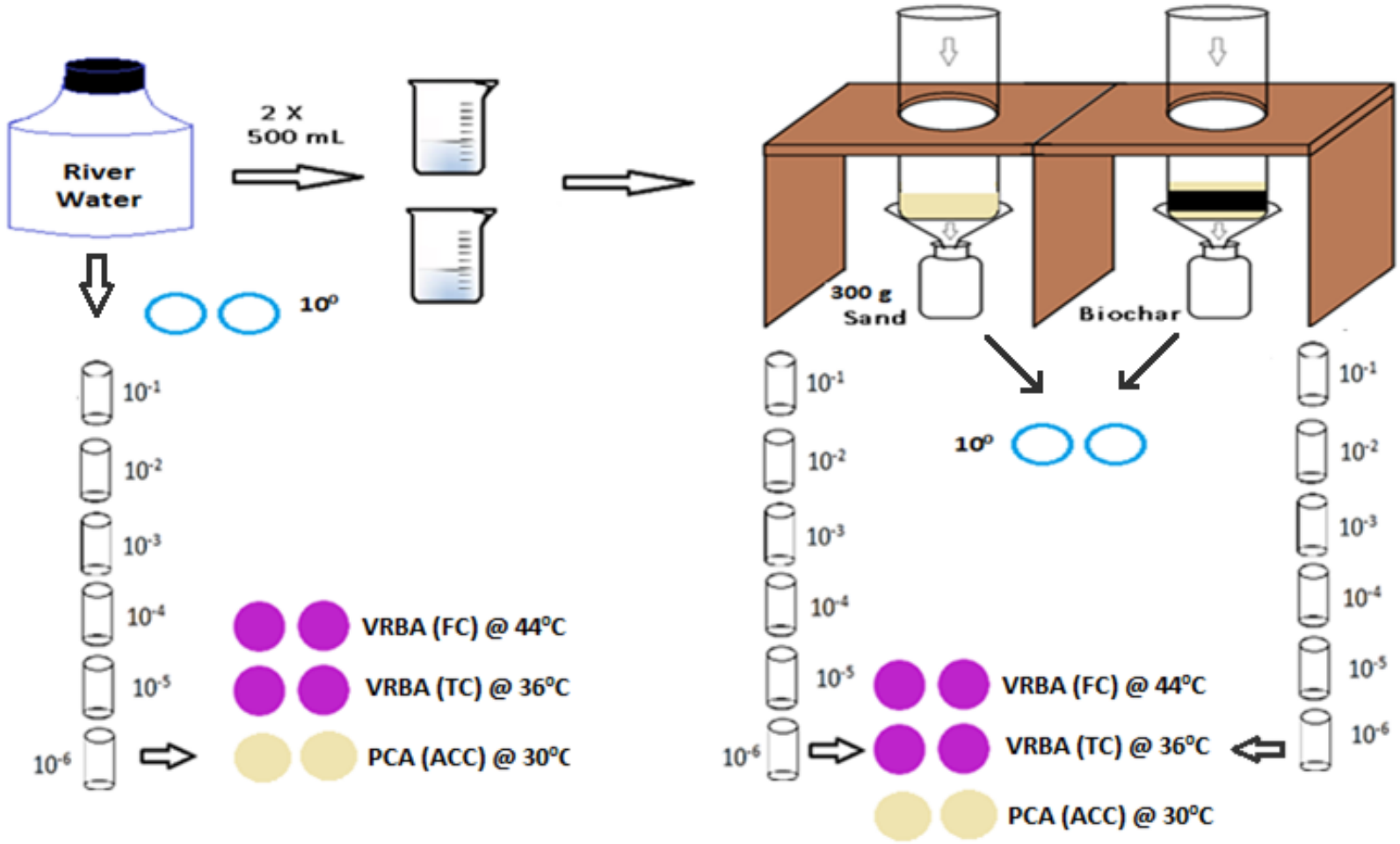

Figure 3 Experimental procedure used in study 1 to determine the log reduction induced post-filtration, using sand and biochar filters.

Total coliform (TC), faecal coliform (FC) and aerobic colony counts (ACC)

TC were determined according to the (SANS) methods 4832 (SANS, 2007a). Violet Red Bile Agar (VRBA) (Merck, South Africa) was used for the preparation of the pour plates, incubated at 36±0.5°C for 24 h. FC were also evaluated by using VRBA (Merck, South Africa), but incubated at 44±0.5°C for 24 h (Schraft & Watterworth, 2005). ACC were determined according to the South African National Standards (SANS) methods 4833 (SANS, 2007b). Samples were serially diluted (10^0 – 10^{-6}) and plated using Plate Count Agar (PCA) (Merck, South Africa). The poured plates were incubated at 30±0.5°C for 48 h (SANS, 2007b).

Physico-chemical parameters

Analyses done on the filtrate included: Chemical Oxygen Demand (COD), alkalinity, TSS and VSS, determined according to Standard Methods (APHA, 2005). The COD was measured using a Spectroquat® Nova 60. The alkalinity was measured by titrating river water against 0.1 M H_2SO_4 and was expressed in $mg.L^{-1}$ $CaCO_3$. Other water quality parameters also measured included: Ultraviolet Transmission Percentage (UVT%), pH, Electrical Conductivity (EC) and turbidity. The pH was measured using a 320 pH meter (WTW, Germany). The EC was measured using a HI 8711 conductivity meter (Hanna Instruments, USA) and expressed in $mS.m^{-1}$. The UVT% was determined using a hand held Sense™ Ultraviolet Transmittance Monitor (Berson, Germany). The water turbidity was measured using an Orion AQ3010 Turbidity Meter (Thermo Scientific, USA).

Biochar

The two biochar types that were evaluated had been produced by a slow pyrolysis process, commonly associated with the production of biochar used for wastewater treatment (Tan *et al.*, 2015). The biomass that had been used for the production of the different biochar types were: (1) wood from Eucalyptus Trees; (2) wood from Pine Trees. The two biochar types had undergone pyrolysis at different temperatures, forming a charcoal like material with varying particle sizes. Pyrolysis temperature was not dependent on the original biomass used, but specifically chosen for this study to ensure variation between the two biochar varieties. Eucalyptus biomass was produced at 700 – 800°C, Pine biomass, however, was subjected to lower temperatures ranging from 400 – 500°C. The biochar produced was sieved and particle sizes of 0.5 – 2.0 mm used for experimental purposes.

RESULTS AND DISCUSSION

Study 1: Evaluating the potential of biochar to be used as a viable filter media for improving river water quality

The physico-chemical analysis results, presented in Table 2, display the variations observed between the three trials (Trials 1 – 3) completed in this study. Changes in the river water composition were determined by measuring the physico-chemical parameters before and after

filtration, for both Pine and Eucalyptus filter media (Table 2). Guidelines have been established regarding certain physico-chemical and microbial water properties for acceptable irrigation water (Table 1). The filtrate collected was compared to the guidelines (Table 1) established by the DWAF (1996), indicative of effective filtration and acceptable water quality.

Table 1 Guidelines regarding quality of water intended for irrigation purposes (DWAF, 1996)

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

Considering Trial 1 (Table 2), Eucalyptus biochar brought about a significant ($p>0.05$) improvement to the COD, UVT% and turbidity, however, the opposite was observed for the Pine filtrate. Pine biochar resulted in a decrease in the quality of the river water, as increases in COD and TSS, decrease in UVT% and a poorer turbidity were measured in the filtrate when compared to the unfiltered river water (Table 2).

Upon completion of Trial 2 Eucalyptus filter media again revealed improved filter capabilities regarding the COD, UVT%, turbidity, TSS, VSS and conductivity, when compared to results obtained for the Pine filtrate (Table 2).

The results recorded for the final trial (Trial 3, Table 2), confirmed the clear advantages of using Eucalyptus biochar over Pine biochar as a filter media. Eucalyptus biochar was again able to improve the COD, UVT%, turbidity and TSS of the unfiltered river water, which Pine biochar was unable to do. More so, Pine biochar produced a less desired filtrate with increased COD, turbidity, TSS and decreased UVT% (Table 3).

When evaluating the individual physico-chemical parameters of all three trials (Trials 1 – 3, Table 2) the COD of the river water before filtration, Trial 2, had the highest COD (120.60 $mg.L^{-1}$) of the three trials, with Trials 1 and 3 showing little variation (61.20 and 64.80 $mg.L^{-1}$ respectively). Post-filtration Eucalyptus filtrate showed considerable improvements in COD content for all three trials, with Trial 2 recording the best reduction (down to 16.2 $mg.L^{-1}$). Similar trends were, however, not observed for the Pine filtrate, as Trials 1 and 3 reported increased COD values compared to the unfiltered river water (Table 2). The water used in Trial 2, which had the highest COD value, showed the only decrease in COD value (from 120.60 to 70. 2 $mg.L^{-1}$) for Pine biochar filtration.

A possible explanation for the differences in COD reduction could be attributed to the different temperatures at which the two biochar filter media were produced. A higher pyrolysis temperature is usually associated with improved removal of organic matter, as was seen with the Eucalyptus biochar which underwent pyrolysis at the higher temperature. However, at lower

pyrolysis temperatures, as used in the production of Pine biochar in this study, less effective removal of organic matter would be expected, resulting in increased COD values in the filtrate (Ahmad *et al.*, 2014; Mohan *et al.*, 2014).

The UVT% for the unfiltered river water was poorest for Trial 2, which also reported the highest COD value of the three trials. Eucalyptus filtration showed improved UVT% for all three trials (Table 2), with Trial 1 reporting an excellent UVT% of 91.50%. Opposite trends were recorded for Pine filtrate, which had poorer UVT% post-filtration in all the three trials. Trial 3 filtrate had the poorest UVT% of only 8.40% (Table 2). This was expected, as the Pine filtrate appeared brown and murky. These variations in UVT% might also be linked to the differences in pyrolysis temperatures, as well as variation in the composition of the different biochar types investigated (Mohan *et al.*, 2014; Tan *et al.*, 2015).

Turbidity values (Table 2) for the river water, showed similar trends as for the UVT%. Improved turbidity values were measured for the filtrate after Eucalyptus filtration, with an increased turbidity recorded for the Pine filtrate. Eucalyptus filtration (Trial 2) showed the greatest improvements to turbidity, reporting a change from 21.80 NTU to 6.61 NTU (Table 2). However, Pine filtration for Trial 3 worsened the turbidity form 14.00 NTU to 42.30 NTU. These results (Table 3) were confirmed by the visual observations made for the Pine filtrate, as described earlier.

Suspended solids measurements were reported separately as Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) (Table 2). The TSS generally decreased for the Eucalyptus filtrate for all three trials, however, only minimally when compared to the unfiltered river water. Unsurprisingly the Pine filtrate showed increased TSS values. Nevertheless, both Pine and Eucalyptus filtrate was able to meet the guideline of 50 $mg.L^{-1}$ for TSS (DWAF, 1996). Similar trends were observed for the VSS, as both filter media types were able to lower the levels of volatile material suspended in the water sampled after filtration (Table 2). In view of the TSS, turbidity, UVT% and COD values measured (Table 2), the unfiltered river water (Trial 2) had the least favourable water quality of the three trials. Hamoda *et al.* (2004) reported similar COD and TSS values (as measured for the river water used in this study) for secondary wastewater used in sand filtration experiments. Similar physico-chemical measurements had also previously been reported for the water of the Plankenburg River, thus highlighting the poor quality of the water associated with this river system (Bester, 2015; Olivier, 2015).

Large changes in pH were reported for the filtrate of both Pine and Eucalyptus when compared to the unfiltered river water (Table 2). The pH changes recorded were generally from $\pm$ pH 6 to $\pm$ pH 9 after filtration, which can be considered problematic when compared to the guideline established by the DWAF (1996) for irrigation water (Table 1). The lowest pH measured after filtration was for Trial 2 (pH 8.90), falling outside the acceptable pH guidelines of 6.5 – 8.4 for neutral irrigation water (DWAF, 1996). When considering that biochar production favours the development of alkali salts (metallic compounds), as suggested by Ahmad *et al.* (2014), an increase in pH for the filtrate is thus expected.

The guidelines (DWAF, 1996) suggests an acceptable conductivity of 40 $mS.m^{-1}$, which was exceeded by both the untreated water (Trials 2 and 3), as well as the filtrate of Pine and Eucalyptus filter media (Trials 1 – 3). The unfiltered river water of Trial 3 had the highest initial conductivity of all the river water samples. Lastly, increases in alkalinity values were recorded for both filter media during all three trials (Table 2). However, Eucalyptus filtrate reported lower increases in alkalinity in comparison to the Pine filtrate. In Trial 2 Pine filtrate showed a 225 mg. $CaCO_3.L^{-1}$ increase in alkalinity, when compared to the unfiltered river water (100 mg. $CaCO_3.L^{-1}$) (Table 2).

When considering the physico-chemical properties (Table 2), it was clear that the unfiltered river water used in Trial 2 was of a poorer quality, when compared to Trials 1 and 3. Furthermore, post-filtration physico-chemical results revealed Eucalyptus biochar to possess superior filtration capabilities, especially regarding UVT%, COD and turbidity reduction, when compared to Pine filtration. Also, when considering Trials 1 – 3, post-filtration, it appeared that using biochar as a filter media would increase the likeliness for a spike in filtrate pH for both filter media tested. This could be considered problematic if a change in water pH is undesirable. Trial 2 revealed that even at the poorest water quality, as measured (Table 2), Eucalyptus biochar was still effective in significantly improving many of the physico-chemical parameters measured, thus, further promoting Eucalyptus biochar as a more effective filtration media than Pine biochar.

Post-filtration microbial analysis

Upon evaluation of the log reductions presented in Fig. 4 (Trials 1 – 3), clear variations between the different filtration media investigated were observed. For the sand filter column (Trial 1) the greatest log reductions were recorded for FC (>1 log), which was significantly better ($p<0.05$) than the log reductions for ACC and TC (<1 log) (Fig. 4). Significantly improved ($p<0.05$) log reductions in ACC, TC and FC were recorded for the filtrate when filtering with Eucalyptus filtrate columns (Fig. 4). Pine filter columns produced significantly less effective ($p<0.05$) microbial reductions for all enumerated microbial groups (ACC, TC and FC) when compared to Eucalyptus filter columns (Trial 1, Fig. 4). Furthermore, results did not differ significantly between the sand filters (control) and Pine filter columns (except for TC, Trial 1), thus, showing the inefficacy of Pine biochar when used as a filter media.

Similar trends were observed between Trials 1 and 2 (Fig. 4), as the sand filter columns were unable to achieve even 1 log reduction in ACC, TC and FC, with significantly better ($p<0.05$) filtration efficacy reported for Eucalyptus biochar filtration (Trial 2, Fig 4). Pine filter media again showed poor filtration potential as results did not differ significantly, ($p>0.05$) reductions were seen for the control (sand filter).

Table 2 Physico-chemical properties of unfiltered river water, as well as Eucalyptus and Pine biochar filter columns

Quality parameter	Trial 1			Trial 2			Trial 3		
	Before filtration	E	P	Before filtration	E	P	Before filtration	E	P
COD ($mg.L^{-1}$)	61.20	14.40	90.00	120.60	16.20	70.20	64.80	7.20	100.80
UVT%	52.40	91.50	16.70	49.60	88.00	12.00	53.10	89.30	8.40
pH	6.52	9.05	9.11	6.31	9.03	8.90	6.50	9.32	9.00
Turbidity (NTU)	12.59	4.83	26.80	21.80	6.61	52.90	14.00	2.89	42.30
TSS ($mg.L^{-1}$)	33.30	25.00	36.00	40.00	23.30	46.66	30.00	21.70	34.00
Conductivity ($mS.m^{-1}$)	36.70	51.60	85.80	54.50	55.60	125.10	45.60	50.00	100.00
VSS ($mg.L^{-1}$)	30.00	15.50	23.00	26.60	12.40	24.00	27.00	12.00	20.00
Alkalinity	75.00	175.00	200.00	100.00	225.00	375.00	100.00	125.00	200.00

*E = physico-chemical parameters measured after Eucalyptus filtration
*P = physico-chemical parameters measured after Pine filtration

A final trial (Trial 3, Fig. 4) again proved Eucalyptus filter media columns to be significantly better ($p<0.05$) as a filter media than sand filter columns and Pine filter columns. Also, Pine filter columns again showed that they had minimal, if any, efficacy regarding microbial reductions, as there were no significant differences ($p>0.05$) reported between the sand filter columns (control) and Pine filter columns (Trials 3, Fig. 4).

When considering the two biochar types tested, sand filter (control) columns resulted in poor microbial reductions for all enumerated groups (ACC, TC and FC) (Fig. 4). The highest log reduction for sand filtration was reported for the FC microbial group in Trial 1, recording 1.36 log. Significantly poorer ($p<0.05$) log reductions were, however, reported for Trials 2 and 3, measuring 0.72 and 0.79 log respectively (Fig. 4) for FC. Similar log reductions were reported by Elliott *et al.* (2008), ranging between 0.5 – 0.7 log reductions for *E. coli* when using standard sand filters only.

The Eucalyptus biochar resulted in significantly better ($p<0.05$) log reductions observed for the Eucalyptus filtrate, when compared to the sand filtrate for all three trials (Fig. 4). Eucalyptus filter columns successfully removed all microbial groups tested for (ACC, TC and FC) for all trials (Fig. 4). As discussed, Eucalyptus filter columns were also able to significantly improve the COD, UVT% and turbidity (Table 2). When considering microbial association with particles suspended in the river water, the effective retention of suspended matter observed may be closely linked to the effective decrease in microbial numbers, as the Eucalyptus filters brought about improvement to the TSS values (Table 2).

Finally, Pine filter columns resulted in poor log reductions for all trials (Fig. 4). Trial 1, however, reported significantly better ($p<0.05$) reductions for TC and FC, with over 1 log reduction for each when compared to Trials 2 and 3, where reductions lower than 1 log were observed. Furthermore, Pine filter columns were not significantly better at reducing FC than the sand filter columns in Trials 1 – 3 (Fig. 4). ACC for Trials 1 and 3 (for both sand and Pine filter columns) had achieved significantly lower ($p<0.05$) log reductions than for TC and FC,

Overall, Eucalyptus filter columns proved to be significantly more ($p<0.05$) effective at reducing ACC, TC and FC than Pine filter columns for all three trials (Fig. 4). Pine filter columns were, at best, only able to reduce FC by 1.5 log, nearly 2 log less effective than Eucalyptus filter columns (Fig. 4). According to Gupta *et al.* (2013) different types of biochar are often associated with their own unique set of absorption characteristics.

Eucalyptus biochar was also produced at a higher pyrolysis temperature (700 – 800°C) than Pine biochar (400 – 500°C). Researches have reported differences regarding surface functional groups of biochar when different biomasses were used to produce biochar, as well as when different pyrolysis temperatures were used. (Ahmad *et al.*, 2012; Li *et al.*, 2013b; Pintor *et al.*, 2012). This might explain the significant differences ($p<0.05$) observed between the Pine and Eucalyptus filter media.

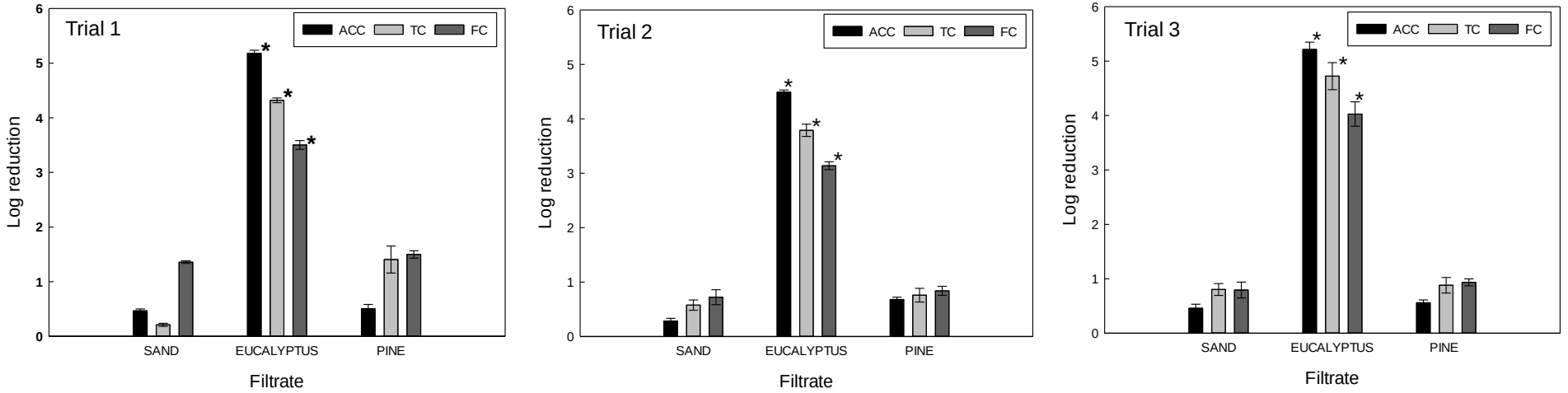

Figure 4 Microbial reductions reported, post-filtration for sand, Eucalyptus and Pine filter columns for Trials 1 – 3. Error bars represent standard deviation calculated at a 95% confidence level. * - indicates no microbial growth detected.

Biochars produced at higher pyrolysis temperatures have been associated with improved adsorption characteristics. This study showed similar trends, as the biochar produced at the higher pyrolysis temperatures clearly showed improved adsorption potential (when only considering pyrolysis temperatures of the biochar) (Alam *et al.*, 2009; Hunt *et al.*, 2010; Meyer *et al.*, 2011). At higher pyrolysis temperatures surface areas are increased in biochar, as there is a release of volatile components such as cellulose and hemicelluloses, promoting the formation of channel structures (Yao *et al.*, 2012; Ahmad *et al.*, 2014). Li *et al.* (2013) suggested that the release of volatile components facilitates the formation of bundles of vascular structures in the biochar, which is responsible for improving the specific surface area, porosity and pore structure, ultimately improving the adsorption properties. This might provide a feasible explanation as to why Eucalyptus biochar showed significantly better filtration properties to that of Pine, regarding changes in physico-chemical and microbial properties of the river water (Trials 1 – 3).

As little is known concerning the true effect of biochar on microbial reductions when it is incorporated as a filter media, comparing results to previous research done can thus be challenging. Therefore, comparing the results reported in this study to alternative filtration methods can be insightful when determining the efficacy of biochar as a viable filtration treatment.

Stauber *et al.* (2006) tested the ability of Biosand filters to reduce the occurrence of *E. coli* in water. Biosand filters essentially consist of a mixture of sand and gravel layers used to filter water for drinking purposes. It was found that the Biosand filters were able to reduce *E. coli* counts by only 0.6 Logs. Considering Trials 2 & 3 sand filtration reduced FC by 0.72 and 0.79 log respectively

(Fig. 4), therefore showing considerable similarity to the results reported by Stauber *et al*. (2006). Upon maturation of the Biosand filters used by Stauber *et al.* (2006) an average of 1.5 – 2 log *E. coli* reduction was reported. However, Eucalyptus biochar filtration (in this study) was able to effectively reduce FC to below detectable levels (Fig. 4), thus possibly showing even more potential than the Biosand filters for microbial reductions.

A study done by Lukasik *et al.* (1999) reported a maximum of 0.5 log reduction for a large variety of different *E. coli* isolates, after 4 L of *E. coli* containing water was filtered through sand filter columns (each containing 1 kg of sand). These results were in line with the current study's findings for sand-only filters (Trials 1 – 3), where a maximum reduction of just over 1 log (1.36 log) was reported for Trial 1, while significantly lower log reductions were reported for Trials 2 and 3 (Fig. 4).

Both sand and Pine filter columns were considered significantly ($p<0.05$) less effective than that of a Eucalyptus filter columns for all enumerated microbial groups. Although the exact filtration capabilities of Eucalyptus biochar still remains greatly unknown, potential exists for its application in water treatment. As minimal differences in log reductions between sand and Pine filter columns were observed (Trials 1 – 3) (Fig. 4), it was concluded that Pine biochar, produced under the conditions specified, was ineffective as a filtration media, as Pine filtrate was of poorer

physico-chemical quality than unfiltered river water. It was clear that different biochar types, as used in this study, produced at different pyrolysis temperatures, displayed very different characteristics regarding filtration efficacy. Additional research will be required to determine the maximum removal efficacy of Eucalyptus biochar, as it remains unknown, as > 99% removal efficacy of ACC, TC and FC under the conditions tested was observed (Fig. 4).

Study 2: Evaluating potential microbial washout from used biochar filters

Figure 5 represents the log values observed in column washout for ACC, TC and FC after previously used columns stood unused for 48 – 72 h. By allowing 500 mL of autoclaved, distilled water to filter through the used Eucalyptus biochar filter columns (used in Study 1), considerable amounts of microbial washout were found for all enumerated groups (ACC, TC and FC), as represented by Trials 1 – 3 (Fig. 5). These findings were noteworthy, as no microorganisms were detected directly after filtration of river water in Study 1 (using river water containing > 3 log. 100 mL^{-1} in microbial levels) for any of the enumerated groups, although the same filters were reused in this study, Study 2, $\geq$ 48 h later.

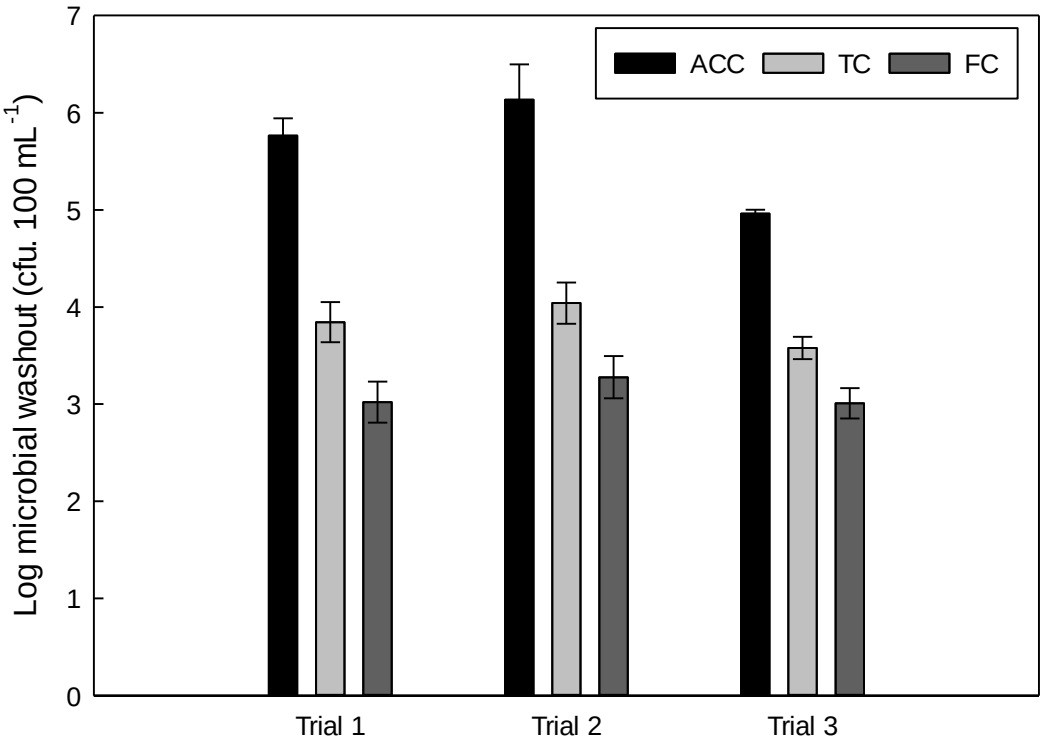

Figure 5 Log microbial washout detected for different enumerated groups when filtering with previously used Eucalyptus filter columns. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

Observations regarding the microbial washout detected for columns used in Trial 1, reported ACC values of 5.76 log, TC 3.84 log and FC 3.02 log per 100 mL filtrate tested. Similar trends were

observed for columns used in Trials 2 and 3. Thus, none of these filtrates met the DWAF (1996) irrigation water guideline of 1 000 cfu.100 mL^{-1}, as all three trials reported > 3 log (per 100 mL) microbial washout for the FC group (Fig. 5).

Factors responsible for the high levels of microbial washout detected, from the same Eucalyptus filter columns used in Study 1 (which initially resulted in filtrate in which no microbial growth was detected post-filtration), had to be considered. Biochar has been reported to contain high levels of nutrients (Marschner *et al.,* 2013), combined with sufficient moisture available (from the original water filtered through), could explain how the survival, and even the replication of microorganisms, was possible after 48 – 72 h. Ideal incubation conditions for ACC are suggested to be 48 h at 30±0.5°C (SANS, 2007b) which, when considering that the used filter columns were kept for ≥ 48 h, at room temperature (25±2°C), close to optimal conditions, could have favoured the survival and growth of ACC. The above mentioned factors thus had the potential to allow the survival and replication of microorganisms, especially the ACC groups. Hence the microbial washout detected in this study and no washout reported in Study 1.

Although more favourable conditions were created for ACC microbial groups, up to 3.27 log FC washout was still observed for Trial 2 (Fig. 5). The lower levels of TC and FC washout could be expected, as the microorganisms (such as *E. coli*) associated with the Coliform groups (TC and FC) are more likely to be present in the intestines of warm-blooded mammals, and are less likely able to multiply in environments outside the digestive tract, as in the base of biochar (Gemmell & Schmidt, 2013; Odonkor & Ampofo, 2013). Research has, however, suggested that given the correct temperature*, E. coli* (which fall within the FC group) have shown potential to exist in soil (Benjamin *et al.*, 2013). This notion is supported by Khalil & Frank (2010), who reported the survival of *E. coli* at lower temperatures (outside the digestive tract), although these lower temperatures (< 25°C) were not considered optimal for their growth and survival (Khalil & Frank, 2010). Thus, not ruling out the possibility that microorganisms, such as *E. coli*, were able to multiply within biochar filters over the incubation time of > 48 h.

The river water originally filtered through the Eucalyptus filter columns (Study 1) had between 3 – 4 log FC present (Fig. 4), similar to the amounts of microbial washout detected in this study (Fig. 5). The ACC for the unfiltered river water did not exceed 5.21 log in Study 1. However, in this study up to 6.13 log ACC were reported, higher than that of the river water used in Study 1, suggestive of possible microbial multiplication over the 48 – 72 h period. Conclusions can be made suggesting that Eucalyptus biochar filter columns showed poor potential to be ‘re-used’ once exposed to river water containing a large variety of microorganisms. These results ultimately provide insight on the viability of Eucalyptus biochar (used as a filter media) to be used more than once, without continuous water flow for ≥ 48 h. Researches have suggested that in order to consider a filter media effective, constant filtration should be observed over a period time. Stauber *et al.* (2006) reported successful removal of *E. coli* over a 15 day period using Biosand filters. Similarly, making use of a standard sand filters, Elliott *et al.* (2008) reported up to 0.50 log

improvement of filtration capabilities (for *E. coli*) when filters were allowed to run for up to 72 h.

This was not the case for Eucalyptus biochar filtration, as decreased filtration potential was observed for used filter that stood unused for as little as 48 – 72 h. Nevertheless, limited research is available on discontinuous filtration systems. This study, as mentioned, tested the filtration capabilities of 'used' Eucalyptus biochar filter columns, when left unused for 48 – 72 h period. The relevance of these experiments conducted were significant when considering biochar as a viable alternative filtration media to improve irrigation water, as in practice water filters might be used discontinuously as the demand for water varies and is dependent on many factors.

CONCLUSION

In this study the potential of two biochar types used as a filter media produced significantly different results. Considerable variation in filtration capabilities were observed between the Pine biochar filter columns and Eucalyptus biochar filter columns tested. Eucalyptus biochar filter columns were significantly more ($p<0.05$) effective than Pine filter columns at improving river water quality. Eucalyptus filtrate revealed improved COD, UVT%, turbidity and TSS, while less successful improvements were brought about by Pine biochar filter columns. Eucalyptus biochar filter columns had the superior filtration capabilities, as no microbial growth was detected for any of the enumerated microbial groups (> 3 log reduction per 100 mL in FC). Pine biochar filter columns were significantly less ($p<0.05$) effective, seldom reporting more than 1 log reduction for any enumerated microbial groups. Unfavourable changes in pH were, however, noted for both Pine and Eucalyptus filter columns. The pH measured for the filtrate was considerably high, ranging from 8.90 – 9.35, exceeding the guideline suggested by the DWAF (1996) for neutral irrigation water (pH 6.5 – 8.5).

Upon completion of the first study only Eucalyptus biochar filter columns were considered for further studies, as Pine biochar showed minimal, if any, potential as a viable filter media. In a second study completed Eucalyptus biochar filter columns had compromised filtration capabilities when allowed to stand for 48 – 72 h, without continuous water flow. High levels of ACC, TC and FC were detected in the filtrate collected after autoclaved water was allowed to filter through the Eucalyptus filter columns used in Study 1. Up to 3.27 log FC microbial washout was detected, which was unexpected, as no microorganisms were detected in the filtrate for the 'virgin' Eucalyptus filter columns used in Study 1. According to the second study biochar filter columns previously exposed to microbial contaminated water, showed compromised filtration capabilities regarding microbial washout, as microbially contaminated water introduced into the Eucalyptus biochar filter columns, had the potential to survive and even multiply in a $\geq$ 48 h time period.

As these biochar filter columns can be considered a novel concept, future research needs to be done evaluating the feasibility of different biochar types to be incorporated as a filter media to improve water quality. In this study the different biochar types obtained for testing were also produced at different pyrolysis temperatures. As pyrolysis temperature is likely to influence biochar

filtration efficacy, the differences observed in filtration efficacy between the Pine and Eucalyptus biochar might not only be attributed to their origin. Optimal pyrolysis temperatures must thus be determined for individual biomass sources before conclusions regarding the best biomass source can be drawn. Determining the volume of water that can be successfully filtered through a biochar filter column with no microbial washout will also need to be addressed in future research.

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Chapter 6

GENERAL DISCUSSION AND CONCLUSIONS

Worldwide the increased demand for fresh water is not only limited to the ever-expanding agricultural sector but is also the result of increased human consumption due to ever-growing populations. Furthermore, changes in rainfall patterns due to global warming, as well as increased pollution of the already limited fresh water sources, has led to a global water crisis.

Global trends promoting healthier lifestyles, has placed emphasis on fresh or minimally processed crops. Fresh produce is most susceptible to poor irrigation water quality and has been linked to numerous outbreaks of foodborne illnesses. Untreated river water, contaminated with faecal waste and pathogenic microorganisms, can act as a vector responsible for the transfer of pathogens to crops. These contaminated crops, if consumed, can ultimately be responsible for causing illness. Many disease outbreaks have been reportedly caused due to consumption of *Salmonella* spp. and *Escherichia coli (E. coli)* present on fresh produce. When considering the reliance the agricultural sector in South Africa has on fresh water from rivers, especially for irrigation purposes, high levels of pathogens can present a serious problem. Research has confirmed that the many rivers in the Western Cape consequently exceed the limit of $\leq$ 1 000 Faecal Coliforms (FC) for every 100 mL of water as established by the World Health Organisation (WHO), as well as the Department of Water Affairs (DWA) (DWAF, 1996).

When considering the high levels of pollution of Western Cape Rivers, emphasis is placed on the importance of river water disinfection prior to irrigation of fresh produce. Various disinfection techniques are available, each possessing characteristic advantages and disadvantages. Chemical disinfectants, in particular chlorine (Cl), peracetic acid (PAA) and hydrogen peroxide (H_2O_2) have been implemented in water disinfection. However, UV irradiation is greatly underutilised in South Africa, as the use of chemical disinfectants is the more recognised disinfection treatments. Cl, being the longest used chemical disinfectant, is generally accepted because of its safety, eases of application and successfully disinfection results. More recently it has been challenged by PAA and H_2O_2, which is also under investigation as they have the added benefit of biodegradability and less formation of harmless by-products.

The overall objective of this study was to evaluate the effect of UV irradiation, in combination with chemical treatments, whilst considering the influence of varying water quality and microbial factors of contaminated river water. The first phase of this research concentrated on the effect Cl, PAA, H_2O_2 and low-pressure (LP) UV irradiation as individual treatments and in combination on the disinfection potential of known microbiological strains at laboratory-scale. The combination of (Cl+UV), (PAA+UV) and (H_2O_2+UV) was of particular importance in this study. *E. coli* strains F11.2 and MJ58 in simple saline solution (SSS) were decided, as they showed resistance to the chemical and UV treatments and therefore gave a more complete picture, supposing the *E. coli* populations found in river water displays similar resistance levels.

Commercially recommended chemical doses of 4 $mg.L^{-1}$, 6 $mg.L^{-1}$ and 2.5 $mg.L^{-1}$ for PAA, Cl and H_2O_2 respectively, showed variations in disinfection effectiveness for strains F11.2 and MJ58. PAA, the most effective disinfectant, was effective at a 15 and a 25 min contact times. However, clear advantages are seen for 25 min chemical contact times. Both (25/PAA) and (25/PAA+UV) were able to fully reduce the microbial levels in SSS, thus determining the effects of combination treatments nullified. The effectiveness of UV irradiation at a dose of 13 $mJ.cm^{-2}$ made drawing a clear conclusion on combination treatments difficult. Furthermore, being sure of advanced oxidation effects for the combination treatments was not clear.

Subsequent studies found that the Plankenburg River was critically polluted and not suitable for irrigation purposes if the water was not treated first. The study included three trials, performed at laboratory scale, in order to assess the influence varying river water quality and variations in microbial populations had on disinfection efficacy. Disinfection treatments using PAA at 4 $mg.L^{-1}$ and H_2O_2 at 2.5 $mg.L^{-1}$ proved to be ineffective when compared to Cl disinfection at 6 $mg.L^{-1}$, what was significantly more effective. Only the contact time of 25 min was used, as learning from the previous study that allowing increased contact times did initiate better microbial reductions. LP-UV irradiation at a dose of up to 13 $mJ.cm^{-2}$ was not effective when poor river water quality was recorded on the particular day. Therefore, when commercial-scale UV treatments should be applied, > 13 $mJ.cm^{-2}$ doses must be considered. LP-UV irradiation, with regards to this study, was primarily investigated to gain knowledge on its disinfection effectiveness for *E. coli* strains in SSS as well as naturally present microorganisms in river water as commercially, medium-pressure (MP) UV irradiation is more commonly used.

Of greater importance for this study, the combination treatments (PAA+UV), (Cl+UV) and (H_2O_2+UV), showed better disinfection results than the individual treatments (even when the individual doses were kept the same). Establishing the exact effects of the combination treatments were challenging, as UV irradiation proved so effective. Poor river water quality did favour (Cl+UV) disinfection over the (PAA+UV) and (H_2O_2+UV) treatments as AOPs were seemingly initiated, nevertheless, dissatisfactory disinfection was still prominent. Controversial to the study in SSS, Cl disinfection was more effective than PAA (from the first part of this study), showing the influence varying water quality had on chemical disinfection. This initiated the thought process that vary water quality greatly influences disinfection of both UV irradiation as well as chemical treatments. Cl residual levels of > 1 $mg.L^{-1}$, more than twice the recommended levels, are of concern when Cl is dosed at 6 $mg.L^{-1}$. Negative connotations have been linked to Cl disinfection offering high residual levels, emphasising the need for more research on lower Cl doses whilst still considering variable water quality.

The second phase of the research, conducted at a pilot-scale, investigating the disinfection effectiveness of Cl, PAA, H_2O_2 and MP-UV (as individual treatments and in combination) revealed considerable variation in disinfection efficacy amongst the different treatments. Three trials were completed as varying water quality was noticed to influence disinfection efficacy, multiple trials thus

allow for better conclusions to be made. Firstly, poor disinfection for all three chemicals was observed, regardless of the water quality of the specific day. Cl proved to be significantly ineffective as a water disinfectant when doses of 3 $mg.L^{-1}$ we used (half the dose used in the previous research section). Considering that in the previous phase of this study Cl was significantly more effective dosed at 6 $mg.L^{-1}$, nevertheless, Cl was still however the most effective of the three chemicals investigated. Individual PAA and H_2O_2 treatments (dosed at 4 $mg.L^{-1}$ and 2.5 $mg.L^{-1}$, respectively) further reinforced the need of combination treatments when using low doses of chemicals, as they were greatly ineffective. As a stand-alone treatment, MP-UV (25 – 30 $mJ.cm^{-2}$) showed much disinfection variation, correlating its dependence on good water quality to effective microbial reductions. Assuming river water quality to be greatly variable, using both chemicals and MP-UV as stand-alone treatments would require higher dose rates to be considered adequate and reliable disinfection methods. Additionally chemical reaction times of longer than 25 min could also be considered for future trials. Lastly, the combination of chemicals and UV: (PAA+UV); (Cl+UV) and (H_2O_2+UV) showed little evidence of AOPs as they were unable to report improved disinfection when compared to the MP-UV treatments alone. Increasing the chemical doses used, can in this case, better induce more effective disinfection as AOPs are more likely to be initiated at higher chemical doses.

A subsequent study investigated the influence photo-repair on MP-UV irradiated river water exposed to a UV dose of 25 – 30 $mJ.cm^{-2}$. At the completion of the study, evidence of photo-repair was established with almost a 16% recovery reported in some instances. Considering the proposed 3 log target reduction, a 15.86% log recovery did still, however, allow for adequately disinfected river water, even if factoring in up to a 15.86% log recovery.

UV-irradiation proved effective in reducing the initial microbial loads in river water for the first part of this study, where an almost 16% recovery after 3 h exposure to visible light would more often than not render UV treatments ineffective in water treatment. Factoring in the microbial and physico-chemical variability of river water, it further adds to the complex phenomena of microbial repair altering the photo-repair potential induced by the photolyase enzymes. Incorporating the potential of photo-repair when evaluating UV disinfection provides a more accurate representation of the actual effectiveness of MP-UV irradiation. Therefore, investigations into higher UV doses would provide better insight on the potential of microbial damage repair. Additionally, all the studies completed in the phase of research on river water went through a standard sand filter as an attempt to standardise the degree of variation in the water.

The last phase of this research was conducted on the possibility of using biochar as an alternative filtration method for the pre-treatment of river water. Biochar filtration, when used as a filtration step, could produce a more uniform and consistent water quality, further optimising chemical and UV disinfection. Three trials were completed for both eucalyptus and pine filters as more accurate conclusion could be made. The efficacy of the disinfection treatments studied in the previous two phases of this study, clearly showed varying microbial and physico-chemical

properties had a direct influence on water disinfection, for both chemical and UV treatments. Eucalyptus biochar filter columns significantly improved ($p<0.05$) the river water quality, with considerably less effective filtration recorded for pine biochar filter columns. Eucalyptus filter columns are able to drastically improve the ultraviolet transmission (UVT%) of the river water from 52.40% to 91.50%, as well as reduce the chemical oxygen demand (COD) from 120.60 $mg.L^{-1}$ to 16.20 $mg.L^{-1}$, also greatly improving the turbidity. Negatively, unfavourable change in water pH can be expected as unfiltered water with a near neutral pH became more basic pH ($\pm$ pH 8 – 9) post-filtration for both pine and eucalyptus biochar filter columns. The improved water quality will be very favourable with regards to UV disinfection if considered to be incorporated as a pre-treatment method in the future. Pine biochar is not recommended for future studies, however, changing the pyrolysis temperature can potentially influence its effectiveness. Additional to improved physico-chemical benefits, eucalyptus biochar is very effective at significantly improving the microbial properties of the river water, even when subjected to extreme water quality. Eucalyptus biochar can be effectively used in river water disinfection, according to this study. Adequate removal of ACC, TC and FC of > 3 log is possible, however, the exact microbial removal potential remains unknown as the total amount of microorganisms present in the river water was removed. Thus, determining if eucalyptus filters will remain as effective with more highly contaminated river water would be recommended for further investigation.

Lastly, the potential of microbial washout was determined for previously 'used' eucalyptus filter columns to gain a more comprehensive impression. These filters are unsuccessful when > 48 h is passed after initial usage, as a > 3 log faecal coliform (FC) washout observed. Additionally, high levels of ACC and TC are also detected in the filtrate collected after the autoclaved water filtered through the eucalyptus filter columns. These results are unexpected, as no microorganisms were detected in the filtrate for the unused eucalyptus filter columns used in the previous part of this research.

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