Scoping Study on Different On-Farm Treatment Options to Reduce the High Microbial Contaminant Loads of Irrigation Water to Reduce the Related Food Safety Risk

Report to the
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edited by

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EXECUTIVE SUMMARY

Recent research has shown that the microbiological quality of South African rivers has become a reason for concern (Britz *et al*., 2013). Insufficient sanitation facilities and inadequate sewage treatment works throughout South Africa have often been implicated as the primary sources of pollution. Farmers are often dependent on these rivers as their only source of irrigation water, and thus their use poses a possible health risk to farm workers and consumers alike.

The extent of this pollution and the link with food safety was investigated in a five year WRC-funded study, which emphasised that many rivers were of an unacceptable microbiological standard and did not meet international faecal guidelines for safe irrigation water. This presents the scenario where consumers unknowingly face a high risk of being infected with harmful organisms when consuming fresh produce. One only needs to recall the recent tragic outbreak of *E. coli* O104:H4 in Germany during 2011 to realise the potential risks. Contaminated (presumably by irrigation water) fenugreek seeds of Egyptian origin were identified as the causative agent which claimed the lives of 47 individuals and led to approximately 4 000 confirmed infections in Germany (EFSA, 2011).

Prevention of river and irrigation water pollution would be the ultimate solution, but in the interim cost effective treatment techniques for irrigation water are required to ensure food safety. Several disinfection techniques exist that are currently used on-farm. These are generally classified into one of three categories, namely: chemical (chlorine, bromine, hydrogen peroxide, peracetic acid or ozone based), mechanical/physical (filtration) and physical/photochemical (ultrasound, UV) disinfection (Raudales *et al*., 2014). The effectiveness of these treatments depends on parameters of water quality, including total dissolved solids, turbidity, pH, total suspended solids and chemical oxygen demand (Jones *et al*., 2014).

Therefore, the aim of this project was to conduct a scoping study on different on-farm treatment options to reduce or remove the high levels of potentially pathogenic micro-organisms from irrigation water. This was achieved by:

1) Conducting a comprehensive literature study and survey of potential on-farm treatment options for irrigated water contaminated with high levels of micro-organisms to enable a treatment option to be selected for the trials in an exploratory study;
2) Conducting an exploratory study of an on-farm treatment option (in this case, ultraviolet (UV) light) by monitoring the water quality throughout the irrigation water cycle;
3) Determining the efficacy of different treatment options (including UV, hydrogen peroxide, chlorine, peracetic acid, hydrogen peroxide/UV, peracetic acid/UV and chlorine/UV) on different *E. coli* strains (reference strains, environmental strains and mixed environmental strains) at laboratory-scale and river water in a custom pilot-scale irrigation water test unit;
4) Proposing the most appropriate treatment options and requirements for further research.

The results from the Scoping Study indicated that resistance variation between strains were evident for all the treatments (Chemical and UV). It was also observed that environmental strains (isolated from rivers and fresh produce) were in general more resistant than reference strains. This once again illustrates the ability of bacteria to adapt to environmental stress.

Treatments tested on river water samples did also indicate that disinfectant efficacy for all treatments was greatly influenced by river water quality. Water quality, measured in terms of physicochemical parameters such as COD, UVT%, TSS, pH, etc., had a direct influence on the available chlorine, and peracetic acid levels during disinfection, as well as on the degree of photo reactivation that can occur after UV irradiation. The chemical treatments (chlorine and peracetic

acid) also had disadvantages in terms of their range of efficacy, DBP's, concerns about the safety and effect on the environment, microbial resistance, cost, long contact times and overall carbon footprint. UV was, however, shown to have potential as an environmentally friendly and safer disinfection treatment for polluted irrigation water.

Certain factors still need to be considered, based on the limitations of this Scoping Study. One of the most important issues to be addressed is how effective UV disinfection of water from other rivers with other physicochemical properties (than the Plankenburg River) would be. Another important question is what would the maximum tolerated limits be for quality parameters such as COD, UVT%, TSS within which optimum UV disinfection (with minimum photo recovery) can be achieved. The use of specific pre-treatment technologies to achieve water with quality parameters below these limits should also be considered for severely polluted rivers.

This Scoping Study focussed only on the microbial standards (*E. coli* < 1000 cfu.100 mL^{-1}) established for water intended for irrigation of fresh produce by the WHO and DWA (WHO, 1989; DWAF, 1996). From a food safety perspective the effect of disinfection on other important food pathogens linked to fresh produce, such as *Salmonella*, *Listeria*, entero-haemorragic *E. coli*, protozoan pathogens (i.e. *Cryptosporidium* and *Giardia*) and viruses also needs to be considered. Included in these considerations is the effect of photo reactivation and dark repair, and how it is minimised by pre-treatment technologies and increased UV dosages. From a practical point of view, it is also important to be able to better collate UV disinfection trials done at laboratory-scale on a collimated beam with what dosages are required in pilot and full-scale systems.

Therefore, the use of ultra-violet (UV) treatment of irrigation water to ensure food safety should be further researched by conducting a study on the technical and financial requirements for an on-farm irrigation water UV treatment system to ensure food safety by

- Choosing and optimising a pre-treatment step (sand/media filtration and/or flocculation/sedimentation process) to standardise the water quality (in terms of physico-chemical characteristics such as COD, TSS, turbidity, UVT%, pH, alkalinity) for different river waters used for irrigation of fresh produce;
- Determining the microbial loads (specifically the aerobic colony count (ACC) population) of different river waters used for irrigation of fresh produce and, by using a collimated beam set-up, making recommendations as to the UV dose required to sufficiently reduce the most resistant micro-organisms;
- Investigating the effect of UV (higher doses) on a wider range of indicator organisms (total and faecal coliforms), the ACC population, *Enterobacteriaceae* and specific pathogens (incl. *Salmonella*, *Listeria*, *E. coli*, Enterococci, Protozoa (*Cryptosporidium* and *Giardia*) and viruses (in terms of disinfection efficiency, photo and/or dark repair) so as to make recommendations as to the required dosages to achieve sufficient reductions of the most resistant micro-organisms implicated in food safety;
- Correlating collimated beam dosage determinations to actual (pilot or full-scale) required dosages;
- Make recommendations as to expanding current guidelines pertaining to the microbiological quality of irrigation water for fresh produce, over and above the faecal coliform guideline levels;
- To perform an analysis of costs (capital and operational) of the selected full-scale pre-treatment technologies and UV treatment of river water of differing qualities.

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1. INTRODUCTION AND OBJECTIVES

There is an urgent need for research into possible on-farm treatment options to help reduce the high levels of microbial contamination in irrigation waters and thereby reduce the associated food safety risk to consumers. Of primary concern during such treatment, is the reduction of pathogens in the irrigation water and that the treatment process be financially feasible and technically appropriate and robust.

A safe and abundant supply of water is not only of paramount importance to everyone but it is inextricably linked to every industry in our country. Water use in South Africa is dominated by agricultural irrigation which accounts for >60% of all surface and ground water used. Over the last few years it has been brought to light by various local research organisations that many of the South African rivers that are drawn from for agricultural irrigation purposes are carrying extraordinarily high pathogenic loads and some of the produce irrigated by this water are minimally processed foodstuffs or products that are consumed raw. The WRC reports – "*A quantitative investigation into the link between irrigation water quality and food safety*" (Britz *et al*., 2012) and "*An investigation into the link between water quality and microbiological safety of fruit and vegetables from the farming to the processing stages of production and marketing*" (Du Plessis & Korsten, 2015) have clearly demonstrated the extent of the problem in terms of geographic distribution and the high microbial loads in rivers used as irrigation water sources.

Several risks have been identified when polluted water is used for crop irrigation. Risks can be short-term and range in seriousness, depending on the potential contact with humans, animals and the environment (e.g. microbial pathogens). Long-term impacts could arise from continued use of polluted water (e.g. chemical effects on soil) (Toze, 2006).

No irrigation water contaminated by untreated or poorly treated faecal waste is risk-free. All such sources of water contain harmful disease-causing organisms and have the potential to make people ill; it is only the concentration of such organisms that varies for different sources of such water. The risks of using such water to produce edible crops should be weighed up against the crises of poor hygiene and hunger.

Apart from the threat to the health of consumers, large outbreaks of associated illnesses will damage the trust of the public, thereby affecting consumer confidence in the local produce as well as the sales of all similar products. These outbreaks could also result in legal challenges that could potentially lead to loss of entry into lucrative export markets as well as possible rejection by the local market. Such consequences would be disastrous for South African agriculture considering that this sector is one of the largest employers of labour in the country and rapidly increasing in economic importance. One needs to look no further than the recent *E. coli* O104:H4 outbreak in Germany and the rest of Europe, in which over 35 people died and over 3 256 people became ill, to illustrate the risk to consumer health and the consequences to international trade and exports. In the above case, Spain alone lost agricultural sales amounting to €200 million per week and more than 70 000 jobs were be lost.

Based on the evidence of microbial pollution in rivers used for irrigation in South Africa (Britz *et al*., 2012) and the potential risk of causing disease in consumers, the pollution either needs to be prevented at source or treated at the point of use. The ultimate solution to the irrigation water problem is properly and reliably treating water pollution at the source. An even better solution is to prevent the pollution itself. There are so many political, financial, social and resource problems, as well as water shortages and environmental limitations that must be taken into consideration that

short-term solutions have become a huge challenge. The important fact is that many of our rivers are heavily contaminated and must be seen as the direct source of contaminated irrigation water.

In the original aims and method, the irrigation water quality was monitored throughout the irrigation water cycle, but this aspect didn't generate significantly reliable results, and thus the Project Team in consultation with the Reference Group agreed to change the original proposed method.

The aim of this project was to conduct a scoping study of different on-farm treatment options to reduce the high levels of potentially pathogenic micro-organisms from irrigation water.

The revised method could be summarised as follows:

- Investigate differences in river water quality;
- Investigate differences in resistance to treatment between reference strains and environmental *E. coli* strains to different treatments of hydrogen peroxide (H_2O_2, chlorine, peracetic acid (PAA), ultra-violet (UV) and combinations of the chemical disinfectants with UV);
- Investigate the occurrence of photo-reactivation and dark repair after UV irradiation.

These investigations were first performed at laboratory-scale, using known single reference strains and environmental strains, in saline solution. This was done to provide insight into optimising treatment conditions. Thereafter, investigations were continued at laboratory-scale with single reference and environmental strains in sterilised river water to determine the influence of the water chemistry on disinfection efficacy and provide further insights in optimising treatment conditions. Investigations then progressed to treatments being carried out at laboratory-scale in river water (with unknown mixtures of micro-organisms, i.e. mixed populations). The thinking behind this was to be able to have a better understanding of the treatment conditions required at pilot-scale, where an unknown mixture of environmental micro-organisms could be present in the water, having a range of resistances to disinfection. The final investigations, using treatment parameters obtained from preliminary laboratory-scale investigations, were performed in river water in a custom built pilot-scale treatment unit.

2. LITERATURE REVIEW OF ON-FARM TREATMENT OPTIONS

2.1 BACKGROUND

Water is an indispensable natural resource for food production. It is fundamental to life and a crucial component in the environment. It is utilised on large scale in food production, industrial areas, hygiene and sanitation and even for power generation (Walmsley *et al.*, 1999; Steele & Odumeru, 2004; CDC, 2014). The nature and rate of economic growth, has an enormous impact on water abstraction and discharge. It is important that water sectors worldwide align the provision of water with the spatial and sectoral growth of a country's economy. Social change must also be taken into account as it brings to light a wide range of challenges including migration between rural and urban areas, growing informal settlements on the margins of cities and questions arise as how to provide these consumers with water in the most cost effective way (CSIR, 2012).

Another problem water managers are faced with, is that in developing countries water storage, distribution, monitoring, treatment and wastewater collection infrastructures are handling loads far above normal capacity, causing it to become outdated and in need of reparation or replacement (CSIR, 2012). The advantage of effective infrastructure maintenance will lead to sustainable water services and efficient distribution of water.

In the agricultural field, water is of critical importance for the production of nutritious, safe and available fresh produce. Globally, approximately 19% of cropland accounts for irrigated agriculture and supplies 40% of the food demand. Worldwide irrigation contributes about 70% of water withdrawals from river systems, and subsequently plays a significant part in the maintenance of global food security (Molden, 2007). Food security, especially in developing countries, however, is threatened as urbanisation, industrialisation and other non-agricultural water requirements continue to overshadow the importance of water used for irrigation (Hanjra & Qureshi, 2010). Economic growth in developing countries may further influence water management policies. These should always be aligned in relation to observed sectorial growth, in order to ensure sensible water abstraction. Moreover, it is now well known that the South African infrastructure for wastewater management is in urgent need of restoration. Lack of maintenance over the years has led to an ineffective system (Ijabadeniyi *et al.*, 2011). Climatic changes in arid regions of the world also add to the declining availability of water for agricultural irrigation.

Sources responsible for poor water quality include carry-over from human settlements, and water-overflow from industrial and agricultural activities (UN-Water, 2013). Effluent from industrial sources is often discarded into near-by rivers and other groundwater resources, thereby contaminating the water and posing a significant risk to food safety (Giddey *et al.*, 2015). Due to the limited availability of water, the use of wastewater for irrigation in urban and peri-urban regions of developing countries is inevitable (Norton-Brandão *et al.*, 2013).

For South Africa water scarcity is a reality and therefore, treatment of wastewater is no longer just an option but a necessity (FAO/WHO, 2008). Studies revealed that within the last decade, the quality of South African river water has decreased notably (Britz *et al.*, 2013). As a result of the increased population growth, people move to the cities for better opportunities and a better standard of living. About 58% of the South African population lives in urban areas and 11.5% in rural areas where basic water services are scarce (DEAT, 2006c). Many people in rural areas do not have access to clean water and sanitation facilities and are forced to use the nearest river water for their daily needs (Gemmell & Schmidt, 2012).

Of all food categories, fresh produce is the main recipient of poor-quality irrigation water (Lee *et al.*, 2014). Raw produce irrigated with untreated river water carries a risk of pathogenic contamination (Pachepsky *et al.*, 2011). Consequently, the increased consumption of fresh produce is linked to more outbreaks of foodborne diseases. Thus, within the South African

context, research highlights the unsuitability of river water for the irrigation of fresh fruits and vegetables (Britz *et al.*, 2012; Rachida *et al.*, 2015).

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

Since an array of political, financial, social and other factors complicate the direct prevention of water contamination, disinfection of irrigation water must receive more attention. Disinfection methods are traditionally divided into chemical and mechanical techniques, but alternative treatments such as ultrasound and ultraviolet (UV) light are options to be investigated. Treatment techniques should always be assessed in terms of financial and practical viability as well as technical feasibility prior to their implementation or recommendation. Thus emphasis should rather be placed on the prevention of pre-harvest contamination by implementing novel treatment strategies.

2.2 GLOBAL WATER SITUATION

The Earth's total water supply is estimated at 1 385.92 million m^3 per year, of which 96% is oceanic saline water (Anonymous, 2014). The remaining are freshwater resources (2.5%) (FAO, 2013) and mainly utilised for drinking purposes and crop irrigation. The world has had an annual water withdrawal of <6 800 m^3 over the last decade (Verma & Fraiture, 2009). Of this 70% was used for agriculture, 20% by industry and 10% for domestic purposes (FAO, 2013).

Global water demand is driven mainly by agriculture and human users (Verma & Fraiture, 2009). It is estimated that the world's population will reach 9 billion by 2050 (UN., 2013). Subsequently global food demand will rise markedly (UN-Water, 2013), because people are likely to consume more meat, fish, dairy and sugar, all of which use more water for production than grain-derived food products (De Fraiture *et al.*, 2007; Verma & Fraiture, 2009).

Water forms part of the water-energy-food security nexus which implies that complex relations between these resources exist (Gulati *et al.*, 2013). However, water scarcity, climate change and the energy crisis affect food security as a global water crisis emerges (Hanjra & Qureshi, 2010). Moreover, constant growth in population and as their income increases so does the demand for water in irrigation, domestic and industrial applications (Hanjra & Qureshi, 2010). According to the UN, water scarcity rather than shortages in agricultural land, will hinder the need for increased food production in the near future (UNDP, 2006). In Australia for instance, the production of cereal and rice in the Murray-Darling Basin decreased by 40% (ABS, 2008). By 2050, a projected increase of 65% in global cereal demand will put enormous pressure on the already limited global water resources (De Fraiture *et al.*, 2007).

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

In the light of continual water scarcity, various sectors will be in competition for the available water and may force water use away from agriculture (Molden, 2007). There will also be an increase in water-related foodborne diseases as water quality declines. These and other factors contribute to the vast challenge of maintaining agricultural production and global food security. Effective water resource management for food security will require novel initiatives as population growth increases.

2.3 WATER SITUATION IN SOUTH AFRICA

Rainfall and climatic variability, surface flow characteristics as well as groundwater replenishment and quality, contribute to what is known as the hydrological cycle and require extensive management to ensure effective, sensible water use (DEAT, 2006b). Based on the United Nations estimated minimum requirement of 1 000 m^3 per person per annum, South Africa is classified as one of the 20 most water scarce countries globally (DEAT, 2011). A more comprehensive projection by the National Water Research Strategy (NWRS) suggests that an insufficiency of water will be reached by 2025 when water requirements are calculated with respect to different scenarios of economic growth (DWAF, 2004b; DEAT, 2011).

Rainfall in South Africa is low, approximately 450-500 $mL.yr^{-1}$ (DEAT, 2006b). Since the local climate ranges from desert to sub-humid, the spatial distribution of rainfall is also highly variable (DWA, 2013). The total mean runoff in the country amounts to 49 000 million $m^3.yr^{-1}$, with only 8.6% of the yearly rainfall being utilised (DEAT, 2006b; DWA, 2013). Although rivers and dams are extensively developed, pollution contributes to a compromised usable yield of surface waters (DEAT, 2006b). South African dams, nonetheless, represent a capacity to the order of 66% of the annual runoff and predominantly supply the water requirements of the country (DWAF, 2004a).

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

2.4 CURRENT AND FUTURE WATER REQUIREMENTS

South Africa is a water scarce country where the demand exceeds the natural water available (UNEPFI, 2009). Water scarcity is a critical issue especially in developing countries, as it can lead to a decrease in food production (FAO, 2007). A clean water supply is of utmost importance as almost every industry relies heavily on water to function productively (Tarver, 2008). The low rainfall, high evaporation rates, growing economy and increasing population, demands the availability of large water volumes (CSIR, 2012).

An accurate understanding of water use requirements is essential for managing water resources wisely. Sectorial water requirements vary with regard to assurance of supply as well as quality, quantity and temporal distribution (DEAT, 2006c). Agricultural irrigation represents a strong seasonality factor in water requirement while the domestic, industrial and mining sectors require a more constant supply (DWAF, 2004b).

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

Considering the relationship between economic growth and water requirement, the NWRS has estimated water requirement based on expected growth in gross domestic product (GDP). A base scenario of 1.5% GDP growth and a high scenario of 4.0% GDP growth up to 2025 imply that local water requirements will increase to 14 230 and 16 814 million $m^3.yr^{-1}$, respectively. For the two scenarios, water availability was calculated as 14 166 and 14 940 million $m^3.yr^{-1}$, respectively, by 2025, resulting in deficits of 234 and 2 044 million $m^3.yr^{-1}$ (DWAF, 2004a; DEA, 2013).

Imbalances between availability and demand and the degradation of surface and groundwater are often experienced in water scarce regions. Due to the increasing water scarcity

problem countries are forced to use low quality water for agricultural purposes. Using questionable quality water for irrigation may affect human health as well as influencing the sustainability of agriculture (Srinivasan & Reddy, 2009). Another problem associated with irrigation water contamination, is the resistance of microorganism. Antibiotic and biocide resistance is increasing and a major cause for concern. Therefore, effective water management in irrigation is required, since the agricultural sector has the highest demand for water (Pereira *et al.*, 2007).

2.5 SOURCES OF CONTAMINATION

2.5.1 Background

Contamination of fresh produce can take place anywhere along the farm-to-fork chain (Jung *et al.*, 2014). This can occur before harvest, during harvest, storage, transportation, during processing, retailing as well as during distribution and even after reaching the consumer (Brackett, 1999; Panigrahy *et al.*, 2011). All these may pose risks if hygienically compromised practices are the norm. It is interesting to note that most pathogens that resulted in recent fresh produce outbreaks are related to faecal contamination (Ravaliya *et al.*, 2014).

It is also important to note that the contamination of fresh produce can be multi-dimensional and include physical, chemical and biological aspects. Although the three forms of contamination may differ with regard to source, regularity of occurrence and detection, they are equally important, often resulting in immediate and/or chronic health implications (Norton-Brandão *et al.*, 2013; Allende & Monaghan, 2015).

Commonly encountered pre-harvest contamination sources include faeces, soil, irrigation water and water used to apply chemicals, green manure, dust, animals and human handling (Beuchat, 1996b; 2006; Johnston *et al.*, 2006). Faeces, handling, processing equipment, containers for transport and storage, animals, dust and water used for washing and rinsing are well known post-harvest sources. Regardless of the possible sources of contamination, effective handling and sanitising practices in the production of fresh produce and minimally processed foods (MPF) must be maintained in order to minimise the risk of infections and disease outbreaks.

2.5.2 Soil as contaminant

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

Upon exposure to animal waste, the profile of microbes within soil may be widened (Whipps *et al.*, 2008). This is problematic since cultivated land may have been previously used for animal farming purposes, thereby exposing the soil to faeces. In addition, fertilizing farmland with animal manure or slurry may add to creating a reservoir of pathogens (Allende & Monaghan, 2015). As a matter of fact, sewage and the manure of cattle and sheep are the primary contributors of *E. coli O157:H7* and *Salmonella* in soil (Olaimat & Holley, 2012). These enteric pathogens are likely to occur in land treated with manure since they may survive in soil for years (Doyle & Erickson, 2008). Faecal contamination from cattle, poultry and pigs may further introduce *Campylobacter jejuni* to land intended for agricultural cultivation (Warriner *et al.*, 2009). Soil can also be contaminated with pathogens during times of heavy rain, as flooding may carry such microbes from contaminated upstream sources (Norton-Brandão *et al.*, 2013). The zoonotic pathogens, as well as *Salmonella*,

survive optimally in clay-based soil of low temperature when manure is still present (Holley *et al.*, 2006).

2.5.3 Irrigation water as contaminant

Globally, surface water resources are extensively utilised for irrigational purposes. This, too, is the case in South Africa where commercial and subsistence farmers draw water from rivers to irrigate a variety of produce (DEAT, 2006a). In this regard, researchers have identified microbiologically unsound irrigation water as the predominant pre-harvest contaminant of fresh produce (Beuchat & Ryu, 1997; Warriner *et al.*, 2009; Pachepsky *et al.*, 2011; Britz *et al.*, 2013). Contamination of such water may result from either point-source or non-point-source origins (Stewart *et al.*, 2008). A point-source problem is easily identified, quantified and resolved and may include sewage and/or industrial discharge practices. Non-point-source factors are commonly not identifiable or quantifiable.

A variety of bacteria, viruses and protozoa have been isolated from irrigation water and subsequently reported to contaminate cultivated produce (Warriner *et al.*, 2009; Rachida *et al.*, 2015). However, as was the case for soil, pathogens in water are predominantly of faecal origin or often even transferred directly from faecal matter. The use of manure as a fertilising practise may easily result in the contamination of irrigation water. Untreated manure may introduce *E. coli*, *Salmonella* spp. and *L. monocytogenes* into cultivated soil, but may further contaminate irrigation water if contact should occur. By means of manipulating feed rations, or treating manure prior to use, the risk of microbiological contamination of irrigation water as well as soil may be reduced (Jamieson *et al.*, 2004).

In developing countries irrigation water contaminated with human faecal matter is a growing cause for concern. This may result from improper sewage treatment systems, the nonchalant release of untreated waste and the establishment of informal settlements close to rivers (Pachepsky *et al.*, 2011; Britz *et al.*, 2013). In the South African context, sanitation facilities in rural areas are often inadequate in relation to the amount of residents present (Barnes & Taylor, 2004). Consequently, rivers have become receivers of waste and waste water while still being used for agricultural irrigation (Matthews, 2006). In addition to the effect of rural areas established close to rivers, various other sources contribute to the pollution of South African rivers. Industrial operations such as wineries and food producing factories for instance, have been reported to discharge manufacturing effluents into local rivers (Lamprecht *et al.*, 2014). Such activities may significantly influence river water quality as it was already shown in 1994, that effluent from a yeast producing company represented a variety of disease-causing microorganisms. These include *E. coli O157:H7* as well as species of *Clostridium* and *Staphylococcus* (Van Der Merwe & Britz, 1994).

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

These impacts on water quality can lead to significant consequences affecting every segment of the South African society, as well as the ecosystems dependent on freshwater resources. Furthermore, South Africa's outdated infrastructure as well as unskilled operators contributes to insufficient water treatment and a potential source contamination (Rietveld *et al.*, 2009). The result is high microbial contaminant loads in river water that pose a risk to human health and safety.

2.6 PREVENTION OF PRODUCE CONTAMINATION

It is nearly impossible to control or prevent contamination of water sources and fields in production areas (Brackett, 1999) as there are just too many variables involved in the contamination of river water and the carry-over of pathogens to fresh produce.

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

As the behaviour and view of food safety differ greatly among food suppliers, the only options to control contamination of fresh produce are to avoid fields where animals have grazed, and to use water free from pathogens for irrigation (Yiannas, 2009; Ijabadeniyi *et al.*, 2011). It is very important for fresh produce suppliers to realise that once the produce has been contaminated with pathogenic microorganisms, sanitisers are unable to decontaminate the food item completely. Instead of trying to decontaminate the food product, contamination should be prevented right from the start (Beuchat, 2006), even before the water is used at the point of irrigation. If all the above-mentioned preventative options are not possible or are difficult to manage, on-farm treatments should be considered as a means to disinfect contaminated irrigation water (Lynch *et al.*, 2009). Farmers should be aware that they have a great responsibility towards consumer safety when supplying fresh produce to the industry, while keeping in mind that the selection of disinfection treatments is dependent on contamination levels of the water, costs, the irrigation mode, the environment, and the education of farm workers and consumers (Britz *et al.*, 2012).

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

Recently, the World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO) made several recommendations as to increase fresh produce safety by means of controlling foodborne pathogens. These recommendations cover an extensive list of factors and include the following: protecting natural water sources from contact with sewage, manufacturing effluent, wildlife activity and animal waste; making use of good manufacturing practices (GMPs) and GAPs, including the use of sanitary agricultural equipment and the provision of facilities promoting personnel hygiene and health habits; and the application of standard operating procedure (SOPs) in order to maintain good hygiene practices throughout production and processing (Olaimat & Holley, 2012). The importance of strict regulations regarding the quality of irrigation water and the use of fertilisers have also been highlighted when considering the safety of fresh produce (Krtinić *et al.*, 2010). Guidance documents produced by the FDA further emphasise the importance of maintaining GAP and GMP within the fresh produce production and processing sectors,

respectively (Lynch *et al.*, 2009). These however, lack regulatory prescriptions and compulsory processing steps for pathogen reduction.

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

Coming back to the importance of water quality, the versatility of this resource in the production of fresh produce must be emphasised. Pre-harvest pesticide application and post-harvest processing and cooling for instance, require the use of water from which pathogens may be transferred to the produce (Greene *et al.*, 2008). Of greater relevance however, is the use of microbiologically contaminated water for irrigational purposes. The introduction of pathogens into irrigation water is multi-factorial and the prevention thereof is an extremely difficult task. Informal settlements developing next to rivers, failing sewage treatment plants and careless waste-water disposal contribute to South African rivers showing high levels of microbiological contamination (Britz *et al.*, 2013). In addition to water quality however, crop type and the method of irrigation used significantly impacts the transfer of pathogens from irrigation water to produce (Allende & Monaghan, 2015). Even though these factors may be managed to an extent, the use of water of poor microbiological quality is not recommended. Accordingly, on-farm treatment of irrigation water is suggested as a means of minimising pathogen transfer to fresh produce.

Knowledge of the field location where produce was grown and what irrigation and harvesting techniques where used can, when put together, improve understanding of why outbreaks occurred and thus help to develop effective on-farm preventative measures of contamination. A possible long term solution is to directly treat contaminated water sources on-farm before using it to irrigate crops (du Plessis *et al.*, 2015).

2.7 ON-FARM TREATMENT OPTIONS

Good quality water for irrigation purposes is becoming scarcer and more expensive to obtain (Newman, 2004; Yiasoumi, 2005). Due to the water quality situation in South Africa, the importance of water treatment prior to irrigation has become essential. Disinfection of water is necessary since it can help control pathogen growth not only in the water but also in the irrigation system and thus reduce the risk of introducing disease to the farm and fresh produce through irrigation (Pehlivanoglu-Mantas & Sedlak, 2006; Lenntech, 2014).

The intention of disinfecting irrigation water is not to produce potable water, but to irrigate crops with water that complies with national guidelines. Treatment of irrigation water is not a priority in developing countries and the use of contaminated irrigation water is a common occurrence (Britz *et al.*, 2012). The call for disinfection has increased with the need to reduce the health risks related to fresh produce (WHO, 2006).

Several factors need to be taken into consideration before the right disinfectant technology can be chosen for sanitising water for irrigation purposes (Lazarova & Bahri, 2005). These include: the sanitising capability on the number and type of microorganisms present; the nature and concentration of the disinfection system; the potential toxicity of the disinfectant at high levels and the effects it might have in water, soil and on plants; it is important to know if by-products are formed when the disinfectant reacts with water and the effects this may have on individuals that come into contact with it; and water quality parameters will influence the sanitising efficacy of the disinfectant. These include the suspended solids content; organic matter within the water; water pH and temperature; as well as the efficiency contact time (NHMRC & NRMMC, 2004); as well as

the cost of the disinfectant, including the installation capital and operating costs (Lazarova & Bahri, 2005; Huang *et al.*, 2011; Jones *et al.*, 2014).

Several disinfection techniques have been established and some are currently used as on-farm treatments. These are generally grouped into one of three categories (Table 2.1) (Raudales *et al.*, 2014). The effectiveness of treatment techniques depend on parameters of water quality and composition. These parameters are highly variable, changing daily or seasonally as a result of natural or human influences (Anonymous, 2014). All methods are not similarly appropriate for the treatment of surface water (Jones *et al.*, 2014). Every decision is unique according to a specific farm-setup and its financial implications. However, the goal is to utilise irrigation water that will not pose risk to consumers and which complies with national standards.

Table 2.1 Disinfection techniques currently used.

Physical/ mechanical	Chemical	Physical/ photochemical	Combination Treatments
Sand/media filtration	Chlorine based	Ultrasound	Chlorine/UV
Ultrafiltration	Bromine	Ultraviolet light (UV)	
	Hydrogen peroxide		Hydrogen peroxide/UV
	Peracetic acid		Peracetic acid/UV
	Ozone		

2.7.1 Physical and Mechanical Methods

Physical methods have been used for many years and are the oldest technologies available for water treatment (Momba *et al.*, 2008; Kesari *et al.*, 2011). Physical treatments are primarily referred to as filtration methods and based solely on the separation of solids from liquids (Yiasoumi, 2005; Lenntech, 2014). For the purpose of disinfecting irrigation, waste and municipal water, filtration methods, which incorporate techniques such as straining, absorption and adsorption, are of particular importance. In addition to removing some microbiological pathogens, filtration processes are generally capable of removing suspended solids as well as unwanted taste compounds, odours and chemicals from water. Such disinfection treatments are often preceded by straining or settlement processes and accompanied by additional disinfection methods in order to increase their efficacy (Yiasoumi, 2005; Momba *et al.*, 2008; Kesari *et al.*, 2011).

2.7.1.1 Sand/media filtration

Background and mode of action

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

Disinfection by means of slow sand filtration occurs when water slowly passes through a bed of porous material. Sand is predominantly used as filtration medium, but materials including pumice have also been used successfully (Zheng & Dunets, 2014). Hendricks (Hendricks, 2006) described such a system as a bio-depth-filtration process occurring in a filter medium, aided by a straining process provided by a biologically active layer. Contaminated water is disinfected as it

moves through the filter, allowing pathogens to be captured in the pore spaces in the medium (Fisher, 2011a). Adding to this, microbial pathogens are further captured in a biofilm layer/s that slowly form on the surfaces of the filtration grains during normal operation (Zheng & Dunets, 2014). Microorganisms present in the biofilm compete with water pathogens for resources or even by direct attacks. This active biofilm is known as the "schmutzdecke" and consists of inorganic and organic particulates, as well as a variety of fungi, nematodes, protozoa and bacteria (Stewart-Wade, 2011). Although the biofilm is regarded as the predominant biological control measure provided by slow sand filtration, the sand bed self should be biologically mature as well. This means that in addition to the physical filtration provided by the sand, biofilm formation deep within the sand bed aids in disinfection by means of antagonistic effects and direct competition.

The typical construction of a slow bed sand filter is shown in Figure 2.1. A water layer of approximately 0.9 m deep must be maintained in order to protect the biofilm from moisture and temperature fluctuations while providing sufficient pressure for movement through the sand (Stewart-Wade, 2011; Zheng & Dunets, 2014). The sand, or medium layer, should be at least 80 cm deep and is supported by a gravel layer with a depth of approximately 15 cm. The latter prevents sand from flowing to the outlet tank while allowing water to move freely. Since the development of the active biofilm layer results in losses of maximum headwater, it must be constantly monitored and removed once an established criterion for maximum head-loss is reached (Stewart *et al.*, 2008).

Slow bed sand filtration has been reported to be effective in controlling a variety of pathogenic microbes. According to Hendricks (Hendricks, 2006), a biologically mature sand filter is generally capable of 2 to 4 log reductions of bacteria, cysts, viruses, oocysts, algae and parasite and nematode eggs. Zheng and co-workers (Zheng & Dunets, 2014) reported however, that some nematodes and viruses, as well as *Fusarium,* can only be partially removed from water and only at slow flow rates. Adding to this, Hugo and Malan (Hugo & Malan, 2006) reported that the filtration process is ineffective in removing nematodes as a result of the large pore size of the sand bed. *Phytopthora* and *Pythium* species are reported to be easily removed (Zheng & Dunets, 2014).

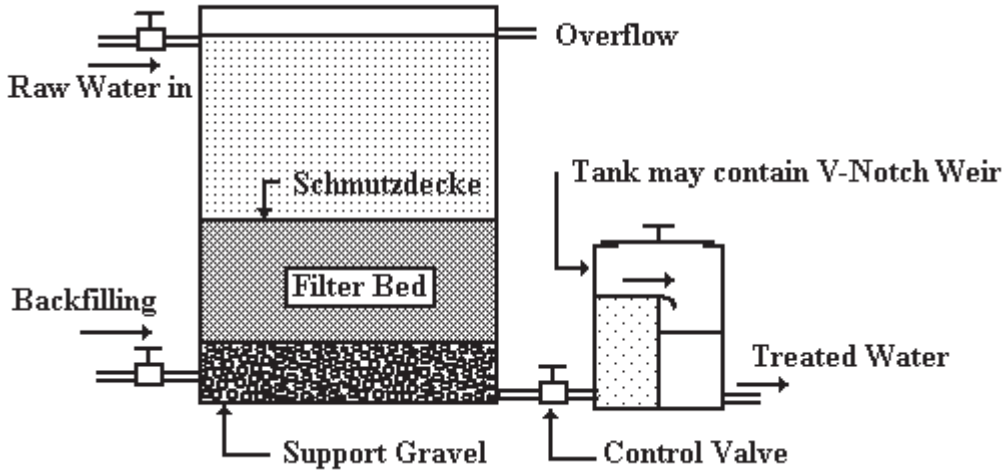


Figure 2.1 Graphic representation of a typical slow bed sand filter (Anonymous, 1996).

Advantages

Slow bed sand filters are relatively inexpensive and are easily built and maintained (Hugo & Malan, 2006; Langenbach *et al*., 2009). It does not require chemicals or energy to achieve disinfection and needs a smaller space for water treatment compared to other natural technologies for pathogen

removal (Langenbach *et al*., 2009). It is capable of preventing waterborne diseases by removing algae, bacteria, protozoa such as *Giardia* and *Cryptosporidium* (Hijnen *et al*., 2007), viruses as well as several *Phytophthora* species from irrigation and drinking water. The success of this treatment method is unaffected by the water's pH (Huisman & Wood, 1974; Runia, 1995; Bitton, 2005). The slow bed sand filtration method is also effective in disinfecting wastewater (Tchobanoglous, 1979; Cleasby, 1990).

Disadvantages

The slow bed sand filtration method is a time consuming process, with retention times of 3-15 h. As a result of this the process and may not be viable for irrigation purposes especially where large volumes of water must be treated (Huisman & Wood, 1974; Droste, 1997; Bitton, 2005). A further requirement is that the water that is to be treated must be of a fairly good starting quality as water with high turbidity levels will lead to algae growth which can rapidly clog the filters (Binnie *et al.*, 2002). A combination of several filter systems is usually necessary to optimally remove pathogens and organic matter from the water (Hugo & Malan, 2006). Even though slow bed sand filters are capable of removing most pathogenic microorganisms from the water, it is not proficient in fully successfully removing plant parasitic nematodes from irrigation water.

When the land is very restricted and expensive, the use of this technology may be eliminated as it as it has a large foot-print, consequently adding to the capital costs of the method (Zheng & Dunets, 2014). Another disadvantage of this method is that when using sand filters on irrigation water containing high levels of particles, the filter's pores may plug too frequently and therefore, regular maintenance and a pre-filtering step are required (Campos, 2008). Also for several days after resanding, the quality of the filtered water is low (Huisman & Wood, 1974; Bitton, 2005).

Conclusion

In conclusion, slow bed sand filtration is a versatile water disinfection process and can also be very effective when combining it with other treatments such as ultraviolet light. Slow bed sand filters are relatively inexpensive and safe to use when compared to other water treatment methods such as chlorination or bromination, are easily built and do not require chemicals or energy to achieve disinfection. It is capable of preventing waterborne diseases by removing algae, bacteria, protozoa such as *Giardia* and *Cryptosporidium*, viruses as well as several *Phytophthora* species from irrigation and drinking water (Hijnen *et al.*, 2007). One of its biggest disadvantages is that the sludge build-up has to be treated with additional disinfection methods before it can be discarded. Another disadvantage of this disinfection method is that for several days after resanding, the quality of the filtered water might be of lesser value. Slow bed sand filtration will thus not be an effective method for irrigation water disinfection if it is used as the sole treatment method.

2.7.1.2 Ultrafiltration

Background and mode of action

Ultrafiltration is a membrane filtration method that was developed in the 1930's. Since its development, the technology has been widely applied for the production of pure water in the biopharmaceutical, food and beverage and biochemical industries (GHD, 2005; Vickers, 2005; Nath, 2006). Today however, ultrafiltration is also utilised for the production of microbiologically safe drinking water. The process has been increasingly applied for the removal of particulate and organic material as well as a wide spectrum of unwanted microorganisms from water (Arnal *et al.*, 2009; Konieczny *et al.*, 2009).

Membrane processes in general can be driven by differences in osmotic pressure and temperature and, in the case of nanofiltration (NF), reverse osmosis (RO), microfiltration (MF) and

ultrafiltration (UF), by differences in pressure (Peter-Varbanets *et al.*, 2009; Fane *et al.*, 2011). Ultrafiltration is thus a separation process, driven by low pressure, during which water and substances with low molecular weight move through a porous membrane to produce what is known as permeate or ultrafiltrate. Larger particles, macromolecules and colloidal substances do not pass through the membrane and are retained as retenate (concentrated solution) (Nath, 2006). Even though substances are primarily retained because of size, factors such as the membrane surface chemistry and particulate substances, as well as electrical charge, may be influential. In Table 2.2 a summary of the operational differences between four membrane processes is given.

With reference to Table 2.2, substances in the size range of 1 000-500 000 Dalton (Da) will permeate the typical membrane used in ultrafiltration, retaining only particles of high molecular weight (Nath, 2006). This implies that the osmotic pressure difference across the membrane is very small and that fairly low pressures are adequate for establishing high flux rates (Table 2.2). Compared to microfiltration, however, higher pressure is required by ultrafiltration as a result of the smaller pore size. Typically, peristaltic pump systems or compressed nitrogen are used to generate the pressure required by the membrane separation system. Figure 2.2 shows an illustration of an ultrafiltration system.

Table 2.2 Operational and technical differences between membrane processes used for water disinfection (Van der Bruggen *et al.*, 2003; Baker, 2004; Ozaki, 2004; Fane *et al.*, 2011).

Parameters	**Micro-filtration**	**Ultra-filtration**	**Nano-filtration**	**Reverse osmosis**
Size of pores	10 nm-1 µm	3-10 nm	2-5 nm	N/D
Particulates retained (MW)	>300 000	1 000-300 000	>150	<350
Pressure exerted (MPa)	0.005-0.20	0.01-0.30	0.30-1.50	1.00-10.00

N/D = No data available

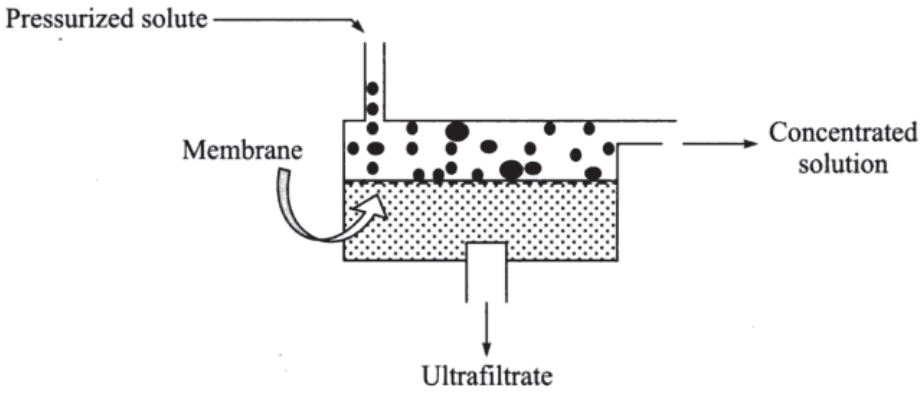


Figure 2.2 Illustration of a typical ultrafiltration membrane setup (Nath, 2006).

Advantages

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

Disadvantages

Despite its widely acclaimed versatility and benefits, the use of physical disinfection processes is also associated with some drawbacks. With regard to filtration, the size of the unwanted microorganisms plays a significant part in the efficacy of the treatment. Viruses typically range between 20-100 nm in size and are very difficult to remove by means of filtration (Zheng & Dunets, 2014).

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

Conclusion

An advantage of ultrafiltration include its ability to reduce the strength of waste present in a solution and at the same time being able to recover valuable by-products such as proteins in the process. Ultrafiltration can also remove most bacteria, viruses as well as protozoan cysts such as *Giardia* and *Cryptosporidium*, as these organisms are generally larger than the membrane pore size. This method is also capable of reducing the turbidity of water and as a result ultrafiltration is often used as a pre-treatment method to remove molecules with high molecular weight from water. Another advantage of ultrafiltration is that no disinfection by-products are formed by this method. Ultrafiltration's biggest disadvantages are its high cost and extremely long operating time. Even though these systems are adjustable to reduce the operating time needed for water purification, this will proportionately increase the total cost of the system. Another disadvantage of ultrafiltration is its inability to remove particles that are smaller than the membrane pores. This poses a problem since pathogens which are smaller than ultrafiltration membrane's pore sizes, cannot be removed

from the water without an additional disinfection method being applied to the water. Thus ultrafiltration of irrigation water will not be an acceptable treatment method, since unsuccessful treatment of water can have a negative influence on consumers.

2.7.1.3 General remarks on physical and mechanical methods

Despite its widely acclaimed versatility and benefits, the use of physical disinfection processes is also associated with some drawbacks. With regard to filtration, the size of the unwanted microorganisms plays a significant part in the efficacy of the treatment (Momba *et al.*, 2008). Viruses typically range between 20-100 nm in size and are very difficult to remove by means of filtration. Slow bed sand filtration also requires a fair amount of maintenance as the thickening active biofilm layer must be removed from time-to-time to maintain sufficient flow of water in the system (Hendricks, 2006). This implies that a new top layer of sand is added to the filter. Following this, a conditioning period of at least 24 h is required before again using the filter. Thereafter, its effectiveness may be slightly impaired for a few days (Bitton, 2005; Mwabi *et al.*, 2012).

Generally, it is recommended that slow sand filtration is used in combination with additional filtration techniques as a means of effectively reducing pathogen and particulate levels in irrigation water (Hugo & Malan, 2006; Zheng & Dunets, 2014). This is important since high levels of turbidity may result in rapid clogging of the small pores within the filter medium. In addition to these disadvantages, slow bed sand filters occupy large surface areas and are associated with high installation expenses. Lastly, it should be mentioned that the slow flow rates required to properly disinfect water, makes slow bed sand filtration an unlikely option for the treatment of large volumes of irrigation water.

Ultrafiltration is a complex process represented by high capital costs and the requirement of expertise for its operation (Mwabi *et al.*, 2012). It has also been reported that, in spite of the continual cross-flow cleaning procedure, ultrafiltration membranes are at risk of clogging and that water pre-treatment is often required (Momba *et al.*, 2008). Another disadvantage of ultrafiltration is the fact that pathogenic microorganisms, especially some viruses, may pass through the pores of the membrane to remain in the treated water (Davey & Schäfer, 2009). This implies that, in addition to treating the concentrated mass retained by the membrane, further disinfection processes are required to ensure water safety (Van der Bruggen *et al.*, 2003). Such treatment significantly contributes to the high operating costs associated with ultrafiltration. The concept of membrane fouling, in which water gradually permeate the membrane at slower rates when pressure remains constant, has also been identified as a major limitation related to UF (Nath, 2006).

2.7.2 Chemical Treatment

Chemical treatments of water systems have been applied for many years and are still used by multiple water industries. Numerous chemicals are available to enhance the microbiological quality of water such as ozone (O_3), bromine (Br_2), chlorine (Cl_2), chlorine dioxide (ClO_2), chloramine (RNHCl), hypochlorites (ClO^-), peracetic acid ($C_2H_4O_3$) and hydrogen peroxide (H_2O_2) (Acher *et al.*, 1997).

Chemical disinfection is based solely on the oxidation potential of the chemical itself that results in lethal damage to the microbes. However, the choice of disinfection agents remains difficult when considering other external factors. These include suspended solids, oxidisable organic and inorganic material, temperature and pH. Together with water quality parameters, the dose ($mg.L^{-1}$) and exposure time (min) are major determinants of disinfection efficiency (Yiasoumi, 2005; Ali, 2010).

Although chemical disinfectants (also known as biocides) are effective in treating contaminated water, it has been shown that they release disinfectant by-products (DBP) into the water (Yiasoumi, 2005). During disinfection, chemical substances react with compounds present in the water leading to the formation of DBPs (Voigt *et al.*, 2013; Lenntech, 2014). Typical DBPs such as di-trichloroacetic acids, trihalomethanes and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone are considered carcinogenic or mutagenic (Woo *et al.*, 2002; Bitton, 2005). These may have detrimental effects on human health (White & McDermott, 2001), however, the risk humans are exposed to, are much smaller than the risks associated with inadequate disinfection (Connellan, 2013).

Biocides rarely have a specific target site and often are effective by acting on multiple target sites (Maillard, 2002) but certain factors can influence the efficiency of a biocide. If *Cryptosporidium parvum* is a problem then chlorine should not be used due to their being resistant to chlorine (Binnie *et al.*, 2002). The correct contact time also needs to be established for effective disinfection with longer contact times needed if high microbial loads are present (Maillard, 2005). The pH of certain biocides or the water being treated can also influence the efficiency of disinfection. Organic matter in a water system may be oxidised by the biocide, therefore less biocide is available to oxidise microbes, as well as being able to protect microorganism (Maillard, 2005). Thus, these factors need to be considered before selecting a biocide for water treatment (Freese *et al.*, 2003; Yang *et al.*, 2008). Other disadvantages are that their manufacturing, storage, transport and use pose a continuous threat to anyone who works with them as well as serious consequences for the environment (Woo *et al.*, 2002; Westerhoff, 2006).

2.7.2.1 Bromine

Background and mode of action

In the past bromine disinfection was mainly used to treat swimming pool and cooling tower water, although there are a few cases in literature where bromine was used for wastewater disinfection. However, it is not recommended for the disinfection of drinking water since it imparts tastes and odours to most water. In the 1990's some wastewater utilities in the USA started using bromine in combination with chlorine as disinfectant treatment (Tate & Arnold, 1990; Freese *et al.*, 2003).

A chemical such as bromine has excellent disinfecting properties since it is a strong oxidiser (Newman, 2004; Punyani *et al.*, 2006). The process entails transforming bromine into hypobromous acid (Droste, 1997; Yiasoumi, 2005; Yiasoumi *et al.*, 2005). This is achieved when sodium bromide is added to sodium hypochlorite. Hypobromous acid is an effective sanitising agent over a wide pH range. At a pH of 8.5, 60% of bromine is still in hypobromous acid form and able to successfully disinfect water. Recycled water commonly used in horticulture contains fluctuating levels of ammonium and other nitrogen-based compounds. Both bromine and chlorine are capable of reacting with these compounds and as a result form broamines and chloramines, respectively. Chloramines are poor biocides, while broamines show disinfection properties comparable to hypobromous acid (Yiasoumi, 2005).

During the oxidation process, oxidising compounds are reduced and lose their activity. It is thus important to maintain a high concentration of bromine during disinfection in the water to ensure that complete disinfection takes place (Newman, 2004). Studies by Freese and co-workers (Freese *et al.*, 2003) showed that bromine disinfection of pathogens present in sewage is almost equal in efficacy to chlorine. In sewage with a pH above four, bromine disinfection was found to be more efficient than chlorine disinfection.

Advantages

Bromine dissolves three times faster than chlorine in water, no dangerous gasses are required during the production of bromine, it's activity is short since it does not bind strongly to water, as a

result, the residual concentrations stay low and no additional substances are necessary to remove bromine after disinfection is completed (Lenntech, 2014). Bromine has similar disinfection properties to chlorine, also a halogen and cost for disinfection is comparable to that of chlorine gas (Tebbutt, 1992; Yiasoumi, 2005). Cost of commercial treatment of irrigation water with bromine for the elimination of pathogens is economically justifiable (Hugo & Malan, 2006). Other advantages include bromine's long shelf life and the fact that it is an effective disinfectant of water over a wider pH range than chlorine (Korslin, 2012).

Disadvantages

Disinfection of water with bromine is pH and COD dependable, requires a contact time of 10 to 30 min and to maintain sufficient disinfection, a lot of bromine needs be added to the water in comparison with chlorine (Lenntech, 2014). A high concentration of bromine is capable of killing most pathogenic organisms, but it is not effective against protozoans such as *Cryptosporidium* and *Giardia* (Freese *et al.*, 2003).

Bromine is also very reactive and corrodes materials for example the pipes and pumps used for disinfection (Lenntech, 2014). During bromine disinfection bromamines and hypobromous acid react with organic matter present in the water and forms carcinogenic disinfection by-products, such as tribromomethanes. These can be harmful to humans as well as the environment and also imparts taste and odours into waters which may affect the taste of fresh produce (Freese *et al.*, 2003; Westerhoff, 2006; Lenntech, 2014). Bromine should be transported, stored and used with care since exposure can lead to eye and mucous membrane irritation.

Conclusion

Even though bromine is cost effective and can be used to kill most microorganisms, it is unable to kill protozoan pathogens. It also produces disinfectant by-products during treatment which may be harmful when consumed. Bromine is also a very reactive disinfectant capable of corroding metal and imparts tastes and odours in treated water. Thus bromine will not be an effective method for disinfection of water used for irrigation purposes on fresh produce.

2.7.2.2 Chlorine

Background

Chlorine is the most common and widely applied water disinfection method. It was first discovered in 1774 in its gaseous state in Sweden (Lazarova & Bahri, 2005). In 1886, the first chlorine disinfection was applied to combat a typhoid epidemic (Schoenen, 2002). To date, chlorine has various applications as it is extremely versatile in water and wastewater treatment for control of pathogens, removal of ammonia, control of taste and odour, colour reduction, destruction of organic matter, hydrogen sulphide oxidation and iron and manganese oxidation. Chlorine is extremely effective against bacteria and to a lesser extent, against viruses and protozoa- *Cryptosporidium* and *Giardia* require higher chlorine doses for elimination (Cheremisinoff, 2002; Wong, 2002; Lazarova & Bahri, 2005).

Chlorine exists in three forms: chlorine gas, hypochlorite (sodium hypochlorite or calcium hypochlorite) and chlorine dioxide (Newman, 2004; Ivey & Miller, 2013). They are generated by different chemical reactions in water (Table 3) and recently, hypochlorites have gradually become alternatives for chlorine gas and chlorine dioxide in water and wastewater disinfection industries. Hypochlorite is commercially available in dry and liquid form and considered much safer than other chlorine sources (Lewis, 2010).

Sodium hypochlorite (NaOCl)

Sodium hypochlorite (liquid bleach) has been used since the 1930s (Newman, 2004). It is most commonly used in industry for domestic, industrial and commercial water applications. Although the transport of NaOCl takes up more space and is more costly to distribute over long distances than dry chlorine, it is far safer to handle and the maintenance is low (Lewis, 2010).

Table 2.3 Sources of chlorine and reactions in water (Newman, 2004).

Sources of chlorine	Formula	Reaction in water
Chlorine gas	Cl_2	$Cl_2 + H_2O \rightarrow HCl + OCl$
Sodium hypochlorite	NaOCl	$NaOCl + H_2O \rightarrow NaOH + HOCl$
Calcium hypochlorite	$Ca(OCl)_2$	$Ca(OCl)_2 + 2H_2O \rightarrow Ca(OH)_2 + 2HOCl$
Chlorine dioxide	ClO_2	$HOCl + HCl + 2NaClO_2 \rightarrow 2ClO_2 + 2NaCl + H_2O$

NaOCl is produced by the addition of Cl_2 to caustic soda (NaOH) and forms sodium hypochlorite (NaOCl), water (H_2O) and salt (NaCl) (Lenntech, 2014). This occurs in the presence of heat and is regarded as a highly exothermic reaction (Newman, 2004; Lewis, 2010). Commercial NaOCl solutions are available in 10-15% (trade %), with 12.5% trade percent most commonly used for water and wastewater treatment. Trade percentage does not reflect the precise chlorine concentration in NaOCl solutions.

The disinfection reaction of NaOCl in water produces hypochlorous acid (HOCl) that contains an oxygen atom with very strong oxidising properties (Lenntech, 2014). Since NaOCl is very effective against bacteria, it is extensively used for water disinfection to eliminate indicator organisms and pathogens including faecal coliforms and *E. coli.* It is less effective against viruses, protozoa and helminths and not effective against *Cryptosporidium* oocysts and *Toxoplasma* oocysts (Voigt *et al.*, 2013). Since it is in liquid form, it is easy to adapt to greenhouse systems for irrigational purposes (Newman, 2004).

Calcium hypochlorite $(CaOCl)_2$

Calcium hypochlorite is available as a powder, tablet or granules (Lewis, 2010). The production involves the addition of chlorine gas to a solution containing calcium oxide (lime) and sodium hydroxide (NaOH) (Wong, 2002; Lewis, 2010). The most common form exists in a powder (HTH = High Test Calcium Hypochlorite) containing 65-70% available chlorine, 4-6% lime and calcium carbonate.

The disinfection reaction in water is shown in Table 3 where hypochlorous acid formed in water dissociates into the hydrogen ion and hypochlorite (OCl^-). Since two hypochlorous acid molecules are produced from one $Ca(OCl)_2$ molecule, this disinfectant it is considered a strong oxidant (Lewis, 2010). This allows $Ca(OCl)_2$ to be very effective against bacteria, algae, slime, fungi and other microorganisms (Newman, 2004). Granular $Ca(OCl)_2$ is soluble in water ideally at room temperature (Lewis, 2010) and is easier to store than NaOCl since it does not require large spaces for bulk tanks. Yet, care should be taken during storage using corrosion-resistant materials.

Mode of action

Chlorine causes significant injury in pathogens such as *E. coli*, *Salmonella thyphimurium*, *Yersinia enterocolitica* and *Shigella* spp. (Leyer & Johnson, 1997; Bitton, 2005). There are various cell locations that are targets for cell injury and ultimately, cell death. Chlorine attacks the bacterial cell

membrane, consequently leading to decreased cell permeability and disruption of many other functions. When cells are exposed to chorine their DNA, RNA and proteins leak out of the cells restricting protein and RNA synthesis as well as potassium uptake, ultimately causing cell death (Bitton, 2011). Chlorine also causes destruction to bacterial nucleic acids and enzymes such as catalase and dehydrogenases (Bitton, 2011). Additional effects of chlorine include the disruption of nutrient transport, inhibition of cell respiration, damage to ion sulphur centres and oxidation of sulfhydryl groups causing the disability of cells to maintain an adequate energy charge to ensure cell viability (Leyer & Johnson, 1997; Bitton, 2005). Generally, Gram-negative microorganisms like *E. coli* are more sensitive to chemical disinfectants than Gram-positives like *Listeria monocytogenes* due to the intracellular space between the two peptidoglycan layers present in Gram-positives.

Disinfectant by-product (DBP) formation

The presence of chlorine residuals after disinfection provides both positive and negative consequences. Residuals include the prevention of pathogen regrowth (Voigt *et al.*, 2013) and also protect irrigation pipes against slime and algae growth. However, chlorine residuals may also have detrimental effects when applied in high concentrations. Disinfectant by-products and residuals are the result of the reaction with organic and inorganic particles naturally present in water sources and can be a great concern for crop safety and consumers of fresh produce (Bouwer, 2002). Water regulations and guidelines set by international and national organisations regulate the presence of chlorine residuals to reduce the risk of DBP formation in water sources (Table 4).

The occurrence of DBPs was first detected by (Bellar *et al.*, 1974) in the USA and (Rook, 1974) in the Netherlands. They noted four trihalomethanes (THMs) in water following chlorination: chloroform, monochlorodibromomethane, dichlorobromomethane and bromoform (Bitton, 2011). In 2010 Richardson (Richardson *et al.*, 2010) discovered over 600 DBP's and typical forms include THM's such as chloroform ($CHCl_3$), bromodichloromethane ($CHBrCl_2$), dibromochloromethane ($CHBr_2Cl$) and bromoform ($CHBr_3$) and haloacetic acids (HAA) such as monochloroacetic, monobromoacetic, dichloroacetic, dibromoacetic and trichlors acetic acids.

Table 2.4 International and national residual chlorine guidelines (WRC, 1998; WHO, 2004; EPA, 2011; DWA, 2013)

Organisation	Residual chlorine values
Water Research Commission (1998) *Guideline for Domestic water supply*	0.3-0.6 $mg.L^{-1}$
US EPA (2004) *Reclaimed water for irrigation*	≤ 1 $mg.L^{-1}$
World Health Organisation (2004) *Guidelines for drinking water quality*	≥ 0.5 $mg.L^{-1}$
Department of Water Affairs (2013) *Wastewater intended for irrigation*	≤ 0.25 $mg.L^{-1}$

Chloroform is the most commonly THM as a result of chlorination and is also a known carcinogen (Freese *et al.*, 2003; Sayyah & Mohamed, 2014). Chloroform can cause cancer if one is exposed to high concentrations over a long period (Freese & Nozaic, 2004). Despite the studies done on THMs and their health effects on humans, no evidence has been found proving that THMs are harmful in the quantities normally found in water (Freese & Nozaic, 2004). Most of these studies were based on rats, mice and rabbits with the highest doses causing no adverse health effects range from 34 to 43 $mg.kg^{-1}$ chloroform per day (Ruddick *et al.*, 1983).

The reaction in surface water between natural organic particles (humic and fulvic acids) leads to the formation of DBP's containing volatile and non-volatile compounds with probable mutagenic or carcinogenic activity (Sayyah & Mohamed, 2014) . Wastewater provides a good substrate for DBP formation due to the high organic content present. Therefore, residual chlorine concentrations of 0.1 $mg.L^{-1}$ (special limit) and 0.25 $mg.L^{-1}$ (general limit) have been set by the Department of Water Affairs (DWA, 2013) for wastewater intended for irrigation. This was to limit the formation of DBPs that might be harmful for human health. A study done by Freese and co-workers (Freese *et al.*, 2003) to eliminate parasitic oocysts (*Giardia* and *Cryptosporidium*), viruses (coliphages) and bacterial indicators (*E. coli* and coliforms) from secondary wastewater resulted in chlorine residual concentrations that did comply with DWA standards.

Factors influencing disinfection efficiency

pH – Chlorine's activity is measured as 'free' residual chlorine and when it dissolves in water, and exists in equilibrium as hypochlorous acid (HOCl) and hypochlorite (OCl^-) (Newman, 2004). A low ratio of HOCl to OCl^- is explained when the pH of a certain solution is also low. Below a pH of 7.5, the predominant species of chlorine exists as HOCl which is regarded as a very strong oxidiser in water (Bitton, 2005). Above pH 7.5, the dominant species is OCl^- having a much lower oxidative capacity than HOCL. The disinfection efficiency at this point will be markedly decreased (Wong, 2002). In fact, HOCl is 80 times more effective against *E. coli* than OCl^-. Only a small change of 0.1 units can cause a change in the HOCl and OCl^- ratio (Anonymous, 2004b). Therefore, the most active form of free chlorine should be maintained and the pH of a solution should be kept between 7.4 and 7.6 (Newman, 2004). Within this pH range, the ratio between oxidative species is suitable to deliver a maximum germicidal effect (Anonymous, 2004a).

The oxidation reduction potential (ORP) of chlorine is also influenced by pH. The ORP is an indication of the disinfectant's oxidising capability and higher ORP values indicates stronger oxidising potential (Park *et al.*, 2004). They examined the effect of different chlorine concentrations (1.0-5.0 $mg.L^{-1}$) and pH values (3.0, 5.0 and 7.0) on the elimination of *E. coli* 0157:H7 and *Listeria monocytogenes*. Lower *E. coli* populations were found at lower pH values. Moreover, results showed that a low pH leads to increased sensitivity of these pathogens. It has been suggested that stronger bactericidal activity at low pH values could be due to the higher ORP. However, *E. coli* was effectively reduced at a wide range pH values (between 2.6 and 7.0) (Park *et al.*, 2004). Therefore, pH is an important factor to consider when using chlorine for disinfection. A pH lower than 8.0 is recommended for chlorine disinfection which falls within the range of pH values characteristic to surface waters (pH 6.5-8.5).

Temperature

Disinfection of chlorine is decreased at lower water temperatures (EPA, 1999; Pickard, 2006) and higher chlorine doses and longer exposure times are needed for effective disinfection (Bitton, 2011). In general, a temperature decrease of 10°C will lower the disinfection rate by 50-60%. The inactivation of parasites and pathogens increases at higher temperatures (Bitton, 2011). Therefore, it can be assumed that river water representing lower temperatures in winter months may yield decreased disinfection compared to disinfection achieved during summer months.

Organic matter and turbidity

Components naturally present in water interfering with chlorine disinfection are organic and inorganic particles as they also exert a chlorine demand. Free chlorine residuals react with organic content in water that leads to the formation of DBPs (Wong, 2002; Pickard, 2006; Sayyah & Mohamed, 2014). Nonetheless, the TSS and organic load (COD, DOC (dissolved organic content)

and NOM (natural organic matter) present in water may lower chlorine efficiency to inactivate pathogens (Ayyildiz *et al.*, 2009).

Organic and inorganic particles increase water turbidity and also protect microorganisms (coliform bacteria) from free chlorine disinfection. This is known as 'particle association' (Pickard, 2006; Ayyildiz *et al.*, 2009; Bitton, 2011; Van Haute *et al.*, 2013). Protection is provided through the stabilisation of cell membranes whereby access to key components for cellular inactivation is restricted for coliform reduction (Winward *et al.*, 2008). The implementation of a pre-filtration step is suggested to eliminate suspended particles in order to enhance the disinfection effectiveness. Other researchers (Ayyildiz *et al.*, 2009; Van Haute *et al.*, 2013) found that the reduction of total coliforms and *E. coli* increased 1.5-2 times when COD levels were decreased by 50% using filter compared to reductions achieved without filtration.

Concentration (dosage) and contact time

Numerous studies have been done at different chlorine dosages and contact times for the inactivation of pathogenic microorganisms (Wong, 2002; Veschetti *et al.*, 2003; Koivunen & Heinonen-Tanski, 2005b). Dosages and exposure times will differ due to the varying water qualities. A study conducted on secondary wastewater (Freese *et al.*, 2003) showed that 6 $mg.L^{-1}$ NaOCl dose was adequate to obtain a 2-3 log reduction for most bacterial indicators after a reaction time of 30 min. Winward (Winward *et al.*, 2008) also studied the effect of chlorine on grey water and observed coliform reductions of 3.8 logs after a 30 min disinfection period and 10 $mg.L^{-1}$ chlorine. A similar study done on reclaimed water evaluated the effect of 0.2-3.0 $mg.L^{-1}$ chlorine for 30 min and observed coliform reductions of 3.5 logs (Li & Zhang, 2013).

Type of microorganism

There is strong variation in the susceptibility of microorganisms to chemical disinfectants (Veschetti *et al.*, 2003; Bitton, 2011; Li & Zhang, 2013). Resistance can differ among non-spore forming bacteria and also within strains of the same species (AWWARF & USEPA, 2005; Bitton, 2011; Cherchi & Gu, 2011). It is important to note that the inactivation kinetics of reference strains, however, is not always the same as those observed with environmental strains (Wojcicka *et al.*, 2007).

Li and co-worker (Li & Zhang, 2013) found that *Salmonella* was more resistant to chlorine disinfection than total coliforms and *Enterococcus.* When NaOCl was compared to peracetic acid disinfection it was observed that NaOCl was more effective at reducing resistant organisms such as faecal streptococci, bacteriophages and *E. coli* (Veschetti *et al.*, 2003). Freese (Freese *et al.*, 2003) observed 2-3 log reductions for bacterial indicators, however, coliphages showed more resistance at the same disinfection parameters. Van Haute and workers (Van Haute *et al.*, 2013) conducted a study on NaOCl disinfection of *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes*. They found that *E. coli* O157:H7 was much more susceptible to chlorine than *Listeria monocytogenes* which is Gram-positive. This is due to structural differences in the bacterial membrane and cell wall composition between Gram-negative and Gram-positive microorganisms (Mir *et al.*, 1997).

Advantages

Chlorine is the most commonly used disinfectant (Koivunen & Heinonen-Tanski, 2005a; Van Haute *et al.*, 2013) and is effective against a wide range of microorganisms (Eckert, 2013). The strong oxidising capacity also reduces odour and taste problems, prevents slime and algal growth and maintains the water quality in distribution systems (Wong, 2002). Chlorine also leaves a residual that prevents microbial recontamination in water systems.

Chlorine is a recommended treatment option for irrigation due to the low installation and operating costs and reliable variability (Freese *et al.*, 2004; Van Haute *et al.*, 2013). It is relatively easy to handle followed by simple dosing. Both sodium hypochlorite and calcium hypochlorite can be used in large-scale operations to treat irrigation water and to date, no other disinfectant has been found to compete with the same overall versatility (Freese *et al.*, 2004; Voigt *et al.*, 2013).

Disadvantages

The main drawback is the formation of DBPs and THMs. These are considered as carcinogenic and mutagenic, although little evidence is available proving the effect on human health specifically (Freese *et al.*, 2004; Koivunen & Heinonen-Tanski, 2005a). Chlorine disinfection is very dependent on water quality and DBP formation occurs due to the reaction between remaining chlorine residuals and organic substances present in the water (Wong, 2002). Therefore, the use of chlorine for fresh-cut produce washing is permitted in European countries.

Hypochlorite solutions are highly unstable since degradation takes place on heat and light exposure. Therefore, safety measures should be in place during storage. Granular hypochlorites are much more stable than liquid hypochlorites (Newman, 2004), however, combustion can occur when the latter are exposed to heat or readily oxidisable organic matter (Freese *et al.*, 2003). With regards to disinfection area, good ventilation should be maintained to prevent harmful health effects.

Conclusions

Chlorine has been used for more than a century as it is a very effective and the most popular disinfectant for water decontamination. The main drawback is the formation of DBPs especially in low quality irrigation water. This disadvantage can be linked to the current state of South African rivers, since rivers are the main source of irrigation applied by farmers. However, the public health benefits provided by chlorine utilisation greatly exceeds the dangers caused by THM. Chlorine is very effective at eliminating a broad range of bacteria such as *E. coli* and total coliforms that are general indicators of water quality in South Africa. The ability to disinfect water with chlorine at farm-scale may be feasible since chlorine can be applied on large scale to make water suitable for use prior to irrigation.

2.7.2.3 Hydrogen peroxide

Background and mode of action

Hydrogen peroxide was discovered in 1818 and first used as a disinfectant in 1891 (Linley *et al.*, 2012). In 1950, the first application of H_2O_2 occurred through the disinfection of drinking water (Lenntech, 2014). It is one of the most versatile oxidants and has a wide application that can be used both alone and in combination with other disinfection treatments. It is used for the disinfection of wastewater (Ronen *et al.*, 2010; Vargas *et al.*, 2013), air, drinking water and soil (Lenntech, 2014). It is generally used to control colours, tastes and corrosion in polluted sources, destructs residual chlorine, reduces the COD and BOD and inhibits microbial growth (Vargas *et al.*, 2013). A commercial form of H_2O_2, hydrogen dioxide (XeroTol), has the ability to kill bacteria, fungi, algae, yeasts and viruses and is often used as an irrigation water disinfectant (McDonnell & Russell, 1999; Newman, 2004).

Hydrogen peroxide is a strong oxidant that easily enters the cell membrane of microorganisms and releases free hydroxyl radicals (OH) and superoxide radicals (O_2^-). These are known as reactive oxidative species (ROS) (Labas *et al.*, 2008); (Vargas *et al.*, 2013; Zheng & Dunets, 2014). The damaging effects of H_2O_2 are referred to as oxidative stress and the radicals, particularly OH radicals that have the greatest potential to destroy (Labas *et al.*, 2008), attack

components of the cell membrane ultimately followed by the destruction of DNA, proteins and lipids (McDonnell & Russell, 1999; Vargas *et al.*, 2013; Lenntech, 2014).

The lethal and sub-lethal effects of these radicals lead to changes in the physical bacterial structure that delay cell growth due to cell membrane oxidation. The disinfection performance of H_2O_2 is determined by factors such as concentration, contact time, pH, catalysers as well as temperature (Lenntech, 2014). Labas and co-workers (Labas *et al.*, 2008) stated that effective H_2O_2 disinfection depends on the concentration and exposure time.

Different concentrations of H_2O_2 are available ranging from 3 to 90% (McDonnell & Russell, 1999). Concentrations commonly used for disinfection range between 3 and 25%, with 25% being used when higher levels of disinfection are required (Fraise, 1999).

Advantages

Hydrogen peroxide disinfection poses little danger to the environment as it degrades into hydrogen and oxygen leaving no residual (EPA, 2006; Lenntech, 2014; Zheng & Dunets, 2014). It is effective at a wide pH range (Fisher, 2011b) and has a higher oxidation potential than chlorine and chlorine dioxide (Newman, 2004). It is known for its versatile effectiveness against microorganisms such as bacterial spores (Linley *et al.*, 2012), yeast, moulds, viruses and bacteria (Cords *et al.*, 2005).

The use of H_2O_2 as a biocide is becoming increasingly common, due to its non-toxic and degradable properties. These properties are considered important when selecting a biocide for environmental use, such as water treatment (Linley *et al.*, 2012). Hydrogen peroxide has been used as a topical disinfection, as well as in the food industry to facilitate sterile packaging (Newman, 2004). It has also been found to be an effective disinfectant of fresh produce due its oxidising capability. It does not produce carcinogenic compounds, as it does not react with organic compounds present in perishables and H_2O_2 has been classified as Generally Regarded as Safe (GRAS), for certain food applications (Joshi *et al.*, 2013).

However, the use of H_2O_2 may result in detrimental quality changes (Joshi *et al.*, 2013). A study was done by Sapers (Sapers, 2001) on the effectiveness of sanitizing agents in lowering *E. coli* loads on apples. Results indicated that similar log reductions were achieved as when chlorine and other commercial sanitizers were used. However, with 5% H_2O_2 concentration (or combinations of H_2O_2 and other commercial sanitisers), higher log reductions were achieved (3-4 log reductions). Both sanitizers were heated to 50-60°C as slightly better results were seen at 50°C when compared to ambient temperature (Sapers, 2001). It is important to note that strain-strain differences were seen in response to the sanitizing agents.

Labas (Labas *et al.*, 2008) found the effect of H_2O_2 on *E. coli* ATCC 8739 at concentrations ranging from 15-300 ppm and long contact times only resulted in very low inactivation levels. Another study a showed that a concentration of 250 $mg.L^{-1}$ H_2O_2 resulted in a 2.2 faecal coliform reduction after 120 min (Orta de Velásquez *et al.*, 2008). Results from a study by Koivunen (Koivunen & Heinonen-Tanski, 2005b) showed that using H_2O_2 in combination with UV radiation slightly increased the log microbial reductions when compared to using UV alone. Therefore, H_2O_2 may be used in combination with other treatments or compounds in order to achieve a more effective microbial reduction.

Disadvantages

The following are disadvantages associated with its use: Its reaction with organic particles in irrigation water via oxidation decreases the disinfection efficacy (Newman, 2004; Zheng & Dunets, 2014) suggested a pre-treatment step such as filtration to eliminate some of these organic particles. This is one of the main observed drawbacks of using H_2O_2 for irrigation water disinfection. Also, peroxides are highly unstable and corrosive, therefore proper safety measures

should be taken during handling and storage (Lenntech, 2014). Fisher (Fisher, 2011a) reported that the handling and storage of H_2O_2 are problematic and can be costly due to the high concentrations required for effective pathogen reduction.

Conclusion

Although H_2O_2 is a very versatile disinfectant, it is unstable and easily impacted by water quality characteristics restricting effective pathogen removal (Vargas *et al.*, 2013). It is capable of killing bacteria, viruses, fungus, algae, yeasts as well as their spores on contact and is frequently used as a disinfectant for wastewater, irrigation equipment and other surfaces. Treatment of wastewater with 50 $mg.L^{-1}$ hydrogen peroxide is enough to reduce faecal coliforms to less than 1 $cfu.100\ mL^{-1}$, but it is incapable of destroying protozoan pathogens such as *Giardia* and *Cryptosporidium*. It is also a dangerous chemical and can have serious consequences if individuals come into contact with it. If used at very high concentrations or in conjunction with other treatment options it may become quite costly. Long contact times and low quality water treatment will not be a feasible option for irrigation water disinfection.

2.7.2.4 Ozone

Background and mode of action

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

When used as disinfectant in water treatment, ozone must be generated on-site owing to its instability and rapid rate of breakdown (Momba *et al.*, 2008). It has been reported that O_3 decomposes in three ways; however, the exact mechanisms are debateable (Hallmich & Gehr, 2010). As O_3 reacts with water, it decomposes into free radicals, hydroperoxyl (HO_2) and hydroxyl (OH) that have strong oxidising properties and serve as intermediates of the reaction (Voigt *et al.*, 2013). The effectiveness relies on the O_3 concentration and contact time together with the susceptibility of target microorganisms by possibly altering the protein bonds in cell membranes and impacting the cell DNA (Hallmich & Gehr, 2010). They also did a study on O_3 disinfection of *E. coli* and found that noticeable changes within the cell only took place after most of the cells became non-viable. This confirms that, in most cases, the cells are destroyed due to the inactivation of the cell membrane followed by DNA damage.

Advantages

Compared to other disinfectants such as chlorine, O_3 requires much lower dosages and shorter contact times (10-30 min) due to its high oxidation potential (Wong, 2002; Hunter, 2008; Voigt *et al.*, 2013). Ozone has been shown to be a powerful disinfectant for the elimination of bacteria and viruses such as coliforms, *E. coli* and *Giardia lamblia* as well as *Cryptosporidium* oocysts (Burns, 2010). Added to the fact that O_3 is a better disinfectant than chlorine, some other advantages include the reduction of colour, odours and the removal of suspended solids (Masten & Davies,

1994). When water is overdosed with O_3, it is not a concern as it decomposes rapidly back into oxygen, leaving no residual that will need to be removed by a post-treatment.

Disadvantages

Several disadvantages are linked to the use of O_3, in particular the high capital cost, since O_3 gas is usually generated on-site (Masten & Davies, 1994; Voigt *et al.*, 2013), as it is unstable during storage and also requires highly skilled staff to manage and operate generation facilities. If not operated properly, health problems may occur during exposure due to leakages (Freese *et al.*, 2003; Gurol, 2005). The rapid decomposition of biodegradable ozone residuals may require an additional disinfectant to control the regrowth of microorganisms or the aseptic transport of disinfected product to the point of use (Percival *et al.*, 2004). Unfortunately, the formation of DBPs from O_3 disinfection has been reported: non-halogenated by-products (aldehydes, ketones and carboxylic acids) and bromates are formed when O_3 reacts with the natural organic matter (Wong, 2002) in the water (Freese *et al.*, 2004; Bitton, 2005; Hunter, 2008).

Conclusion

Ozone is a powerful disinfectant that can be used to treat drinking water as well as effluents for irrigation purposes. In good quality water, low doses are needed to destroy bacteria, viruses and protozoan parasites such as *Cryptosporidium* and *Giardia*. This makes ozone one of the most effective disinfectants, since it is capable of successfully treating wastewater and destroying protozoan parasites that might be present in the water. Some of ozone's biggest disadvantages are its high cost and instability. As a result of its instability, ozone has to be prepared on-site, thus increasing the chance of individuals being exposed to it. Ozone disinfection is also affected by various factors such as pH and temperature and can form disinfection by-products. Also, when injecting ozone into a closed pipe system, such as used in irrigation, the addition of a gas will be detrimental to the water distribution within the system. Thus when taking all of these factors in to account, it can be concluded that ozone will not be the most efficient disinfection method to treat irrigation water.

2.7.2.5 Peracetic acid

Background and mode of action

Freer and Novy were the first to discover the germicidal effect of peracetic acid (PAA) in 1902 and subsequently reported "the excellent disinfection and cold sterilisation" actions of PAA (Kitis, 2004). In 1951 Greenspan and MacKeller (Greenspan & MacKellar, 1951) determined the PAA's bactericidal, fungicidal and sporicidal concentrations at 0.001, 0.003 and 0.3% ($v.v^{-1}$), respectively.

Apart from water disinfection, there are also environmental disinfection applications in cooling towers, ion exchangers, membrane hollow fibres as well as combined sewer overflows. Industrial disinfection applications of PAA are commonly found within the beverage, medical, pharmaceutical and food processing industries (Stampi *et al.*, 2001; Kitis, 2004) and only within the last 20 years PAA's efficiency towards water/or wastewater disinfection has been discovered (Dell'Erba *et al.*, 2007). Previous studies showed that PAA was effective in the removal of indicator and pathogenic microorganisms in wastewater (Stampi *et al.*, 2001; Salgot *et al.*, 2002; Wagner *et al.*, 2002). In another study PAA was used for the growth control of water pathogens in irrigation water (De Luca *et al.*, 2008; Parke & Fisher, 2012).

Peracetic acid ($C_2H_4O_3$) is a combined mixture of acetic acid and hydrogen peroxide in a watery solution (Dell'Erba *et al.*, 2007; Lenntech, 2014). The reaction occurs in the presence of a catalyst such as sulphuric acid and at a pH below 2, this colourless and bright solution exhibits a sharp odour mainly due to the acetic acid component (Kitis, 2004; Lenntech, 2014). On commercial level, PAA is available in quaternary equilibrium solutions consisting of: CH_3COOH,

H_2O_2 and PAA in water. The commercial form of PAA (10-15%) is much more stable than PAA solutions with higher and lower strength solutions (Kitis, 2004). It has been used in many water disinfection studies (Koivunen & Heinonen-Tanski, 2005b; Luukkonen *et al.*, 2014).

PAA has great disinfection capability against enteric bacteria and to a lesser extent in descending efficiency, against viruses, bacterial spores and protozoan cysts (Stampi *et al.*, 2001; Koivunen & Heinonen-Tanski, 2005a). It has a high oxidation potential of 1.81 electronic volts (eV) and is a stronger disinfectant than hydrogen peroxide, chlorine dioxide, chlorine and bromine (Newman, 2004; Lenntech, 2014).

There is limited research available on the exact mode of PAA disinfection, but the reaction takes place in a similar way to peroxides and other oxidants (Hallmich & Gehr, 2010). Peracetic acid's disinfection capability is based on the generation of ROS, such as superoxide radicals (O_2^- or HO_2) and hydroxyl radicals (HO) (Flores *et al.*, 2014) which conduct the oxidative stress within the microorganisms, ultimately aimed at the disruption of the DNA molecule.

Disinfectant by-product (DBP) formation

Peracetic acid is readily decomposed into harmless by-products such as acetic acid, oxygen and water (Koivunen & Heinonen-Tanski, 2005b; Zanetti *et al.*, 2007; Kobylinski & Bhandari, 2010; Lenntech, 2014) and does not release mutagenic or toxic DBPs (Hagedorn *et al.*, 2003; Crebelli *et al.*, 2005; Koivunen & Heinonen-Tanski, 2005b). However, the possibility of their occurrence cannot be ruled out.

Monarca and co-workers (Monarca *et al.*, 2001) isolated the by-products from river water after being treated with PAA and predominantly detected the presence of non-mutagenic carboxylic acids. The latter are formed when PAA oxidises organic particles present in water and sometimes the formation of aldehydes also occur, but are eventually broken down into carboxylic acids and carbon dioxide (Hagedorn *et al.*, 2003; Crebelli *et al.*, 2005; Dell'Erba *et al.*, 2007). Research on municipal wastewater reported that high dosages of PAA will introduce significant amounts of genotoxic by-products into the water that may be hazardous for human and environmental exposure (Zanetti *et al.*, 2007). PAA is very effective at low concentrations which subsequently will limit DBP formation or chemical residues in effluents (Veschetti *et al.*, 2003; Crebelli *et al.*, 2005). Koivunen and co-workers (Koivunen & Heinonen-Tanski, 2005b) detected PAA residues of 1-2 $mg.L^{-1}$ in water following disinfection but stated that low residual concentrations do not cause any harmful ecological effects since these residues are diluted rapidly after disinfection.

Factors influencing disinfection efficiency

pH – Although PAA's activity has a low dependence on the pH, it is more effective at a lower pH (Kitis, 2004). The undissociated form (CH_3COOOH) of PAA initiates the biocidal activity towards microorganisms (Colgan & Gehr, 2001; Kitis, 2004). Peracetic acid has a pKa of 8.2 (i.e. pH above 9) and its dissociated form (CH_3COOO^-) mainly occurs at alkaline conditions which has shown to decrease its disinfection efficiency. However, at a pH from 5-8 it was shown that the disinfection efficiency of PAA was not affected and Sanchez-Ruiz (Sanchez-Ruiz *et al.*, 1995) observed that coliform removal at pH = 7 was 2-3 logs greater than at pH = 10. Likewise, PAA performance against coliforms was greater at neutral or mild acidic conditions (Baldry *et al.*, 1991).

Temperature – PAA has strong antimicrobial properties functioning over a wide range of temperatures (0-100°C) and its disinfection capability increases with temperature (Profaizer *et al.*, 1997). Full-scale studies on wastewater in Brazil and Italy reported that PAA disinfection efficiency was higher than NaOCl at warm temperatures (Stampi *et al.*, 2001). Similarly, results by Profaizer

and co-workers (Profaizer *et al.*, 1997) have shown bacterial inactivation at 20°C was 1.7 times greater than at 10°C.

Organic matter – There is evidence that high organic contents (including BOD, COD and TSS) leads to decreased PAA efficiency in treated wastewater (Koivunen & Heinonen-Tanski, 2005b; Zanetti *et al.*, 2007; Flores *et al.*, 2014). Julio and co-workers reported that PAA efficiency was 91% before filtration compared to 99% after filtration (Julio *et al.*, 2014). Thus a pre-treatment (filtration) is recommended prior to PAA disinfection (Luukkonen *et al.*, 2014) to remove substances such as COD and TSS present in the water.

Contrary to previous findings (Lazarova & Bahri, 2005), De Luca (De Luca *et al.*, 2008) found that PAA disinfection remained constant at low levels of TSS ranging between 11-40 $mg.L^{-1}$. Similar results were also found by (Stampi *et al.*, 2001) where TSS levels up to 100 $mg.L^{-1}$ resulted in good PAA disinfection.

Concentration and contact time – Gehr (Gehr *et al.*, 2002) found that PAA dosages of 2-6 $mg.L^{-1}$ led to the removal of faecal coliforms from primary effluents to below 1 000 cfu.100 mL^{-1} after 60 min. They found that exposure times longer than 60 min did not have beneficial consequences. Julio and workers (Julio *et al.*, 2014) subsequently increased the dosage to 10-20 $mg.L^{-1}$ PAA to primary treated effluent and obtained a 5.1 faecal coliform log reduction after a reaction time of 15 min. Koivunen and co-workers (Koivunen & Heinonen-Tanski, 2005b) disinfected primary effluent with 5-15 $mg.L^{-1}$ PAA for 27 min and reduced total coliforms with 3-4 logs (initial counts – 4.4×10^4 cfu.100 mL^{-1}).

Secondary wastewater required lower PAA concentrations of 0.6-4 $mg.L^{-1}$ to achieve faecal coliform reductions to below 1 000 cfu.100 mL^{-1} (Gehr *et al.*, 2002). Koivunen (Koivunen & Heinonen-Tanski, 2005b) also found that 2-7 $mg.L^{-1}$ PAA and 27 min reduced total coliforms by 3 logs (<500 cfu.100 mL^{-1}) in secondary (initial coliform counts – 4.8×10^5 cfu.100 mL^{-1}). Similar results showed a maximum total coliform and *E. coli* reduction, ranging between 4.5-5.5 logs, after 15 $mg.L^{-1}$ PAA was added to secondary wastewater for 38 min (Antonelli *et al.*, 2013). Luukkonen (Luukkonen *et al.*, 2014) reported that a dose of 1.5-2 $mg.L^{-1}$ and a contact time of 10-15 min was acceptable for effective bacterial reduction in tertiary wastewater. A PAA concentration of 1.5-2 $mg.L^{-1}$ is deemed economically viable (Profaizer *et al.*, 1997).

South African standards for irrigation water (≤1 000 faecal coliforms.100 mL^{-1}) (DWAF, 1996; DWA, 2013) are the key determinants of the correct concentrations and exposure times for the desired disinfection. Most studies found that PAA was able to achieve a reduction level of 3-5 logs in total coliforms, faecal coliforms, and *E. coli* with PAA concentrations ranging from 5-10 $mg.L^{-1}$ applied at different contact times.

Advantages

The main advantage of PAA is that it produces little to no DPBs when compared to other chemicals like chlorine or ozone (Monarca *et al.*, 2000; Veschetti *et al.*, 2003; Kitis, 2004; Crebelli *et al.*, 2005) but rather is decomposed into harmless by-products, oxygen and acetic acid. Peracetic acid however, does leave low levels of residuals that have the advantage in preventing the regrowth of pathogens after disinfection. Freese (Freese *et al.*, 2003) and Rossi (Rossi *et al.*, 2007) reported that microorganisms could not repair after PAA damage, at least not within the first five hours of disinfection.

The disinfectant has strong bactericidal properties functioning over wide pH ranges, temperatures and solids concentrations (Profaizer *et al.*, 1997). It is a relatively stable disinfectant when stored under appropriate conditions, easy to handle and does not require expensive capital

investment. The use of PAA as a disinfectant is economically feasible as it is very effective at low concentrations and short contact times (Freese *et al.*, 2004; Kitis, 2004).

Disadvantages

The decomposition of PAA to acetic acid may increase the organic content leading to increased COD levels. This compound serves as a food source for microorganisms that may result in microbial regrowth but the latter will not occur if PAA residuals are present. Another drawback is the high cost, partly due to the limited availability. The high cost of PAA is estimated to decrease with increased demand and mass production capacity, especially within the water disinfection industry (Freese *et al.*, 2003; Kitis, 2004). But they also reported that the application of PAA as disinfectant has increased since 2003. Lastly, the organic content of solutions influence PAA efficiency, therefore a pre-treatment step prior to disinfection is strongly required.

Conclusion

An overall conclusion can be made: the wide PAA antimicrobial activity (sporicidal, fungicidal, virucidal and bactericidal) is drawing increased attention from the water disinfection industry. The application of PAA in low to moderate dosages offers advantages in terms of cost and insignificant by-product formation. Altogether, its broad spectrum of activity, functioning over a wide pH range, the absence of toxic residues and short contact times, are acceptable reasons to consider the investment of such a disinfectant for water disinfection. Therefore, the disinfection of irrigation water is feasible as short contact time's yield in effective disinfection leading to less productive time wasted during farming.

2.7.2.6 General remarks on chemical treatment

The chemical methods are based on the oxidation potential of chemicals which are capable of damaging the cell walls of microorganisms through oxidation and eventually result in cell death. The oxidation potential is not the only factor that has to be taken into consideration when deciding which chemical disinfection agent to use, since water quality parameters play a significant role in the germicidal properties of these disinfectants (Yiasoumi, 2005). These parameters which may influence the efficacy of disinfectants are highly variable. Depending on these environmental parameters, the dose of disinfection governs the efficacy of the treatment. The dose of disinfection is a combination of the disinfectant concentration and the contact time (Freese *et al.*, 2003; GHD, 2005; Lazarova & Bahri, 2005; Yang *et al.*, 2008; Ali, 2010).

Even though chemical disinfectants generally deliver good results, it has been shown that they release disinfection by-products (DBPs) into the water (Yiasoumi *et al.*, 2005). Some of the by-products are carcinogenic, mutagenic or even teratogenic and as a result have raised public concern over the possible adverse health effects on humans (Woo *et al.*, 2002; Bitton, 2005).

Other disadvantages of chemical disinfectants are that their manufacturing, storage, transport and use pose a continuous threat to anyone who works with them as well as serious consequences for the environment (Acher *et al.*, 1997; Yiasoumi *et al.*, 2005).

In practice chemical methods especially the chlorine options are the most commonly used for disinfection purposes? Even though all the available chemical methods have specific limitations they are efficient disinfectives and under certain conditions can be even considered environmentally acceptable.

2.7.3 Physical/Photochemical Methods

2.7.3.1 Ultrasound

Background and mode of action

Ultrasound is a cyclic sound pressure of mechanical vibrations with a frequency greater than the upper limit of human hearing, 20 Hz (Hunter, 2008; Oyib, 2009). During the 1970s, it was reported that good disinfection using ultrasound for the removal of heat resistant bacterial spores was found (Naddeo *et al.*, 2014). Since then many studies have been published on the commercialisation of ultrasound for the bacterial inactivation of wastewater. Hunter (Hunter, 2008) observed a 4 log reduction in viable bacterial cells and other studies noted reductions of total aerobic bacterial counts and free bacterial counts (*E. coli* and *Enterococci*) in irrigation and wastewater (Hulsmans *et al.*, 2010). The extensive use of ultrasound on wastewater treatment has shown that pollutants and pathogenic bacteria were reduced successfully. Cui and co-workers (Cui *et al.*, 2011) conducted a study on the disinfection of *E. coli* in primary sludge and also found a 90% reduction at a high dose of 20 Hz. The applications of ultra sound are numerous. Typically, it is used to penetrate a medium to measure the reflection signature or for the removal of trapped gasses, cleaning of microscopic contamination, ultrasonic humidifier as well as the disruption of biological cells (Oyib, 2009).

The physical ultrasonic inactivation of bacterial cells is caused by a phenomenon called cavitation (Cameron *et al.*, 2008; Hunter, 2008; Kesari *et al.*, 2011). This is caused at high frequencies ranging from 20-100 kHz and is better known as 'power ultrasound' (Kesari *et al.*, 2011). Cavitation can be defined as the formation, growth and subsequent collapse of microbubbles over a very short period of time. The high pressure (50 000 kPa) shock-wave generated during bubble collapsing, is the main inactivation technique in microorganisms (Hunter, 2008) as this produces free radicals (OH, HO_2 and O) (Furuta *et al.*, 2004) with strong oxidative powers. At the same time, high temperatures (5 500°C) are generated during bubble collapsing; however, increased temperatures are not the main cause of cell inactivation. The structural design of microorganisms plays a significant role in the inactivation efficiency of ultrasound. Hulsmans reports (Hulsmans *et al.*, 2010) that the exact method of bacterial inactivation is unknown, but suggests three main antimicrobial inactivation steps: mechanical, chemical and heat effects caused by cavitation (Naddeo *et al.*, 2009). During cavitation the pressure gradients from collapsing bubbles cause bacterial cell wall damage due to mechanical fatigue (Cameron *et al.*, 2008). It has also been found that the cell chemical structures are oxidised by radicals, causing complete disintegration of the cell wall (Cameron *et al.*, 2009).

Advantages

Ultrasound is an alternative to chemical disinfectants that neither leads to the generation of DBPs nor contributes additional chemical compounds (Kesari *et al.*, 2011). From an operational view, ultrasound is a simple method with high bacterial inactivation yields (Naddeo *et al.*, 2014) as it removes a variety of microorganisms during water and wastewater disinfection. The efficacy can also be improved by combining it with other disinfection treatments such as chlorine, ozone and ultraviolet.

Ultrasound treatment has been shown to effectively eliminate faecal coliforms and *E. coli* under specific operational conditions (Cameron *et al.*, 2009). Hulsmans (Hulsmans *et al.*, 2010) did a study on water contaminated with *E. coli* (with initial loads of 4.8×10^4 and 2.0×10^4 cfu.mL^{-1}) and after 180 min of ultrasonication, a 2 log reduction (>99%) was observed. In other studies (Cameron *et al.*, 2009; Naddeo *et al.*, 2014) on water disinfection it was found that *E. coli* and total coliforms were optimally removed at low frequencies (20-40 kHz), high densities and sonication

times of 3-15 min. The ultrasonic inactivation of protozoa (*Cryptosporidium* and *Giardia*) from irrigation water, have been shown when applied to different types of wastewater (Sangave & Pandit, 2004; Mahamuni & Adewuyi, 2010).

Disadvantages

Despite the research done on laboratory scale and the potential of ultrasound for water disinfection, little is known about its application at industrial scale (Naddeo *et al.*, 2014). The energy demand for ultrasound is high and therefore, not recommended for large volumes of water (Hulsmans *et al.*, 2010). Ultrasound has the ability to remove all pathogens during disinfection, however this requires high ultrasonic intensities that will lead to increased costs and extended contact times consequently limiting its use for large-scale disinfection. Researchers have found ultrasound is more effective with combined treatment options like ultraviolet and heat treatment (Hunter, 2008; Naddeo *et al.*, 2009).

Conclusion

Disinfecting large amounts of irrigation water at farm-scale is thus not at the present time feasible as it is expensive and requires high energy loads. It also needs extended disinfection periods. For effective elimination of microorganisms ultrasound is rather recommended as a pre-treatment in disinfection processes as it works more effectively in combined disinfection setup.

Ultrasound is effective in treating almost all types of wastewater and is also capable of removing pathogenic bacteria as well as protozoa from irrigation water. Ultrasound treatment is also effective in reducing algae and fungi such as *Phytophthora* from irrigation water without the formation of any disinfectant by-products.

A disadvantage of using ultrasound treatment is that extended contact times are required for disinfection to occur, increasing the cost of this already expensive treatment method even further. Ultrasound efficacy is dependent on various water quality parameters especially turbidity and is then not always effective in killing all microorganisms. When taking all advantages and disadvantages of ultrasound treatment into consideration, it is clear that this method will at present not be the most effective method for treating irrigation water.

2.7.3.2 Ultraviolet light

Background and mode of action

Ultraviolet (UV) light forms part of the electromagnetic spectrum and represents wavelengths ranging from 100-400 nm (Dai *et al.*, 2012). Thus, UV light is that part of the electromagnetic spectrum with wavelengths longer than x-rays and shorter than visible light. Four spectral areas namely, UV-A (long-wave, 315-400 nm), UV-B (medium-wave, 280-315 nm), UV-C (short-wave, 200-280 nm) and vacuum UV (100-200 nm) have been identified and used to characterise UV light according to wavelengths (Bitton, 2005; Anonymous, 2012c). The sub-categories differ considerably with regard to their application. Today, however, this technique is used increasingly on a global scale for the disinfection of work surfaces, air and contaminated sources of water (Hallmich & Gehr, 2010; Gayán *et al.*, 2014).

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

pathogenic strains may hinder the effectiveness of UV light irradiation. These factors should be thoroughly considered when evaluating disinfection efficacy.

UV-C is most effective between 254-260 nm for the inactivation of microorganisms (Rodríguez-Díaz *et al.*, 2009). Within this range, inactivation occurs via oxidation processes in the cell, also known as photolysis, when UV light is absorbed by the pyrimidine bases in RNA and DNA (Bolton & Cotton, 2008a). These nucleotide bases are known as thymine or cytosine in DNA, and cytosine or uracil in RNA. As UV light is absorbed by the cell, chemical pyrimidine dimers are formed between two bases (Poepping *et al.*, 2014) and inhibits the formation of new DNA or RNA chains. These dimers interfere with cellular processes such as DNA replication during cell production (mytosis) as well as transcription of DNA to RNA for protein synthesis (Eischeid & Linden, 2007; Hunter, 2008; Rodriguez *et al.*, 2014). Cyclobutane pyrimidine dimer (CPD) is the main photoproduct formed during photolysis from two neighbouring thymine bases (Eischeid & Linden, 2007).

UV disinfection makes use of monochromatic, low pressure and medium pressure lamps and the latter produces much higher UV intensities than low pressure lamps (Wong, 2002). Low pressure and medium pressure lamps emit light within the UV-B (280-315 nm) and UV-C range (200-280 nm). DNA absorbs UV light significantly at a maximum of 260 nm therefore, both medium pressure and low pressure lamps can be used for disinfection (Eischeid & Linden, 2007). The UV dose requirements of bacteria, bacterial spores, viruses and protozoa vary and the term 'dose' can be defined as follows (Bolton & Cotton, 2008a):

Dose ($mJ.cm^{-2}$) = UV intensity ($\mu W.cm^{-1}$) x UV time (seconds)

Currently, UV dose can only be manipulated in bench scale experiments at laboratory scale using a collimated beam device (Bolton & Cotton, 2008d). The output of the lamp is directed onto a horizontal surface where the sample is placed for irradiation. The most common doses used for the elimination of pathogens are 16 $mJ.cm^{-2}$, 30 $mJ.cm^{-2}$, 40 $mJ.cm^{-2}$ and higher. Two types of UV apparatus are mainly used on industrial scale: 'Flow through-open channel systems' are used for wastewater disinfection and 'in-pipe, closed systems' are used for drinking water and discharge effluents (Acher *et al.*, 1997; Lazarova & Bahri, 2005). The UV dose is significantly affected by the flow rate and water quality. Only a few seconds of UV light exposure are required for the inactivation of microorganisms (Hunter, 2008); however, this is greatly influenced by the flow rate of water travelling through the UV chamber. High flow rates are directly correlated to shorter exposure times and low UV doses and vice versa (EWP, 2014). Also, the effect of water quality on UV disinfection is complex as various water characteristics may influence UV efficiency, such as UV transmittance, suspended solids, temperature, pH and water hardness. Most importantly, UV transmittance is the predominant influence on UV efficiency, as it affects the light penetrating pathogens (EWP, 2014). Other factors such as BOD (biochemical oxygen demand), COD (chemical oxygen demand), suspended solids and turbidity are also coupled to UV transmittance and lower the extent of UV light water penetration (EWP, 2014).

As mentioned there are mainly two types of UV disinfection methods available namely a flow-through open channel system mainly utilised for wastewater disinfection, and an in-pipe closed channel system mainly utilised for drinking water disinfection (Acher *et al.*, 1997; Lazarova & Bahri, 2005; Anonymous, 2012a). These days most wastewater treatment plants use in-pipe closed systems to treat discharged effluents. As most treated effluents are re-used for irrigation, it is transported in pipes after being treated, to the point of use (Buijs, 2012).

When an open channel system is used, UV modules which are stainless steel frames that manifest the low intensity, low-pressure UV lamps are immersed in the water that flows through the channel (Acher *et al.*, 1997). Low-pressure UV lamps have a peak monochromatic emission at a

wavelength of 253.7 nm (Bitton, 2005). The number and size of UV modules needed is dependent on the flow rate of the water to be disinfected, the water quality as well as the disinfection requirements (Acher *et al.*, 1997). Sophisticated controls as well as carefully designed inlet conditions are of great importance to ensure that the lamps stay submerged under water and flow is evenly distributed to prevent short circuiting and loss of disinfection performance (Anonymous, 2012c). Most open channel systems require that lamps be manually cleaned by an operator, thus each lamp has to be removed by hand (Anonymous, 2012a). In open channel UV units the gravitationally fed water flows almost laminar to the UV lamps due to the low velocity of the fluid (Anonymous, 2012b; Buijs, 2012). As a result microorganisms pass through the area with the lowest UV intensity without receiving sufficient UV light exposure.

The in-pipe closed system utilises high intensity medium pressure lamps in a closed area which is installed in the effluent header pipe just before discharge (Acher *et al.*, 1997; Anonymous, 2012c). Medium-pressure UV lamps have a peak polychromatic emission at wavelengths ranging from 185 to 400 nm (Bitton, 2005; Anonymous, 2012c). In-pipe closed channel systems have different requirements for inlet design than open channel systems (Anonymous, 2012a). Water flows in a linear flow at relatively high flow rates, and flow is always evenly distributed inside the chamber, resulting in optimum disinfection performance. Since the flow rate is very high, the irradiation time is relatively short, thus high intensity UV lamps are necessary to insure the minimum UV dose required is applied (Acher *et al.*, 1997). The use of high intensity lamps enables the treatment of wastewater effluents in a relatively small area (Anonymous, 2012a). Closed channel systems have an automatic cleaning system that cleans everything that might have been deposited on the quartz lamp sleeves capable of reducing the UV light intensity transmitted into the water (Anonymous, 2012a). The high output from these lamps allows for the use of fewer lamps than in an open channel system to achieve the same amount of disinfection, significantly enhancing reliability and at the same time reducing maintenance costs. Another advantage of closed channel systems is that less head-loss occurs in comparison with open channel designs (Zimmer & Slawson, 2002; Anonymous, 2012a).

Taking into consideration all of the advantages of closed channel systems in comparison with open channel systems, the use of monochromatic emitting low-pressure mercury UV lamps in water disinfection has mainly been replaced by polychromatic emitting medium-pressure mercury UV lamps that has a much broader spectrum to efficiently kill all pathogenic microorganisms (Zimmer *et al.*, 2003).

Exposure of microorganisms to UV irradiation results in damage to the nucleic acids as well as other components of the cell (Hijnen *et al.*, 2006). Even after UV irradiation some microorganisms are capable of retaining certain metabolic functions such as enzyme activity. Since most microorganisms are exposed to UV irradiation from sunlight on a daily basis, many microorganisms have over time developed mechanisms to compensate for the damage done to them by UV irradiation. Nucleotide excision repair, also referred to as dark repair, and photo-reactivation are the two main pathways available to repair UV damaged DNA or RNA (Zimmer (Zimmer & Slawson, 2002; Hijnen *et al.*, 2007).

According to Zimmer and Slawson (Zimmer & Slawson, 2002), certain microorganisms are capable of repairing damage done to cells following exposure to low-pressure UV irradiation. They compared the efficacy of low-pressure UV lamps with medium-pressure UV lamps. It was found that *E. coli* underwent photo-repair after exposure to low-pressure UV lamps (doses of 5, 8 and 10 $mJ.cm^{-2}$), but no repair was evident after it was exposed to medium-pressure UV lamps (doses of 3, 5, 8 and 10 $mJ.cm^{-2}$) at the same or even lower doses. They (Zimmer *et al.*, 2003) also found no evidence of repair to *Cryptosporidium parvum* following low doses, 1 and 3 $mJ.cm^{-2}$, of both low and medium pressure UV lamps.

In general, microorganisms' resistance to UV follows the same pattern as with chemical disinfectants which are as follows: protozoan cysts > bacterial spores > viruses > vegetative bacteria (Bitton, 2005; Lazarova & Bahri, 2005). Thus, since Zimmer and his co-workers (Zimmer *et al.*, 2003) found that UV is effective against a protozoan organism, it can be assumed that medium-pressure UV will be effective in killing all pathogenic microorganisms without any repair to cells after exposure.

Based on UV disinfection and repair mechanisms, a UV dose of 30 $mJ.cm^{-2}$ will be sufficient to produce reclaimed water virtually free from pathogens and is an adequate method of disinfection of secondary effluent for agricultural irrigation purposes (Yoon *et al.*, 2007).

Advantages

Numerous studies have shown that UV disinfection effectively eliminates the presence of enteric bacteria, bacterial spores, viruses and oocysts without producing any DBPs or other chemical residues that may lead to DBP formation (Rajala *et al.*, 2003; Bolton & Cotton, 2008c; Spellman, 2014). The absence of DBP formation is one of the main advantages of UV compared to traditional disinfectants that release by-products into the water. Ultraviolet disinfection alters the water quality by degrading the natural organic matter (NOM) and micro-pollutants present in the water (Meunier *et al.*, 2006). When UV is compared to exposure times needed for chemical disinfection, much shorter contact times, in fact, only a few seconds are needed for effective disinfection (Spellman, 2014).

Ultraviolet irradiation is a physical process, therefore, it eliminates the generation, transport and storage of toxic or hazardous chemicals consequently representing lower costs compared to chemical disinfection (Spellman, 2014). Ultraviolet units, as well the installation thereof, are expensive but the operational cost to sustain the apparatus is fairly low as little much maintenance is required. The application of UV disinfection is suitable for small- as well as industrial-scale water disinfection facilities.

Chemical disinfection with chlorine is not effective against *Cryptosporidium* and *Giardia* protozoan microorganisms (Lazarova & Bahri, 2005; Hijnen *et al.*, 2006). Even though ozone is effective against these protozoan pathogens, it is not a viable treatment option since ozone is very unstable and it is almost impossible to predict how it would react with organic matter that might be present in the water (Freese *et al.*, 2003; Selma *et al.*, 2008). These days UV irradiation is regarded as a disinfection method that is extremely effective against most pathogens such as algae, bacteria, fungi, moulds, nematodes eggs, protozoa, viruses, yeasts as well as water moulds such as *Phytophthora* that could be transmitted through water (Yiasoumi *et al.*, 2005; Hijnen *et al.*, 2006; Bolton & Cotton, 2008a).

Disinfection of water and wastewater with UV irradiation for all kinds of purposes has many advantages. These advantages include no production of carcinogenic, mutagenic or toxic by-products (Bitton, 2005; Guo *et al.*, 2009; Buijs, 2012). Ultraviolet irradiation prevents the occurrence of taste and odour problems that can occur on-site or in the final water after treatment; No volatile toxic chemicals are needed for treatment; UV equipment and the water contact chamber requires a minimal amount of space and can usually be retrofitted into existing water treatment plants (Bitton, 2005; Bolton & Cotton, 2008a). Ultraviolet apparatus is relatively inexpensive with low capital and operating costs compared to other treatment methods that are effective in killing protozoan organisms, UV equipment is easy to operate and water treated with UV requires only a few seconds contact time to be properly disinfected and it does not affect the treated water's quality in anyway (Bolton & Cotton, 2008d).

Disadvantages

Despite the advantages associated with UV irradiation, there are also some drawbacks regarding its use. The initial implementation cost of a UV system is expensive. The fluid should be penetrable (low organic content) to UV light otherwise, penetration of the UV rays will not be effective to reduce high bacterial numbers (Hunter, 2008; Spellman, 2014). Similarly, non-homogenous fluids containing certain amounts of suspended solids greatly affect the efficiency of UV light as it directly relates to water turbidity that associates negatively with effective disinfection. Spellman (Spellman, 2014) reported that UV disinfection using low pressure lamps is not as effective when suspended solids levels in the water exceed 30 $mg.L^{-1}$. Studies have mentioned the possibility of photo-reactivation or dark repair of microorganisms occurring at sub-lethal UV doses under the desired conditions (Freese *et al.*, 2004; Lazarova & Bahri, 2005). Another one of UV's disadvantages is that it has no disinfectant residual in treated water and as a result certain susceptible microorganisms can become viable again if it was treated by low-pressure UV lamps in an open channel system. This problem can be overcome by treating water with medium-pressure UV lamps in an in-pipe closed system (Zimmer & Slawson, 2002; Zimmer *et al.*, 2003; Bitton, 2005; Bolton & Cotton, 2008c). Other disadvantages of UV are that it is not always possible to accurately measure the UV dose, so operators have to rely on secondary measurements such as sensor readings, UV transmittance a well as water flow rates. Disinfection reliability also decreases in high turbidity effluents and as a result water for irrigational purposes mostly has to be pre-treated by sand- or ultrafiltration before it can be treated with UV (Lazarova & Bahri, 2005). Since UV lamps contain mercury, breakage of lamps can in certain cases result in a mercury hazard (Bolton & Cotton, 2008a). The amount of mercury contained in these UV lamps are minute and if it breaks are usually contained within the quartz sleeve and might only come into contact with the water due to negligence (Van Kamp, H. 2014, Winelands UV Technology, Stellenbosch, South Africa, personal communication, 20 January). Another disadvantage of UV lamps is that in case of power outages water can be under disinfected (Bolton & Cotton, 2008c).

Conclusion

Ultraviolet irradiation is regarded as a disinfection method that is extremely effective against all pathogens such as algae, bacteria, fungi, moulds, nematodes eggs, protozoa, viruses, yeasts as well as water moulds such as *Phytophthora* that could be transmitted through water. Ultraviolet disinfection of wastewater effluents is an economically competitive alternative to other chemical and physical methods of irrigation water treatment and has a contact time of only a few seconds. Even though UV irradiation experiences reduced disinfection performance in water with high levels of suspended solids, turbidity and organic matter, these problems can easily be resolved by pre-treating the water with sand- or ultrafiltration methods. Medium-pressure UV light is capable of killing bacteria, viruses, protozoa as well as water moulds, without the possibility of reactivation occurring. Another factor making UV treatment such a viable option for irrigation water disinfection is that it does not produce any carcinogenic, mutagenic or toxic by-products or change the chemical characteristics of the water being treated. Ultraviolet irradiation also prevents the occurrence of taste and odour problems that can occur on-site or in the final water after treatment.

2.7.3.3 General remarks physical/photochemical treatments

Ultraviolet disinfection is an effective method of removing a variety of organisms. However, its greatest limitation is associated with water quality (Gurol, 2005), especially water turbidity (Freese *et al.*, 2004). Therefore, applying a pre-treatment, such as filtration (sand filters), is strongly recommended (Newman, 2004).

With regard to the microbiological aspects, the phenomenon of DNA repair mechanisms, which include dark and photo-reactivation, may significantly influence disinfection efficacy. Various

researchers have reported that photo-reactivation specifically is of great concern (Guo *et al.*, 2009; Wang *et al.*, 2011; Guo *et al.*, 2012). In addition, microorganisms may associate with particles suspended in water, subsequently being shielded from the damaging effects of UV rays (Walters *et al.*, 2014). Other, less common, disadvantages of UV disinfection include: mercury hazards in water should the mercury containing lamps break; compromised disinfection resulting from interruptions in power supply and inadequate disinfection resulting from the phenomenon of lamp warm-up (Bolton & Cotton, 2008b).

Ultraviolet installation cost is high which can limit the use of UV disinfection by non-commercial farmers. However, comparing this to the continual costs associated with chemical disinfection, UV disinfection combined with a filtration step can be recommended. Ultraviolet light disinfection is used in multiple European countries and from a South African point of view; the application of this method for water disinfection has potential as a future treatment option.

2.7.4 Combination Treatments

As for any other method, UV irradiation cannot be regarded as a flawless, unsurpassable technique with respect to water disinfection. While each of the individual technologies are associated with their own limitations, research indicates that the application of combination treatments often result in considerable benefits, often more than a summation of the individual effects (Blume *et al.*, 2002; Plummer *et al.*, 2002; Lotierzo *et al.*, 2003; Zoutman *et al.*, 2011; Norton-Brandão *et al.*, 2013; Tawabini *et al.*, 2013).

2.7.4.1 Advanced oxidation processes

Background and mode of action

Advanced Oxidation Processes (AOPs) are increasingly utilised in the different sectors of the water industry including the production of drinking water and the treatment of water containing waste (IJpelaar *et al.*, 2010; Tawabini *et al.*, 2013; Sherchan *et al.*, 2014). The technology of AOPs is based on the combination of UV light with secondary oxidants, such as ozone, hydrogen peroxide and peracetic acid, in order to destruct disinfection by-products, microorganisms and other organic micro pollutants present in water (Caretti & Lubello, 2003; IJpelaar *et al.*, 2010; Teksoy *et al.*, 2011; Wols & Hofman-Caris, 2012). This occurs through the formation of high concentrations of hydroxyl radicals ($OH^{\bullet}$) which are very reactive, non-selective oxidising agents (Timchak & Gitis, 2012; Tawabini *et al.*, 2013). Advanced oxidation processes including UV are seen to be most beneficial, due to the fact that UV is regarded as a disinfection method that is extremely effective against most pathogens such as algae, bacteria, fungi, moulds, nematode eggs, protozoa, viruses, yeasts as well as water moulds such as *Phytophthora,* which are often considered limitations of the chemical compounds (Chlorine, ozone, hydrogen peroxide), in that they are not always active against the entire range of organisms (Koivunen & Heinonen-Tanski, 2005a; Hijnen *et al.*, 2006; Gayán *et al.*, 2014). Therefore, combination treatments should be considered as a means to improve the efficacy but also the scope of organisms targeted for disinfection.

Advantages

Various studies have reported on the bactericidal effect of H_2O_2 (Ksibi, 2006; Labas *et al.*, 2008; Rizvi *et al.*, 2013) but as a result of fairly low efficacy and disinfection rates the chemical is not commonly applied as primary disinfectant in water treatment (Koivunen & Heinonen-Tanski, 2005a). However, the combination of UV light and H_2O_2 as an AOP promotes disinfection and has been proven to be capable of destroying not only a variety of pollutants, but also spores and vegetative cells (Koivunen & Heinonen-Tanski, 2005b; Alkan *et al.*, 2007; Mamane *et al.*, 2007). This particular combination is often encountered in literature and utilises UV light for direct

disinfection, but also for the generation of hydroxyl radicals in water (Timchak & Gitis, 2012). Such treatment thus combines the effects of direct photolysis and advanced oxidation via indirect photolysis (Pereira *et al.*, 2007).

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

In the limited literature available on the disinfection capacity of UV in combination with PAA, it was found that the combination of PAA and UV radiation resulted in significant increases in efficacy, compared to the use of PAA (2-8 mg.L^{-1}) or UV alone (Caretti & Lubello, 2003), albeit at very high UV doses (up to 195 mJ.cm^{-2}). Koivunen & Heinonen-Tanski (2005a) also concluded that the combined PAA/UV (7-15 mgPAA.L^{-1} and up to 8-38 mJ.cm^{-2}) disinfection efficiency and reliability was improved. Similar results were found for coliphages, where inactivation was achieved in a shorter time than PAA alone (Rajala-Mustonen *et al.,* 1997), although turbidity was seen to be a factor in decreasing the efficiency.

Disadvantages

Although the combined effect of UV/H_2O_2 has been shown to be synergistic, the presence of organic particles in irrigation water can reduce the disinfection efficacy, by either reaction with the H_2O_2 (Newman, 2004; Zheng & Dunets, 2014) or by reducing the UVT% (Hunter, 2008; Spellman, 2014). Koivunen & Heinonen-Tanski (2005a), however, did not find significant increases in disinfection efficiency for H_2O_2/UV compared to UV alone. The combination does also not alter the fact that peroxides are highly unstable and corrosive, therefore safety measures in storage and handling are still important (Lenntech, 2014). The implementation of UV/H_2O_2 systems can still be relatively expensive as the initial capital cost is still required and the H_2O_2 remains a regular cost.

Conclusions

Referring to these studies, one can conclude that the combination of UV/H_2O_2 and UV/PAA as AOP show potential as effective disinfection treatments of microbiologically contaminated water.

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

2.8 CONCLUDING REMARKS

South Africa is a water scarce country facing an undeniable national water challenge, not only in terms of availability, but also in terms of the quality of its fresh water sources. Fresh produce production is an important component of the RSA's agriculture, food security situation as well as its economic viability. As a result of the varying rainfall patterns many farmers are forced to use river water to irrigate their crops. These rivers are often contaminated with high microbial loads and are thus of questionable quality for irrigation.

It is well known that about 80% of the world's diseases are caused by contaminated surface waters (Pandey, 2006) as substantiated by many reports on foodborne disease outbreaks linked to fresh produce items that were traced to faecal contaminated irrigation water (Britz *et al*., 2012). In developing countries such as South Africa, due to water shortages, water is mainly extracted from rivers for the irrigation of fruit and vegetables. South African river water is deteriorating rapidly. Various studies have confirmed the presence of high levels of faecal coliforms, *E. coli*, other bacterial pathogens, a range of viruses and protozoa present in our river waters that are generally used to irrigate crops.

Should contaminated surface water be used for agricultural irrigation, however, significant risks of produce contamination and subsequent foodborne disease outbreaks become a reality. As a matter of fact, microbiologically polluted irrigation water has been identified as key source of fresh produce contamination. In this regard, decontamination, or disinfection, of river water prior to its use for agricultural irrigation is fundamental in minimising the risk of disease outbreaks following the consumption of such products. Thus, it is relevant to investigate the use of different disinfection techniques in order to be able to make recommendations as to which processes require further investigation, in the search for effective disinfection of microbiologically polluted irrigation water.

Conclusions pertaining to disinfection options reached from the Literature Review:

1) *Mechanical and physical treatment* methods such as *sand/media filtration* and *ultrafiltration* are well researched, established and commercialised technologies. A multitude of different configurations can be installed for the separation of a variety of types and sizes of constituents in different water sources. Treatments can range from pre-treatment to final polishing steps. Disadvantages include the long time required (sand/media filtration) and high costs (ultrafiltration). These technologies can be scoped and installed according to specific water quality required. The specific process is thus chosen based on the incoming water quality and the quality of water required post-treatment. Further research into these technologies, when comparing disinfection efficacy of other methods, is not required;
2) *Chemical treatment methods* such as *bromine*, *ozone*, *chlorine* and *hydrogen peroxide* are well researched disinfectants, and much is known of the efficacy in irrigation water. In most cases there are concerns over the corrosive nature of the chemicals, the potential risks during transport, storage and handling and the formation of disinfectant by-products in the water. High concentrations and long contact times are sometimes required and the residual in the water could be harmful to the environment. The residual can, however, sometimes be

an advantage as it extends further protection in the water after its initial addition. The efficacy of these chemical disinfectants is also dependent on the pH, COD and TSS of the water. Chemical disinfectants are also not always equally effective against all micro-organisms. Research has shown that certain micro-organisms build up resistance to certain chemical disinfectants, which has implications on the safety of the water for use, concentrations required for disinfection, cost and further resistance. It is thus imperative that these often used chemical disinfectants (*hydrogen peroxide* and *chlorine*) are compared to alternative disinfection methods and that the increasing resistance of micro-organisms to these methods be further investigated;

3) The *chemical treatment* of irrigation water with *peracetic acid*, however, should be investigated further. The wide antimicrobial activity (sporicidal, fungicidal, virucidal and bactericidal) could prove effective. Peracetic acid could be effective at low dosages, a wide pH range, does not result in significant formation of disinfectant by-products and does not require excessively long contact times. The potential of PAA as a disinfectant in irrigation water and possibly in combination with other treatments should be investigated;
4) *Ultrasound* has the ability to remove all pathogens during disinfection, but is dependent on various water quality parameters especially turbidity and also requires high ultrasonic intensities that will lead to further increasing the costs (high capital and operating cost also) and extended contact times, consequently limiting its use for large-scale disinfection of irrigation water;
5) *Ultraviolet* irradiation has been shown to be extremely effective against all pathogens such as algae, bacteria, fungi, moulds, nematode eggs, protozoa, viruses, yeasts and water moulds. Several advantages exist over other treatments, such as the fact that no disinfectant by-products are formed, only very short contact times are required and no chemicals are added to the water. Although UV efficacy is reduced by high levels of suspended solids, turbidity and organic matter (which can be resolved by proper pre-treatment filtration processes), the incorrect scoping and design (including UV transmission, flow rate and required log reductions required (of a specific target organism) is the main reason resulting in poor UV efficiency. Two factors, however, require further investigation, namely the determination of the correct dosages required for disinfection for a variety of waterborne microorganisms (rather than dosage required to reduce laboratory strains of specific bacteria) and the phenomenon of photo-reactivation and dark repair by bacteria after exposure to UV light;
6) It has been shown that *combination treatments* such as UV/H_2O_2 and UV/PAA treatments often result in improved disinfection in comparison to the use of UV light alone. It may therefore be valuable to assess the potential of UV/H_2O_2 and UV/PAA combination treatments for the disinfection of river water. Such experiments should investigate the influence of different water quality parameters and provide insight as to whether or not the technique could present increased disinfection of river water on a larger scale in comparison to UV light alone. This, however, needs to be weighed up against the use of chemicals, the concerns around their handling and their effect on the environment and inducing resistance to these disinfectants.

2.8.1 Recommendations

The treatment of irrigation water is essential to eliminate pathogens to reduce the risk exposed to consumers by fresh produce items. Physical (slow sand filtration and ultrafiltration), chemical (bromine, chlorine, hydrogen peroxide, ozone and peracetic acid) and alternative (UV and ultrasound) approaches are available to eliminate harmful pathogens present in irrigation water.

However, due to the complexity and variability of surface water properties not all methods are equally suitable for disinfection purposes. Water properties including pH, dissolved solids content, turbidity and colour, for instance, may influence disinfection efficiency and are subject to rapid change with human interference or environmental events. Furthermore, disadvantages such as the formation of harmful disinfection by-products and the extreme resistance of some microorganisms toward certain chemicals has been shown. Also, when chemical disinfectants are used additional risks to handlers, as well as to the environment, are introduced. Thus, each disinfectant technology has specific operational and environmental requirements that reflect several advantages and drawbacks.

Therefore, before a disinfection method is selected or compared to another, the quality of water and the quality variations over a fixed period must be determined in order to ensure a continuous effective treatment. Furthermore, when the efficacy of a disinfection treatment method is determined, the type of organism present in a water system must also be determined as each will react differently to whatever treatment is envisaged. *Escherichia coli* is the common indicator organism, used for detecting faecal contamination from human and animal sources. Water guidelines and regulations are thus based on the presence and concentration of faecal coliforms, including *E. coli*. Reference strains have often been used in studies to test the efficacy of certain treatment options, however the growth kinetics are not always the same when compared to those of environmental strains. Naturally occurring environmental strains have been shown to be better adapted to adverse environmental conditions. Hence, using standard reference strains during treatment optimisation studies may lead to inaccurate results and in return incorrect parameters may be selected (UV doses or chemical concentrations and contact times).

Reference strains and single isolated environmental strains can be used in preliminary laboratory studies to select treatment conditions for further investigation at pilot-scale. Investigations and comparisons of disinfection efficiency (log reductions achieved) should be done between established treatment methods (chlorine and hydrogen peroxide), alternative disinfectants (peracetic acid and UV) and combinations of the chemical disinfectants and UV, in terms of differences in resistance to treatment (between environmental and references strains) and photo-reactivation/dark repair, in order to make recommendations as to the required treatment conditions to achieve specific log reduction targets.

Once these questions have been addressed, special attention should also later be given to cost implications of treating large volumes of water, the impact of using chemical disinfectants on the environment and only using coliform indicators as target organisms to determine efficiency. The effect of the selected treatment method should also be determined using other potential waterborne pathogens.

3. EXPERIMENTAL PROCEDURES (PILOT-SCALE)

3.1 Original and revised aims and method

3.1.1 Original aims

The original aim of this study was to conduct a scoping study on different on-farm treatment options to reduce or remove the high levels of potentially pathogenic micro-organisms from irrigation water, this would be achieved by:

1) To do a comprehensive literature study and survey on potential treatment options for irrigation water contaminated with high levels of micro-organisms to enable a treatment option to be selected for trials in an exploratory study;
2) To conduct an exploratory study on an on-farm treatment option (as identified in the literature study and survey of treatment practices) by monitoring the water quality throughout the irrigation water cycle;
3) To study the effect of the on-farm treatment option on the biofilm formation/reduction within the irrigation system;
4) To propose most appropriate treatment options and requirements for further research.

3.1.2 Original method

A. Comprehensive literature study:

A comprehensive literature study will be conducted focusing on 1) the extent of microbial pollution in irrigation water; 2) potential food safety risks associated with these microbial loads; 3) review of current and potential treatment options, focusing on: a) chemical treatment options (chlorine based, hydrogen peroxide, etc.); b) mechanical/physical treatment options (filtration, sedimentation, etc.); and c) non-chemical and non-physical (UV, ultrasound, etc.).

The literature study will include a survey of “treatment practices” currently implemented by farmers to manage the contamination present in the water sources available to them for irrigation purposes.

The literature study will give guidance as to the most feasible treatment option in terms of technical appropriateness and financial feasibility. This will then determine the treatment option to be studied further in an exploratory feasibility study to prove the hypothesis reached in the literature study.

B. Site and Produce selection:

A full-scale commercial farm using irrigation water sourced from a polluted river will be chosen with reference to the following parameters:

1) microbial loads of the river system;
2) type of vegetable or fruit crop being irrigated;
3) type of irrigation system being used;
4) irrigation usage period and irrigation intervals;

C. Monitoring the water quality throughout the Irrigation Water Cycle:

A study will be conducted to determine the water quality parameters (chemical and microbiological) at various stages of the irrigation water cycle. Water quality parameters to be determined will include the following:

1) temperature;
2) pH;
3) conductivity; and
4) chemical oxygen demand (COD);
5) Total soluble solids (TSS)

Microbiological parameters to be determined will include:
1) total Coliforms;
2) *E. coli*;
3) *Cryptosporidium*;
4) *Giardia*; and
5) *Vibrio cholerae*.

The chemical and microbiological parameters will be measured at various stages of the irrigation water cycle to validate the treatment option's efficacy in reducing the microbial load. The stages of the irrigation water cycle which will be monitored are:
1) river water;
2) storage reservoir (if applicable);
3) before sand filter;
4) after sand filter/before "treatment option";
5) after "treatment option";
6) at point of irrigation.

The above monitoring will also be done at various intervals during the growing season of the specific crop and over various growing seasons (i.e. over two years) to determine the robustness of the equipment and to determine any variations in efficacy.

D. Effect of the "treatment option" on biofilm formation/reduction within the irrigation system

In order to determine the effect of biofilms in the irrigation system on the overall microbial load, special attention will be given to the formation or reduction of the biofilm. This will be done as part of the microbiological testing regime to be conducted in C. above.

After the first year of the project, due to preliminary results obtained during the exploratory study on an on-farm treatment option, by monitoring the water quality throughout the irrigation cycle, the Project Team in consultation with the Reference Group agreed to change the original objectives and method. The original contract, objectives and method was amended on 1 April 2014, for the rest of the project period.

3.1.3 Revised aims

Therefore, the amended aim of the study was to conduct a scoping study on different on-farm treatment options to reduce or remove the high levels of potentially pathogenic micro-organisms from irrigation water, this would be achieved by:
1) Doing a comprehensive literature study and survey on potential treatment options for irrigated water contaminated with high levels of micro-organisms to enable a treatment option to be selected for the trials in an exploratory study;
2) Conducting an exploratory study on an on-farm treatment option (as identified in the literature study and survey of treatment practices) by monitoring the water quality throughout the irrigation water cycle.

3a) Determining the efficacy of different treatment options (which included hydrogen peroxide, peracetic acid, chlorine and UV) on different *E. coli* strains (reference strains, environmental strains and mixed environmental strains) at laboratory-scale.
3b) Conducting an exploratory study on the efficacy of the different treatment options on a custom pilot-scale irrigation water test unit.
4) Proposing appropriate treatment options for further research.

3.1.4 Revised method

A. Comprehensive literature study:

A comprehensive literature study will be conducted focussing on 1) the extent of microbial pollution in irrigation water; 2) potential food safety risks associated with these microbial loads; 3) review of current and potential treatment options, focussing on: a) chemical treatment options (chlorine based, hydrogen peroxide, etc.); b) mechanical/physical treatment options (filtration, sedimentation, etc.); and c) non-chemical and non-physical (UV, ultrasound, etc.).

The literature study will give guidance as to the most feasible treatment option in terms of technical appropriateness and financial feasibility. This will then determine the treatment option to be studied further in an exploratory feasibility study to prove the hypothesis reached in the literature study.

B. Site and Produce selection:

A full-scale commercial farm using irrigation water sourced from a polluted river will be chosen with reference to the following parameters:
1) microbial loads of the river system;
2) type of vegetable or fruit crop being irrigated;
3) type of irrigation system being used;
4) irrigation usage period and irrigation intervals;

C. Monitoring the water quality throughout the Irrigation Water Cycle:

A study will be conducted to determine the water quality parameters (chemical and microbiological) at various stages of the irrigation water cycle. Water quality parameters to be determined will include the following:

1) temperature;
2) pH;
3) conductivity;
4) turbidity and UVT (%);
5) chemical oxygen demand (COD);
6) Total soluble solids (TSS)

Microbiological parameters to be determined will include:

1) aerobic colony count;
2) total Coliforms;
3) *E. coli*;

The chemical and microbiological parameters will be measured at various stages of the irrigation water cycle to compare the current treatment option efficacy with a selected alternative treatment, in reducing the microbial load. The stages of the irrigation water cycle which will be monitored are:

1) river water;
2) storage reservoir (if applicable);
3) before sand filter;
4) after sand filter/before “treatment option”;
5) after “treatment option”;
6) at point of irrigation.

The above monitoring will be done at various intervals during the growing season of the specific crop to determine the robustness of the equipment and to determine any variations in efficacy.

D. Efficacy of different treatment options on different *E. coli* strains at laboratory-scale.

A laboratory study will be conducted to determine the efficacy of different treatment options (including UV, hydrogen peroxide, chlorine, ozone) on different *E. coli* strains (reference strains, environmental strains and mixed environmental strains) at laboratory-scale.

This will be done by selecting ATCC *E. coli* reference strains, environmental *E. coli* strains isolated from various rivers and river water samples (mixed environmental strains) and comparing the efficacy of different treatment options (options could include: UV, hydrogen peroxide, chlorine, peracetic acid, commercial products or ozone) at different concentrations/doses, exposure times and for different contamination levels.

Microbiological parameters to be determined will include:

1) aerobic colony count;
2) total Coliforms;
3) *E. coli*;

- Determine optimum treatment combinations (concentration: contact time or dosage);
- Determine difference in terms of dosage requirements between Ref. strains and Env. Strains for specific log reduction;
- Determine differences in resistance to treatment option and compare/correlate to antibiotic resistance profiles;
- Determine if response of single environmental strains is similar to mixed environmental strains (i.e. river water). This will be useful in determining dosages necessary on-farm.

E. Design and construction of a pilot-scale irrigation water test unit

A pilot-scale irrigation water test unit will be designed and constructed at a selected site. The pilot-scale irrigation water test unit will be designed in such a way that river water samples can be treated reproducibly by various methods (options could include: UV, hydrogen peroxide, chlorine, peracetic acid, commercial products or ozone). The design will be such that the efficacy of the methods can be compared, but that differences in water quality and their effect on the efficacy of the methods can also be quantified.

F. Exploratory study on the efficacy of the different treatment options on a pilot-scale irrigation water test unit

The pilot-scale irrigation water test unit will allow the efficacy of various treatment options (options could include: UV, hydrogen peroxide, chlorine, peracetic acid, commercial products or ozone) to be simulated in a reproducible manner (triplicate analyses of river water samples, comparative analyses of treatment options with identical river water samples).

Water quality parameters to be determined will include the following:

1) temperature;
2) pH;
3) conductivity; turbidity and UVT (%);
4) chemical oxygen demand (COD);
5) Total soluble solids (TSS)

Microbiological parameters to be determined will include:

1) Aerobic colony count;
2) Total Coliforms;
3) E. coli

In the original aims and method, the irrigation water quality was monitored throughout the irrigation water cycle, but this aspect didn't generate significantly reliable results, and thus the Project Team in consultation with the Reference Group agreed to change the original aims and method.

The revised method could be summarised as follows:

- Investigate differences in river water quality;
- Investigate differences in resistance to treatment between reference strains and environmental *E. coli* strains to different treatments (H_2O_2, chlorine, PAA, UV and combinations of the chemical disinfectants with UV);
- Investigate the occurrence of photo-reactivation and dark repair after UV irradiation.

These investigations were first performed at laboratory-scale, using known single reference strains and environmental strains, in saline solution. This was done to provide insight into optimising treatment conditions. Thereafter, investigations were continued at laboratory-scale with single reference and environmental strains in sterilised river water to determine the influence of the water chemistry of efficacy and provide further insights in optimising treatment conditions. Investigations then progressed to treatments being carried out at laboratory-scale in river water (with unknown mixtures of micro-organisms, i.e. mixed populations). The thinking behind this was to be able to have a better understanding of the treatment conditions required at pilot-scale, where an unknown mixture of environmental micro-organisms could be present in the water, having a range of resistances to disinfection. The final investigations, using treatment parameters obtained from preliminary laboratory-scale investigations, were performed in river water in a custom built pilot-scale treatment unit.

Therefore, the main body of this report focuses on the Pilot-scale investigations (methods described below). The preliminary laboratory-scale studies can be found in Appendices 1-5.

3.2 General materials and methods

Laboratory-scale studies
Experimental procedures for laboratory-scale studies are described in Appendix A2.

Pilot-plant site description and sampling
Larger-scale disinfection experiments were performed at a pilot-scale water treatment unit positioned on the bank of the Plankenburg River at an industrial site (33°56'15.4''S,18°50'53.0''E) in Stellenbosch, South Africa. The unit allowed for water to be pumped (at *ca*. 3.5 $m^3.h^{-1}$) from the

river, through a sand filter (3 Bag sand filter; filtering down to *ca*. 100 μm), to any of three 2 500 L holding tanks. Once filled, the water within each tank was continuously recirculated. During chemical treatments the respective chemicals were added directly to the holding tanks, after which proper recirculation was allowed. For UV treatments water was pumped directly from the holding tanks through the in-line UV system. The pilot-scale system was designed to allow flow rates in the range of 30 to 200 litres per minute (LPM). The sampling points are indicated in Fig. 3.1a (Study 1 and 2) and Fig. 3.1b (Study 3).

For Study 1 the sampling points were situated before the sand filter (1), after the sand filter before exposure to UV light (2) and following exposure to UV light (3) (Fig. 3.1a). One control sample was taken from point 2 (after the sand filter) where after another sample was taken at point 3 following treatment at the respective UV doses. For Study 2 (Fig. 3.1b), a control sample was taken from point 1, and another sample at point 2 after UV exposure. Up until this point no chemical treatment had been applied. The relevant chemical was then added after the samples 1 and 2 had been taken after which a 25 min reaction time allowed. After this chemical treatment, the holding tank containing the treated water was opened and water was allowed to run through the UV system. A sample of the chemically treated water was then taken at point 3. Lastly a sample was taken at point 4, which was the water exposed to the combination treatment for chemical and UV light exposure (Fig 3.1b).

Water was sampled in sterilised bottles and was drawn from the sampling taps shown in Figures 3.1a and 3.1b. The bottles were kept in insulated cooler boxes to ensure that the water remained at lower temperatures (4-8°C) during transport to the laboratory where microbial analyses were performed.

Experiments were performed in late summer (Study 1), autumn (Study 2), winter (Studies 2 & 3) and early spring (Study 3) which can be considered highly variable in terms of rainfall since the Western Cape falls within a winter rainfall area.

Pilot-scale MP UV treatment

Experiments were performed using a Berson InLine 40+ UV disinfection system (Berson, The Netherlands). This utilises a B810H medium-pressure (MP) UV lamp installed perpendicular to the flow of water in the piping network. Light was emitted in the range of 220 to 580 nm.

On the day of each trial flow rates required to deliver the desired UV doses were calculated with reference to the UV transmission percentage (UVT%) of the river water at the time. The computerised UV system, having an advanced in-line sensor, allowed the operator to adjust the flow rate, in units of $m^3.h^{-1}$, and quantified the delivered UV dose in the desired units of $mJ.cm^{-2}$. The flow rate was adjusted on the digital interface of the UV system in order to establish the value that corresponded to the respective UV doses. The system, however, was not capable of automatically regulating the set flow rate and this was performed manually by manipulating a valve installed in the piping system before the UV lamp. The flow rate was measured by means of an in-line floater device and a scale with units of LPM. Thus, the set flow rate shown on the display of the computer system was converted from $m^3.h^{-1}$ to LPM and adjusted sequentially to expose the water to the predetermined doses.

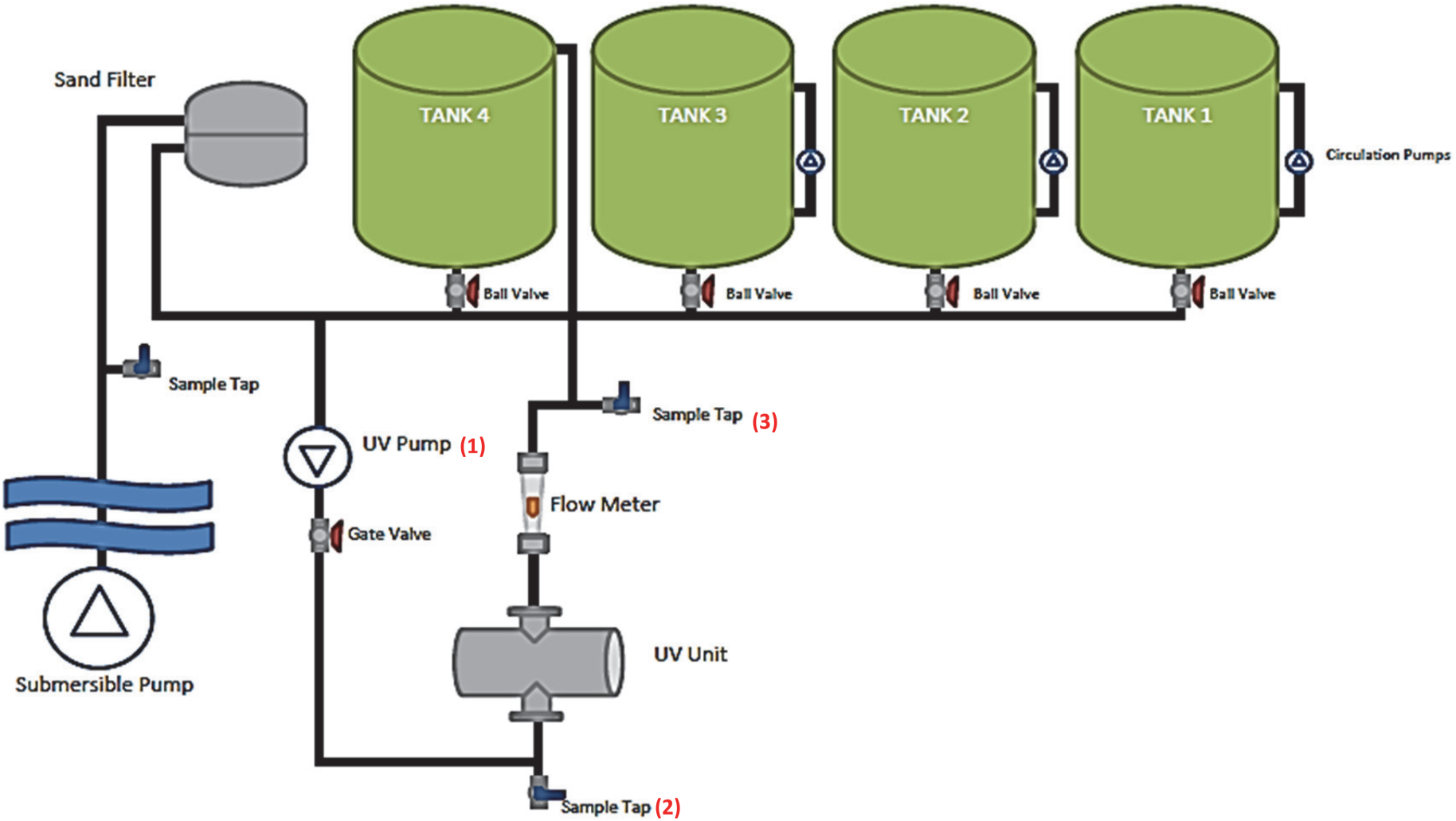


Figure 3.1a. Diagrammatic representation of the pilot-scale MP UV system used in this study, with sampling points for Studies 1 and 2.

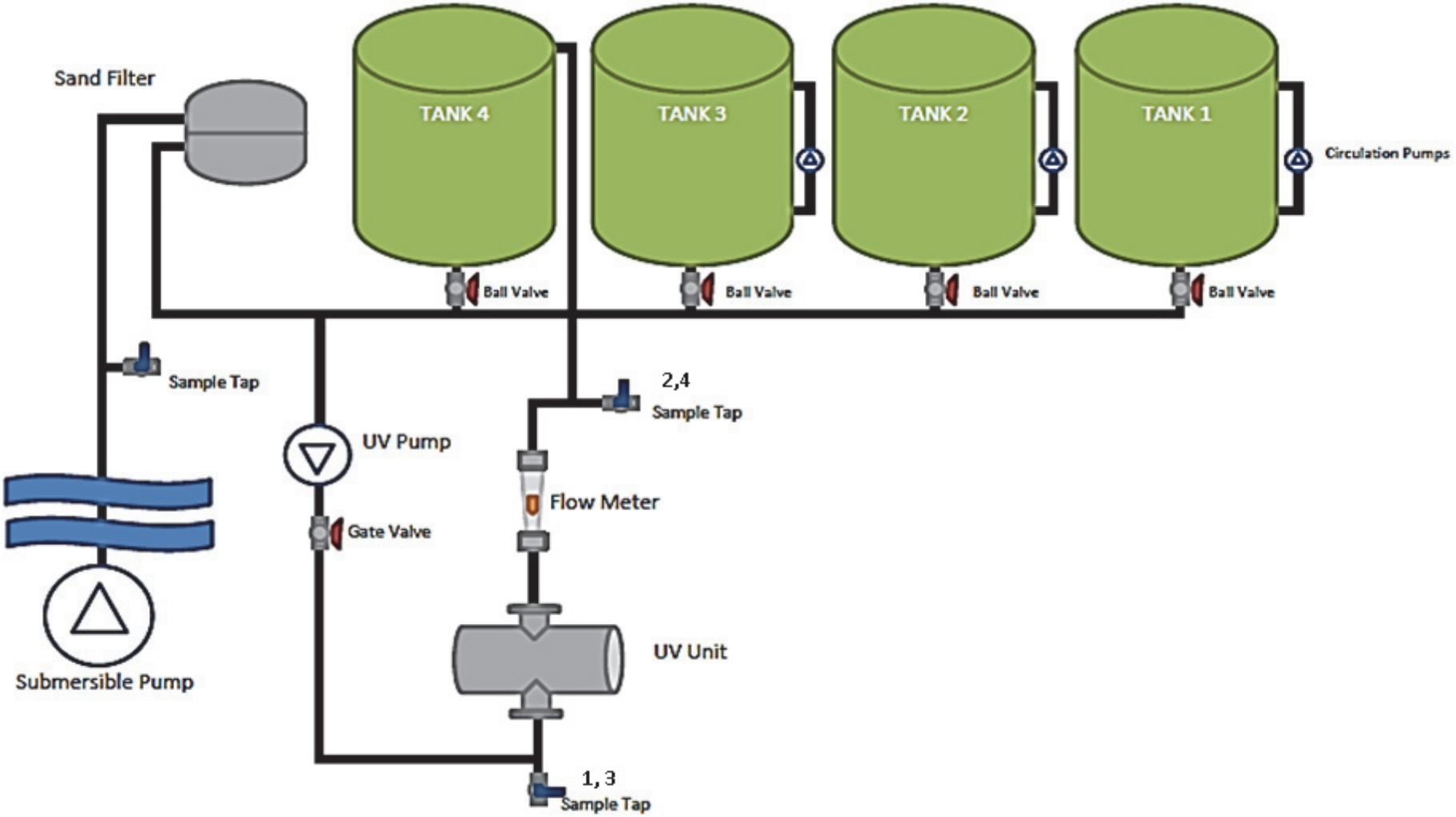


Figure 3.1b. Diagrammatic representation of the pilot-scale UV system used in this study, with sampling points for Study 3.

Photo-repair following MP UV irradiation (Studies 2 and 3)

The potential for photo-repair (photo-reactivation) following specific UV treatments was investigated using a closed system and light emitted at an intensity of 3.5 kLux as measured using a portable Jaz spectrometer (Ocean optics, USA). For this purpose, two 10 W fluorescent lamps (STR-GX3006A, C10W, Eurolux, South Africa) were mounted on the top section of a closed container (Fig. 3.2). The UV irradiated water samples were exposed to the fluorescent light in 500 mL glass beakers while being agitated using a magnetic stirrer and bar. Water temperature was maintained at 23 ± 1°C throughout the period of allowed for reactivation.

Regrowth was expressed in terms of log-reactivation and the percentage recovery was calculated using the following equation as defined by Lindenauer & Darby (1994):

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

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

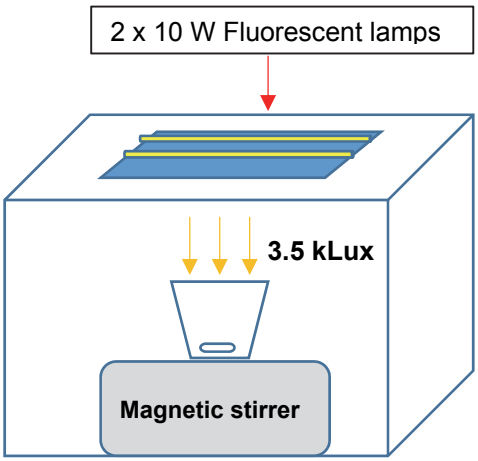


Figure 3.2 UV irradiated water samples exposed to fluorescent light in a closed container.

Treatment Solutions

XY-12®, a liquid sodium hypochlorite (NaOCl) sanitiser representative of 8% (m.v^{-1}) available chlorine (Ecolab, South Africa) were used for chlorine disinfection. A commercial form of PAA was used: Tsunami 100, composed of 31% acetic acid, 15% peroxyacetic acid and 11% hydrogen peroxide (Ecolab, South Africa). Sterile sodium thiosulfate ($Na_2S_2O_3$, 1% (m.v^{-1})) was used after each time interval according to the method of Mazzola *et al*., (2006) to quench the residual action of PAA and NaOCl before plating. For the treatments involving the use of hydrogen peroxide (H_2O_2), pre-calculated volumes of a 30% (v.v^{-1}) H_2O_2 solution (Merck, South Africa) was added to the tanks.

Microbiological analysis

Before (control) and after all specific disinfection and photo/dark-repair experiments a dilution series (10^0-10^{-6}) was prepared. Enumeration procedures were performed subsequently. For each treatment dilution series were performed in triplicate.

Total and Faecal coliform enumeration

Total coliforms (TC) and faecal coliforms (FC) were enumerated according to the South African National Standards (SANS) method 4832 (SANS, 2007a). Violet Red Bile Agar (VRBA) (Merck, South Africa) was used to prepare duplicate pour plates which were inverted and incubated at 35 and 44°C for 24 h to determine TC and FC, respectively.

Aerobic colony count (ACC)

The total aerobic population was enumerated according to the SANS method 4833 (SANS, 2007b). Duplicate pour plates were prepared using Plate Count Agar (PCA) (Merck, South Africa), inverted and incubated at 30°C for 48 h.

Water quality analysis

Physicochemical parameters of river water were determined prior to treatments according to *Standard Methods* (APHA, 2005). These included chemical oxygen demand (COD), total suspended solids (TSS), volatile suspended solids (VSS), alkalinity, conductivity and water temperature and pH. A DR2000 spectrophotometer (Hach, USA) was used to measure COD and results were expressed as mg $O_2.L^{-1}$. A portable HI 8733 conductivity meter (Hanna Instruments, USA) was used to measure conductivity in units of $mS.m^{-1}$. The UV transmission percentage (UVT%), turbidity and total dissolved solids (TDS) content of the water were also determined. Furthermore, total and faecal coliforms and the total ACC population were enumerated in order to quantify the level of microbiological contamination occurring in the untreated river water. Following these analyses, the estimated values were compared to guidelines set by DWAF (1996) for water intended to be used for the irrigation of fresh produce (Table 3.1).

Ultraviolet transmission percentage (UVT%)

The UVT% of river water samples was determined using both a UVT-15 UV% Transmission Photometer (HF Scientific, USA) as well as a hand held Sense^{TM} T UV-Transmittance Monitor (Berson, The Netherlands). Deionised water was used for calibration, representing a UV transmission of 100%.

Turbidity

The turbidity of untreated river water was determined as Nephelometric Turbidity Units (NTU), using an Orion AQ3010 Turbidity Meter (Thermo Scientific, USA), as described by the manufacturer. Solutions of known turbidity were used to verify that the instrument was calibrated.

Total Dissolved solids (TDS)

The TDS content of water samples were calculated from the measured values for conductivity using the following equation (DWAF, 1996):

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

Table 3.1 Limits for physicochemical and microbiological qualities of water intended for the irrigation of fresh produce as suggested by DWAF (1996).

Water quality parameter	Guideline limit
Faecal coliforms	1 000 cfu.100 mL^{-1}
Chemical Oxygen Demand (COD)	NS
Conductivity	40 $mS.m^{-1}$
pH	6.5-8.4
Total Suspended Solids (TSS)	50 $mg.L^{-1}$

NS – Not Stipulated

3.3 Research study design (Pilot plant studies)

In **Study 1** (*MP UV*) the effect of MP UV irradiation on highly contaminated river water was investigated. The only pre-treatment applied was a commercially available sand filter (filtering down to *ca*. 100 μm). River water was irradiated using doses of 13, 17, 24 $mJ.cm^{-2}$. Samples were taken before (Fig. 3.1a point 2) and after (Fig. 3.1a point 3) UV treatment where after TC, FC, and the ACC were enumerated to determine the log-inactivation achieved at the respective doses. The results of microbiological and water quality analyses were compared to guidelines for fresh produce irrigation water quality set by DWAF (1996) (Table 3.1) and were used to interpret the effectiveness of the respective treatments. For each trial the experimental procedure was performed in triplicate.

In **Study 2** the potential of photo (*UV photo-repair*) and dark-repair (*UV photo + dark-repair*) following MP UV irradiation were investigated. River water was irradiated using doses of 13, 17, 24, 40 and 2 x 20 $mJ.cm^{-2}$ (sequential irradiation at 20 $mJ.cm^{-2}$) , respectively, and exposed to different conditions of light and dark in order to observe and analyse possible regrowth.

3.3.1 Study 2.1: Photo-repair following pilot-scale MP UV irradiation (UV photo-repair)

The potential for photo-repair (photo reactivation) was investigated following pilot-scale (MP) UV disinfection. River water was irradiated at UV doses of 13 and 24 $mJ.cm^{-2}$, transferred to 500 mL glass beakers (one for each dose) and subsequently exposed to artificial light at an intensity of 3.5 kLux. The samples were stirred using a magnetic stirrer and bar (Fig. 3.2). Total coliforms (TC) were enumerated before (time -1.0 h) and after (time 0 h) UV treatment and after 1, 3 and 5 h of exposure to the fluorescent light. A control sample (untreated river water) was handled in the same manner. Regrowth was expressed in terms of log-reactivation and the percentage recovery was calculated.

3.3.2 Study 2.2: Photo and dark-repair under varied conditions following pilot-scale MP UV disinfection (*UV photo + dark-repair*)

The effect of an increased MP UV dose and a modified irradiation technique (compared to Study 2.1(*UV photo-repair*)) on the extent of photo-repair were investigated. Also, the potential for dark-repair was evaluated following the respective treatments. River water was irradiated using UV doses of 1 x 40 and 2 x 20 $mJ.cm^{-2}$, respectively. These treatments were chosen in order to evaluate the effect of using multiple UV lamps (simulated by the 2 x 20 $mJ.cm^{-2}$ treatment) in

comparison to the use of one stronger lamp (simulated by the 40 mJ.cm^{-2} treatment) to optimise disinfection and minimise the potential for regrowth following larger-scale UV disinfection. The irradiated samples were exposed to the following conditions post UV treatment: Artificial light as in Study 2.1 (*UV photo-repair*)(3.5 kLux); ambient light (inside laboratory); complete darkness (closed container). For the first condition (3.5 kLux light) samples were treated as described in Study 2.1 (*UV photo-repair*). The irradiated river water was also exposed to the lighting found in the laboratory which was a combination of artificial and natural lighting with intensity in the range of 1.0 to 2.0 kLux (measured using the Jaz spectrometer). River water was transferred to 500 mL beakers (one for each dose) and left on a work-bench inside the laboratory. To investigate dark-repair, water was again transferred to 500 mL beakers which were moved to a closed container, resulting in complete darkness. Total coliforms were again enumerated before (time -1.0 h) and after (time 0 h) the UV treatments and 1, 3 and 5 h following exposure to the different conditions described above. In all instances, regrowth was expressed as log-reactivation and the percentage recovery (for light and dark-repair) was calculated using the equation defined by Lindenauer & Darby (1994).

In **Study 3** (*UV + UV/Chemicals*) the efficacy of UV light, chemical treatment and the combination thereof for the disinfection of microbiologically polluted river water at a pilot-scale level were investigated. In the following trials, river water was exposed to the minimal UV dose that could be achieved at the given day due to variations in the water quality: UV doses of 18, 19 and 30 mJ.cm^{-2}.

The efficacy of Peracetic acid (PAA)(4 ppm), Chlorine (Cl)(3 and 6 ppm) and Hydrogen Peroxide (H_2O_2)(2.5 ppm) was investigated to determine the most effective thereof. A contact time of 25 min was allowed for each of the respective chemicals to react with the river water in the 2 500 L holding tanks before UV treatment and sampling took place.

In addition, the potential of photo-reactivation after chemical/UV combination treatments was also investigated for PAA, Cl and hydrogen peroxide (H_2O_2). For this purpose a UV dose of 30 mJ.cm^{-2} was used in all instances, while a contact time of 25 min was allowed for each of the respective chemicals. A standard recovery period of 3 hours was allowed after treatment to allow photo-recovery to occur.

3.3.3 Study 3.1: PAA and PAA/UV combination treatments (PAA + PAA/UV)

The river water was exposed to commercial PAA (Tsunami 100, Ecolab South Africa) at 4 ppm dose for 25 min and at a UV dose of 18 mJ.cm^{-2}, of which three repeats (trials) were done on three alternative days. The samples obtained in each trial was analysed in triplicate.

The effect of photo reactivation was also tested at 4 ppm dose for 25 min and at a UV dose of 30 mJ.cm^{-2}, of which two repeats (trials) were done on two alternative days. The samples obtained in each trial was analysed in triplicate.

3.3.4 Study 3.2: Cl and Cl/UV combination treatments (Cl + Cl/UV)

Various trials were also conducted where river water was exposed to a UV doses of 19 and 30 mJ.cm^{-2} at Cl (XY 12 – hypochlorite, Ecolab South Africa) concentrations of 3 and 6 ppm for 25 min. For the Cl 6ppm (25 min) and UV dose of 19 and 30 mJ.cm^{-2}, respectively, two trials were done on alternative days of each. For the Cl dose at 3 ppm (25 min) and UV dose of 19 and 30 mJ.cm^{-2}, respectively, one trial was completed of each UV dose on alternative days. (As mentioned, the UV dose applied in each trial was the minimum that was possible on any specific day due to the ever-changing river water quality.)

The effect of photo reactivation was also tested at 3 ppm dose (25 min) and at a UV dose of 30 $mJ.cm^{-2}$, of which two repeats (trials) were done on two alternative days. The samples obtained in each trial was analysed in triplicate.

3.3.5 Study 3.3: H_2O_2 and H_2O_2/UV combination treatments (H_2O_2 + H_2O_2/UV)

H_2O_2 as a viable option on its own for irrigation water treatment has been ruled out mostly as a result of the potential costs involved in treating large volumes at high concentrations. (The results of lab-scale studies done on the effect of H_2O_2 treatment alone on river water and *Escherichia coli* strains in saline are summarised in Appendix A4.1. The effect of UV / H_2O_2 combinations on *E. coli* strains in saline are summarised in Appendix A3). The effect that a combination of H_2O_2 and UV irradiation could have on the disinfection of river water (including the effect on photo-reactivation) was, however, investigated at pilot-scale. Disinfection was tested at a 2.5 ppm H_2O_2 dose (25 min) combined with a UV dose of 30 $mJ.cm^{-2}$, of which two repeats (trials) were done on two alternative days. The samples obtained in each trial was analysed in triplicate.

Sampling on the pilot-plant consisted of a control sample that was taken before UV treatment at sample point 1 (Fig 3.1b) before any treatments were performed on the river water, this was to establish the initial microbial load of the water. The river water was exposed to the same UV dose to be used on the chemically treated water to establish the individual effectiveness of the UV alone (Sample point 2, Fig 3.1b). Thereafter the required amount of chemical was added to the filled 2500 L holding tanks and allowed to react for 25 min while the tank contents was mixed continuously. After the reaction time was completed, the treated water was sampled at sample point 3 (Fig. 3.1b) before it passed through the UV system at the required UV dose. After UV treatment, a sample was drawn at sample point 4, representing water exposed to the combination treatment. The samples obtained in each trial was analysed in triplicate. A 1% Sodium thiosulfate solution (Merck, South Africa) was used to quench the action of the chemicals before microbial analyses were conducted. The response of the microbial population present in the water, following exposure to different UV treatments, chemical treatment and the combination of UV and chemical treatments was expressed in terms of log-inactivation.

4. RESULTS AND DISCUSSION

UV irradiation is a well-established alternative method of disinfection which is frequently used for the treatment of water and wastewater. It has increased in popularity in recent times because it is non-chemical, environmentally friendly technology (Hu *et al*., 2005). As method of water disinfection, UV irradiation is not entirely flawless. A major limitation of the process is the fact that no residual is offered, consequently providing bacteria with an opportunity to increase post-disinfection (Guo *et al*., 2011). In this regard, the ability of microorganisms to reverse UV-induced structural damage has been reported and extensively studied in the literature (Guo *et al*., 2009; Vélez-Colmenares *et al*., 2011). Two methods of repair have been identified. The first involve light-mediated photo-reactivation/photo-repair which occurs as a result of photolyase enzymes. The second repair mechanism is dark-repair, which could occur in the absence of light as a result of nucleotide excision repair (NER) performed by the UvrABC exinuclease (Truglio *et al*., 2006). Dark repair is generally considered to be significantly less influential, particularly over the short term (Guo *et al*., 2011).

Two types of UV lamps, low and medium-pressure mercury vapour lamps (LP and MP), are most frequently used and differ with regards to the wavelengths at which they emit light. Low-pressure lamps were traditionally used while MP UV lamps have only been developed in the last two decades. These emit light at 254 and a range of 200 to 400 nm, respectively (Poepping *et al*., 2014). In comparison to LP lamps, MP lamps also emit light at a higher intensity, consequently being more effective for the purpose of disinfection. As a result, the use of industrial UV disinfection systems utilising MP lamps have increased during recent times (Quek & Hu, 2008a). Several researchers have found MP UV light to be more effective in limiting photo-reactivation following disinfection, compared to LP UV irradiation (Oguma *et al*., 2002; Zimmer & Slawson, 2002; Kalisvaart, 2004; Hu *et al*., 2005; Quek & Hu, 2008a). As mentioned by Quek & Hu (2013) the former authors suggest that a possible explanation is the fact that MP UV lamps emit light of a broader spectrum. This implies that the irradiation may result in greater formation of dimers as well as additional damage to amino acids, important enzymes and possibly also photolyase. Low-pressure UV light, on the other hand, will primarily result in the formation of dimers due to its monochromatic emission at a wavelength of 254 nm (Quek & Hu, 2013).

The aim of the current study was to investigate the potential of MP UV irradiation for the decontamination of microbiologically polluted water from the Plankenburg River. Pilot-scale disinfection was followed by laboratory-scale experiments in which the influence of damage-repair (post-inactivation) was investigated. The series of studies focussed on: the effect of MP UV dose on the inactivation of the microbial population occurring in the Plankenburg River; the influence of water quality and microbiological properties on disinfection efficiency; the impact of photo and dark-repair on the efficacy of different MP UV doses and varied irradiation protocols.

4.1 Laboratory-scale studies

Results for laboratory-scale studies are described in Appendices A3-A5.

4.2 River water quality

The quality characteristics of water that was sampled from the Plankenburg River were evaluated before disinfection in each of the pilot-scale trials done in Studies 1-3. The microbiological and physico-chemical properties determined in Studies 1 and 2 are presented in Table 4.1 and 4.2. River water analyses done as part of Study 3 (*UV + UV/Chemicals*) are presented in Tables 4.3 & 4.4 (for physico-chemical properties) as well as in Table 4.5 (for microbiological properties).

Although these studies were conducted within a six month period, the large variations observed overall for important quality parameters such as the COD content (9-175 $mg.L^{-1}$) and

UVT% (35.1-71.3%), demonstrated the effect that environmental factors such as season and rainfall can have on water quality. Water was expected to be concentrated with reference to pollutants as the water level and flow-rate of the river was consistently low, at the beginning of Study 1 (summer). Incidentally, the UVT % values recorded were also the lowest of all the values recorded during Studies 1-3. After the first rains (autumn), the river water could possibly have a spike in pollutants as the runoff water entering the river system increased. With the increased flow rate of the river, settled debris and sediment could also be disturbed from the river bed. As there is a continuation of rainfall over a period of time the pollutants would become diluted and a decrease in pollution levels of the river system would be observed (winter). This was also demonstrated in the UVT% values that were recorded during Study 3 (*UV + UV/Chemicals)*, which were mostly above 50%. It must also be noted that if it sampling was done just after heavy rain, due to the land run-off, the river water would naturally be more turbid as more than usual amounts of soil will be carried by the water in the river. Seasonal variability in water quality should thus, be kept in mind when evaluating the efficacy of disinfection treatments.

The river water pH values recorded during all trails (pH 6.5-7.7) were all within the ”neutral” class 2 pH range (pH 6.5-8.4) specified by DWAF (1996), which implies that irrigation water would have no detrimental effect on crop quality and yield. Since a fairly neutral pH is also required for chlorine disinfection, the water pH was considered of acceptable standard for chlorine disinfection (Study 3.2 (*Cl + Cl/UV*)).

The conductivity measured during all trials conducted in Studies 1-3 were, except for Study 2.2 (*UV photo + dark-repair*) and Study 3 (*UV + UV/Chemicals*) Trials 2 and 11, above the DWAF target of 40 $mS.m^{-1}$ for irrigation water. The same was observed for other river water trials conducted as part of this study (Appendix Tables A3.2; A4.3.2; A4.4.2). Electrical conductivity is an indication of the presence of dissolved salts (containing carbonates, nitrates, chloride, sodium, potassium, etc.) in the water, all of which can carry an electrical current. Dissolved organic compounds will not affect conductivity as it does not dissociate into ions (DWAF, 1996).

Table 4.1 Water quality characteristics of water from the Plankenburg River before exposure to MP UV irradiation (Study 1 (*MP UV*)).

Trials	**1**	**2**	**3**
UVT%	36.0	35.1	37.6
COD ($mg.L^{-1}$)	96.30	46.80	63.00
Turbidity (NTU)	24.50	15.84	25.60
TSS ($mg.L^{-1}$)	29.00	18.00	25.00
VSS ($mg.L^{-1}$)	25.00	14.00	19.00
pH	7.23	7.42	7.29
Alkalinity (mg $CaCO_3.L^{-1}$)	118.00	118.00	131.00
Conductivity ($mS.m^{-1}$)	60.00	47.00	48.00
Total coliforms (TC) (log cfu.100 mL^{-1})	7.07	6.87	6.94
Faecal coliforms (FC) (log cfu.100 mL^{-1})	6.41	6.23	6.29
Aerobic colony count (ACC) (log cfu.100 mL^{-1})	7.94	7.68	8.75

Table 4.2 Water quality characteristics as measured for water from the Plankenburg River prior to performing photo-repair experiments (Studies 2.1 (*UV photo-repair*) and 2.2 ((*UV photo + dark-repair)*.

Studies	**Study 2.1**	**Study 2.2**
UVT%	49.9	50.0
COD ($mg.L^{-1}$)	27.70	18.90
Turbidity (NTU)	12.06	11.50
TSS ($mg.L^{-1}$)	11.00	15.00
VSS ($mg.L^{-1}$)	5.00	10.00
pH	7.32	7.75
Alkalinity (mg $CaCO_3.L^{-1}$)	118.75	112.20
Conductivity ($mS.m^{-1}$)	53.0	36.30
Total coliforms (TC) (log cfu.100 mL^{-1})	6.10	5.25

Table 4.3 Physico-chemical characteristics of river water measured before each of the combination treatment Trials (no. 1-9) in Study 3 (*UV + UV/Chemicals*). (Treatments that the water were subjected to in each trial is summarised in the Table subscript.).

Trials	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
UVT %	45.5	56.4	48.7	71.3	56.0	66.3	71.3	55.5	56.0
Turbidity (NTU)	8.20	9.50	12.66	6.80	7.22	7.25	6.80	7.43	7.22
Conductivity ($mS.m^{-1}$)	42	40	50	50	57	50	50	58	57
pH	6.6	6.6	7.3	7.3	7.3	7.5	7.3	7.3	7.3
Alkalinity ($mg.L^{-1}$ $CaCO_3$)	62.5	75	100	75	87.5	75	75	75	87.5
COD ($mg.L^{-1}$)	130	136	68	11	57	9	11	98	57
TSS ($mg.L^{-1}$)	8	10	15	3	9	4	3	8	9
VSS ($mg.L^{-1}$)	6	9	12	1	6	1	1	4	6
Chlorine residual	NA	NA	NA	<0.5	<0.5	1.42	1.62	1.63	1.54

Trial no: 1 – PAA (4 ppm)/UV 18 $mJ.cm^2$; 2 - PAA (4ppm)/UV 18 $mJ.cm^2$; 3 - PAA (4 ppm)/UV 18 $mJ.cm^2$;
4 – Cl (3 ppm)/UV 30 $mJ.cm^2$; 5 - Cl (3ppm)/UV 19 $mJ.cm^2$; 6 - Cl (6 ppm)/UV 30 $mJ.cm^2$;
7 - Cl (6ppm)/UV 30 $mJ.cm^2$; 8 - Cl (6 ppm)/UV 19 $mJ.cm^2$; 9 - Cl (6 ppm)/UV 19 $mJ.cm^2$;
Trial notes: Trials 4 & 7, and trials 5 & 9 were conducted on the same day

Table 4.4 Physico-chemical characteristics of river water measured before each of the combination and reactivation treatment Trials (no. 10-15) in Study 3 (*UV + UV/Chemicals*).

Trials	**10**	**11**	**12**	**13**	**14**	**15**
UVT %	57.4	51	44.7	50.2	44.7	57.4
Turbidity (NTU)	6.08	8.93	11.46	17.96	11.46	6.08
Conductivity ($mS.m^{-1}$)	57	35	46	50	46	57
pH	6.6	7.0	6.7	6.5	6.7	6.6
Alkalinity ($mg.L^{-1}$ $CaCO_3$)	50	75	175	57.5	175	50
COD ($mg.L^{-1}$)	38	14	59	175	59	38
TSS ($mg.L^{-1}$)	ND	ND	62	52	62	ND
VSS ($mg.L^{-1}$)	ND	ND	22	18	22	ND
Chlorine residual	0.49	0.77	NA	NA	NA	NA

Trial no. 10 – Cl (3 ppm)/UV 30mJ.cm^{-2}; 11 – Cl (3 ppm)/UV 30mJ.cm^{-2}; 12 – H_2O_2 (2.5 ppm)/UV 30mJ.cm^{-2}; 13 - H_2O_2 (2.5 ppm)/UV 30mJ.cm^{-2}; 14 – PAA (4 ppm)/UV 30mJ.cm^{-2}; 15 – PAA (4 ppm)/UV 30mJ.cm^{-2};
Trial notes: Trials 10& 15, and trials 12& 14 were conducted on the same day and all trials were subjected to 3 hours exposure to fluorescent light prior to UV treatment.

Table 4.5 Microbial load of river water measured before each of the combination and reactivation treatment Trials (no. 1-15) in Study 3 (*UV + UV/Chemicals*).

Trials	ACC	TC	FC
		$cfu.100mL^{-1}$	
1	7.6×10^5	2.9×10^4	1.2×10^3
2	1.7×10^6	6.9×10^4	1.4×10^3
3	6.2×10^5	5.4×10^4	3.8×10^3
4	1.53×10^5	1.58×10^4	2×10^3
5	5.8×10^5	5.1×10^4	1.8×10^4
6	3.2×10^5	3.3×10^4	2.9×10^3
7	1.53×10^5	1.58×10^4	2×10^3
8	3.3×10^5	4.7×10^3	1.9×10^3
9	5.8×10^5	5.1×10^4	1.8×10^4
10	2.1×10^5	3.35×10^4	3.1×10^3
11	6.4×10^4	3.52×10^4	7×10^3
12	2.8×10^6	7.7×10^5	1.5×10^5
13	1.4×10^6	1.57×10^5	1.53×10^5
14	2.8×10^6	7.7×10^5	1.5×10^5
15	2.1×10^5	3.35×10^4	3.1×10^3

Over the long-term the presence of dissolved salts in irrigation water can influence soil salinity profiles, which might in turn affect salt-sensitive crop yields. The river water of most trials did, however, fall within the 40-90 $mS.m^{-1}$ range which is considered acceptable for use on moderately salt-sensitive crops maintained with low-frequency irrigation (DWAF, 1996).

Alkalinity measurement is an indication of the capacity of water to neutralise acid. This buffer capacity is mostly the result of dissolved carbonates and bicarbonates. The alkalinity values for all Trials in Study 3 (*UV + UV/Chemicals*) except Trial 12 (&14) were within the acceptable range of 0-100 $mg.L^{-1}$ $CaCO_3$ (Tables 4.3 & 4.4). The buffer capacity of the river water used in

these trials were, in general, lower than that reported for the UV trials in Studies 1 & 2. This was probably due to seasonal variance. Laboratory-scale studies comparing PAA disinfection in river water and saline (alkalinity = 12mg.L^{-1} $CaCO_3$) (Appendix Table A4.2.1; Figures A4.2.4 & A4.2.5) did indicate that alkalinity might have a negative influence on the disinfection efficiency of very low concentrations of PAA. Later lab-scale studies on river water disinfection did however indicate that COD rather than alkalinity might be the main limiting factor (Appendix Table A4.4.2; Fig. A4.4.3).

When considering Study 3 (*UV + UV/Chemicals*) Trials 1, 2, 5 (&9), and 8 it was observed that COD did not necessarily influence UVT %. These water samples all had similar UVT % values (55.5-56.4%), but differed significantly with regard to their COD content (57-136 mg.L^{-1}). The same was also true for Study 1 (*MP UV*) Trials 1, 2 and 3, which had very little variation in UVT% values (35-37%) combined with larger variations in COD content (46-96 mg.L^{-1}). There is currently no guideline stipulating acceptable COD values for agricultural irrigation water. However, in terms of revisions made to the national water act (DWA, 2013) regarding disposal of waste water either through land irrigation or through discharge into a natural water source, maximum COD levels have been specified as COD< 75 mg.L^{-1}. The fact that the COD levels of the river was, in some trials, above these levels would imply that the waste streams entering the river, whether from point or non-point sources, and were above legal limits.

In contrast to the large variations observed in COD content, Study 3 (*UV + UV/Chemicals*) Trials 1, 2, 5 (& 9), and 8 also had fairly similar TSS values, which indicated that the COD content was influenced more by the dissolved contents that the suspended (microbial) matter. Similar observations were made for Study 3 (*UV + UV/Chemicals*) Trials 12 (&14) and 13, where no correlation between UVT%, COD values and TSS content could be seen.

In Study 1 (*MP UV*) the results of the microbial analyses showed that extremely high levels of TC, faecal coliforms (FC) and heterotrophic bacteria were present in the water on each of the respective days (Table 4.1). Even for FC, the size of the population always exceeded 1 000 0000 cfu.100 mL^{-1} (6 log) with the maximum (6.41 log cfu.100 mL^{-1}) observed in Trial 1. In terms of faecal coliform levels the water did not comply with limits set for water intended to be used for fresh produce irrigation (DWAF, 1996) (Table 3.1). Total coliforms (TC) and the Aerobic colony count (ACC) reached maximum levels of 7.07 (Trial 1) and 8.75 log (Trial 3) cfu.100 mL^{-1}, respectively, again indicating the severity of microbial contamination in the water (Table 4.1). Only TC were measured as part of the photo repair trials in Study 2.1 (*UV photo-repair*) and Study 2.2 (*UV photo + dark-repair*) (Table 4.2). Log values were slightly lower to that observed for TC in Study 1. In the trials done as part of Study 3 (*UV + UV/Chemicals)*, microbial analyses (Table 4.5) indicated that ACC, TC and FC levels were lower than that determined for Studies 1 and 2 (UV photo-repair). This was attributed to seasonal variance, as microbial levels could have been lowered as a result of pollutant dilution during rainy spells.

Limitations on microbial growth occurring in sediments in winter time as a result of lower temperatures might also have had an influence. It was, however observed that even in Study 3 (*UV + UV/Chemicals)*, FC levels were still above the acceptable limits for irrigation of fresh produce. This was in agreement with those of other researchers who reported that the Plankenburg River is extensively contaminated with faecal bacteria (Paulse *et al*., 2009; Ackerman, 2010; Huisamen, 2012).

In Study 1 (*MP UV*) it was found that reductions of up to 3.41 log would be required to yield microbiologically acceptable irrigation water. In support of this, Britz *et al*. (2013) recommended a target reduction of 3 to 4 log based on an investigation of microbiological contamination of Western Cape Rivers and the limits set by DWAF (1996) and WHO (1989). The urgent need for an affordable, safe and effective method of water disinfection is thus apparent.

4.3 Study 1: Microbial disinfection of river water with MP UV irradiation (*MP UV*)

As mentioned in Section 4.1, the UV transmission was poor throughout all three the trials conducted as part of Study 1 (*MP UV*), with a maximum of 37.60% detected in Trial 3. In view of the small UVT% differences between trials (35 - 37.6%) it was expected that on each of the days of treatment, similar efficiency of MP UV irradiation would be observed. Laboratory-scale studies done on the same water samples under low pressure (LP) UV irradiation at doses of 5, 7 and 10 mJ.cm^{-2} proved to provide inadequate log reductions (Appendix figures A3.9-A3.11; Table A3.4). Since LP UV irradiation has some technical limitations for industrial applications, more efficient commercially available MP UV irradiation was applied in the pilot-scale studies at higher doses. The effect that MP UV irradiation had on microbial levels were determined at doses of 13, 17 and 24 mJ.cm^{-2}.

The log reductions obtained in microbial numbers during the three trials conducted as part of Study 1 (*MP UV*) are presented in Figures 4.1, 4.2 and 4.3. A summary of the maximum log reductions achieved in all trials are presented in Table 4.6 (at the highest dose tested) and Table 4.7 (for FC at all doses)

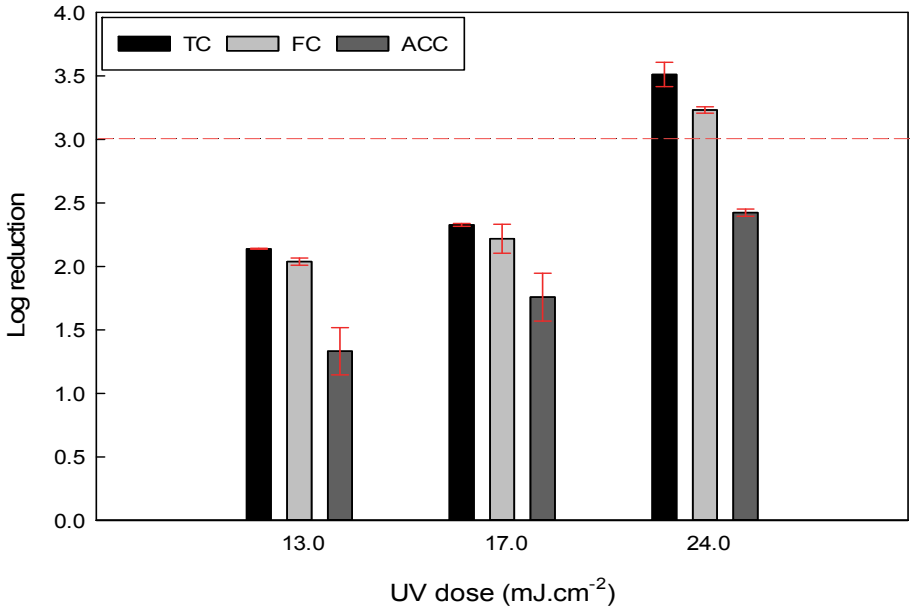


Figure 4.1 Reductions achieved for TC, FC and ACC following pilot-scale MP UV irradiation at three doses (13, 17 and 24 mJ.cm^{-2}) during experimental Study1 (*MP UV)*, Trial 1. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

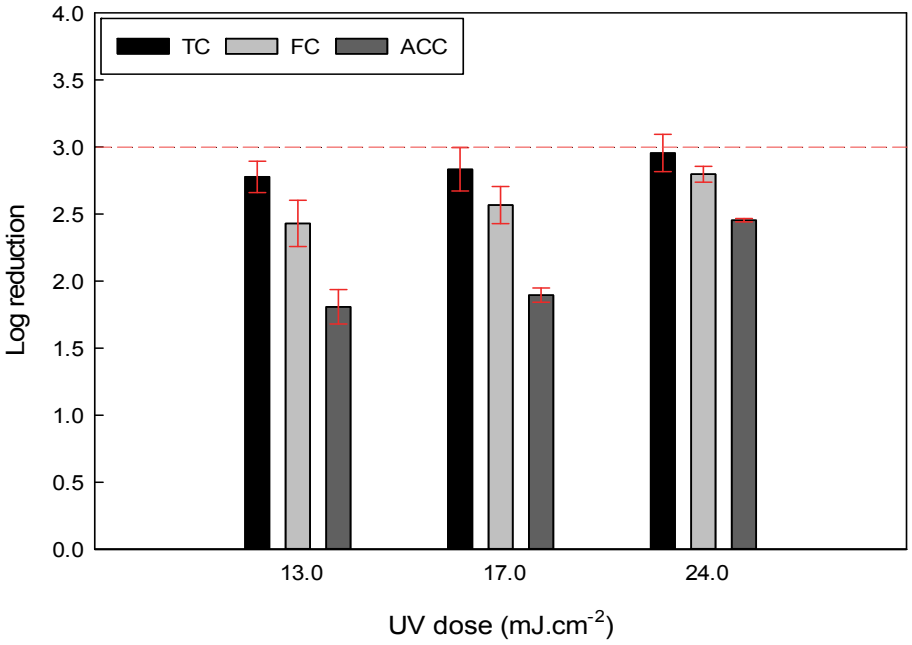


Figure 4.2 Reductions achieved for TC, FC and ACC following pilot-scale MP UV irradiation at three doses (13, 17 and 24 mJ.cm^{-2}) during experimental Study 1 (*MP UV)*, Trial 2. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

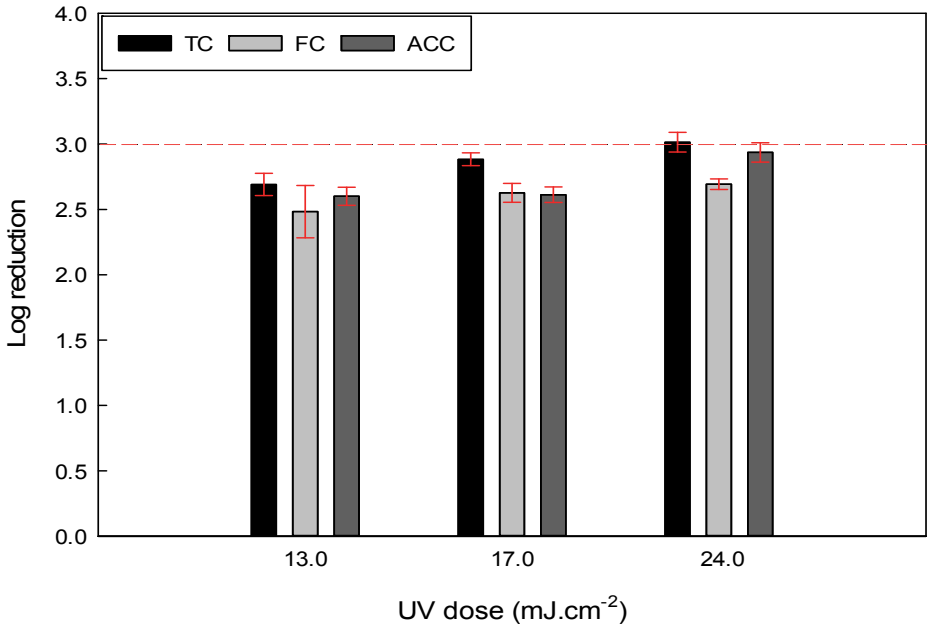


Figure 4.3 Reductions achieved for TC, FC and ACC following pilot-scale MP UV irradiation at three doses (13, 17 and 24 mJ.cm^{-2}) during experimental Study 1 (*MP UV)*, Trial 3. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

Table 4.6 Log reductions (± SD) achieved for TC, FC and ACC following MP UV treatment at a dose of 24 mJ.cm^{-2} during three respective trials.

	Maximum reduction (Log)		
Microorganisms	**Trial 1**	**Trial 2**	**Trial 3**
TC	3.51±0.10	2.96±0.14	3.01±0.07
FC	3.23±0.03	2.74±0.04	2.69±0.04
ACC	2.42±0.03	2.45±0.01	2.94±0.07

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

Table 4.7 Levels of FC (± SD) detected in river water following MP UV irradiation at three doses.

	Log cfu.100 mL^{-1}		
UV dose	**Trial 1**	**Trial 2**	**Trial 3**
13 mJ.cm^{-2}	4.37±0.05	3.80±0.17	3.81±0.22
17 mJ.cm^{-2}	4.19±0.09	3.67±0.14	3.67±0.06
24 mJ.cm^{-2}	3.21±0.01	3.44±0.04	3.60±0.04

The results presented in Figures 4.1, 4.2 and 4.3 indicates that microbial inactivation was enhanced as the UV dose was increased from 13 to 24 mJ.cm^{-2}. This effect was more pronounced in the first experimental Trial (Fig. 4.1) than in Trials 2 and 3 (Figures 4.2, 4.3). In Trial 1 a UV dose of
24 mJ.cm^{-2} resulted in statistically significant ($p<0.05$) log reductions that were 1.37, 0.89 and 1.09 log greater than those achieved at 13 mJ.cm^{-2}. Maximum reductions were recorded as 3.51, 3.23 and 2.42 log, respectively, for TC, FC and ACC (Table 4.6).

The effect of elevating the applied UV dose in Trial 2 was less prominent than in Trial 1 (Fig. 4.2). Although higher reductions were achieved at 13 and 17 mJ.cm^{-2} compared to that seen in Trial 1, no significant differences ($p>0.05$) in log reductions of the enumerated groups (TC, FC and ACC) were observed at 17 mJ.cm^{-2} in comparison to 13 mJ.cm^{-2}. Furthermore, the difference in reductions achieved at the highest (24 mJ.cm^{-2}) and lowest (13 mJ.cm^{-2}) were also not significant for TC ($p=0.09$). As observed in Trial 1, the ACC population was the most resistant group (although more sensitive at 13 and 17 mJ.cm^{-2} compared to ACC in Trial 1) with significantly lower log reductions ($p<0.05$) observed for ACC in comparison to TC and FC. As observed in Trial 1, log reductions achieved for TC and FC were in some instances not statistically different ($p>0.05$).

Similar to observations made in Trial 2, slight differences in reductions were recorded for TC, FC and ACC in Trial 3, following irradiation at 13, 17 and 24 mJ.cm^{-2} (Fig. 4.6). At a dose of 24 mJ.cm^{-2}, the mentioned groups were reduced by 3.01, 2.69 and 2.94 log, respectively (Table 4.6). This was significantly higher ($p<0.05$) than observed at 13 mJ.cm^{-2}. At the maximum dose (24 mJ.cm^{-2}) reductions achieved in Trial 3 were very similar to that achieved in Trial 2, with the exception of ACC. A significant difference ($p<0.05$) of 0.49 log in reduction of the latter group was observed, with the treatment in Trial 3 being more effective. In contrast to Trials 1 and 2, the total ACC population was not the most resistant in this trial (Trial 3). At doses of 13 and 24 mJ.cm^{-2} ACC were reduced to a greater extent than FC, while similar reductions were achieved at a dose of 17 mJ.cm^{-2} for the respective groups (FC and ACC) (Fig. 4.3). The TC population was again the most sensitive.

The differences in UVT% recorded prior to each of the three trials were very small (Table 4.1). Even if it could have influenced the UV irradiation efficacy the fact that the Berson InLine 40+ UV system accounted for any variability in UVT% (defined as a function of the absorption coefficient) it can be said with certainty that the same UV dose was delivered in each of the respective trials. It was thus expected that similar reductions would be observed between trials. This was not the case as significant differences ($p<0.05$) in log reductions were recorded for the different populations in some instances (Table 4.6). This indicated that, irrespective of the applied UV dose, additional factors may impact the efficiency of UV disinfection. Specifically, the influence of water quality and microbiological properties may be important.

In terms of the influence of water quality it is important to consider that particulate substances have been suggested to influence UV disinfection efficiency as a result of "shading" (which include light scattering, refraction or reflection) (Walters *et al*., 2014). A phenomenon known as encasement has also been shown to be influential. Incidentally, research has identified a positive correlation between the size and concentration of suspended particles and the efficiency of UV irradiation in contaminated water (Whitby & Palmateer, 1993; Örmeci & Linden, 2002). In this regard, microbial populations are well-known to be able to associate with particles occurring in their surrounding environment (Fries *et al*., 2008; Droppo *et al*., 2009). When associated with particles, microorganisms may be better protected compared to those freely suspended in the water. The former may obtain valuable nutrients from the substances to which they adhere while also being protected from a range of environmental stress factors (Sinton *et al*., 1999; Davies & Bavor, 2000).

Walters *et al*. (2014) evaluated the effect of particle association and suspended solids on the inactivation of faecal bacteria using UV light. Their work identified a reversed correlation between the total suspended solids (TSS) concentration and the rate of bacterial inactivation. Also, *E. coli* which were associated with smaller particles (particle diameter (d_p) ≤ 12 µm) were inactivated two times faster in comparison to those attached to particles with size $12 < d_p \leq 63$ µm. In the current study, the greater reductions achieved for TC and FC in Trial 1 (Table 4.6) could, however, not be related to lower levels of suspended solids (Table 4.1). Furthermore, the effect of TSS could also not be observed when comparing reductions achieved in Trials 2 and 3. Even though the TSS concentration was 7 $mg.L^{-1}$ lower in Trial 2 than 3, very similar reductions were achieved for TC and FC, respectively (Table 4.6).

UV efficiency has also been discussed with reference to the influence of turbidity. It is stated that high turbidity levels may contribute to absorption and, importantly, blocking of UV light. Pathogens may consequently be protected against the harmful UV rays (Jones *et al*., 2014). Nonetheless, the correlation between UV efficiency and the turbidity level in water is inconsistent. This results from the fact that substances which contribute to turbidity represent highly variable properties in terms of UV blocking and absorption (Jones *et al*., 2014). Generally, however, increased levels of turbidity result in lower efficiency of UV disinfection (Spellman, 2003; Qian, 2011). In this study, the influence of turbidity on UV lethality could also not be clearly observed. The water treated in Trial 2 was representative of the lowest turbidity (15.84 NTU), as well as TSS, yet reductions achieved for TC, FC and ACC, respectively, were never significantly higher ($p<0.05$) in comparison to the other days. Also, while the water treated in Trial 1 was very turbid (24.50 NTU), and representative of the highest TSS concentration (29.00 $mg.L^{-1}$), significantly higher log reductions ($p<0.05$) were reached for TC and FC in comparison to that achieved in Trials 2 and 3.

From these results it is evident that the study of the influence of scattering or blocking effects (imparted by particulate material) is more complex than simply referring to differences in concentrations of the influential particles. In this regard, it is apparent that it was difficult to correlate the effectiveness of the respective treatments with variation in water quality in Trials 1 to 3. Owing to this, and the fact that UV transmission was fairly similar throughout the days of

disinfection, it might be that the differences in water quality were too small to have a clearly visible influence on UV disinfection. The impact of the characteristics of the microbial community occurring in the river water (on each day) may, however, be of great importance. While the level of microbial contamination of the water may vary daily, the presence or absence of particularly resistant strains or species may greatly influence disinfection efficiency.

It is well-known that the effectiveness of UV disinfection is largely dependent on the extent of DNA damage induced by the treatment, as well as the degree of subsequent DNA repair (López-Malo & Palou, 2004). DNA damage and repair, in turn, are influenced by environmental, process and microbial factors which may be prominent prior to, during or following UV irradiation (Gayán *et al*., 2014). As discussed, it was difficult to correlate the physico-chemical properties of the water with UV lethality. However, owing to the fact that the water was sampled from the Plankenburg River (on different days) it was certain that the representative microbial populations were diverse. Both intrinsic and extrinsic microbial factors could, therefore, affect the treatment efficiency (Gayán *et al*., 2014). Intrinsic factors refer to properties such as genetic material conformation and cell and genome size, while extrinsic factors refer to influences from outside, including growing conditions, growth phase, environmental stressors and conditions for recovery. Variation in UV resistance will, therefore, vary between microorganism type, species and strains present in the treated substance (Gayán *et al*., 2014). This is clearly observed when looking at the results presented in Figures 4.3 to 4.5 and Table 4.3.

For TC, FC and the total aerobic population variation in reductions were observed between different days (trials). For TC and FC, specifically, better inactivation at a dose of 24 $mJ.cm^{-2}$ were observed in Trial 1, compared to that recorded for Trials 2 and 3 (Table 4.6). The total aerobic population, however, was maximally inactivated in Trial 3. Thus, while the coliform community in Trial 1 was the most sensitive, the total ACC population in the same sample was the most resistant in comparison to other days of treatment (Table 4.6). It is, therefore, evident that within the same water sample great variation in the UV sensitivity of different types of microorganisms may be observed. Nonetheless, coliforms irradiated in Trials 2 and 3 showed greater resistance to UV light (in comparison to those in Trial 1), as was the case for the total ACC population in Trials 1 and 2 (compared to that in Trial 3) (Table 4.6). These populations possibly represented greater levels of intrinsic resistance to the harmful effects of UV irradiation if the influence of water quality was not to be considered.

On the other hand, extrinsic factors could also have contributed to the differences in UV resistance of the microbial groups. While actively growing microorganisms are more sensitive to UV light, stimulation of RpoS (s^{38}) (a stress response factor) may impart added resistance if co-protective reactions are instigated (Child *et al*., 2002; Berney *et al*., 2006; Bucheli-Witschel *et al*., 2010; Van der Veen & Abee, 2011). In this regard, the extent of pollution contributed by waste from informal settlements and industrial operations may vary on a day-to-day basis. It is therefore possible that the differences in the observed reductions achieved for TC, FC and ACC, respectively, (on different days of treatment) may be attributed to variation in the amount and type of pollution.

Referring to the effect of MP UV irradiation at a dose of 24 $mJ.cm^{-2}$ it was observed that the heterotrophic bacteria population was more resistant in comparison to coliforms in each of the respective trials (Table 4.6). This was expected as ACC is inclusive of all bacteria (WHO, 2003). In a review of the literature, Gayán *et al.* (2014) states that vegetative bacteria are most sensitive to UV disinfection, followed by yeast cells, spores of bacteria, viruses and lastly protozoa. In this regard, factors such as cell size and pyrimidine levels within the microbial DNA become important (Oteiza *et al*., 2010; Fredericks *et al*., 2011; Gabriel, 2012). Also, it has been stated that gram-positive bacteria show increased resistance in comparison to the gram-negatives (Gayán *et al*., 2014). It was therefore expected that the ACC population in the river will be more resistant than

the coliforms since the former will always include some of the more resistant microorganisms mentioned above. As confirmation, Britz *et al*. (2013) reported that samples taken from the Plankenburg River frequently tested positive for the presence of *Staphylococcus* and *Listeria* spp., which are more resistant, gram-positive bacterial species. Although the guideline limit for safe irrigation of fresh produce only include acceptable levels for faecal coliforms, it is important to note that levels of important food pathogens such as *Staphylococcus,* and *Listeria* spp are not addressed in current guidelines. This is of particular concern, as organisms such as *Listeria* spp also has very low infective doses.

The results in Table 4.6 also demonstrate that FC were reduced to a lesser extent in comparison to TC. However, the difference in the log reductions achieved for these two groups at 24 mJ.cm^{-2} were, in most instances, statistically insignificant ($p>0.05$). Considering the guideline limit suggested for FC (Table 3.1), and the final concentrations recorded after UV irradiation trials 1, 2 and 3 (Table 4.7) UV irradiation were not sufficient in rendering the water acceptable for fresh produce irrigation. Although the suggested 3 log target reduction was met in some instances (Table 4.7), the extreme levels of faecal contamination observed at the start of each trial (Table 4.1) were too high to allow for sufficient reductions.

Limited research has been published regarding the use of MP UV irradiation in river water disinfection. Quek & Hu (2008a) reported that MP UV doses ranging from 4.5 to 9.0 mJ.cm^{-2} were required to produce a 4 log reduction of *E. coli*. The experiments were, however, conducted using seeded *E. coli* strains and sterile distilled water, which explains the greater lethality observed in comparison to that of the current research. Guo *et al*. (2009), however, reported a reduction of < 2.5 log when TC were enumerated following MP UV irradiation of a wastewater sample at 15 mJ.cm^{-2}. This result exemplifies the restraining influence of water quality (and the characteristics of a naturally occurring microbial population) on the efficiency of UV disinfection and was more in line with that found in this investigation.

4.4 Study 2.1: Photo-repair following pilot-scale MP UV irradiation (*UV photo-repair*)

Ultraviolet light is widely acknowledged and used as method of water disinfection, mainly due to its known effectiveness against a range of pathogenic microorganisms without leading to the formation of harmful disinfection by-products (Linden *et al*., 2003; Locas *et al*., 2008; Vélez-Colmenares *et al*., 2011; Guo *et al*., 2013). However, UV-induced DNA damage may be repaired by microorganisms through the action of photolyase in the process of photo-reactivation, which was investigated in the current study. The phenomenon is well-known and has been thoroughly scrutinized by various researchers. The majority of studies, however, were conducted on laboratory-scale using low-pressure (LP) mercury vapour lamps (Guo *et al*., 2011; Hu *et al*., 2012; Guo *et al*., 2012; Guo *et al*., 2013). The significance of the current research is therefore immense, as the effect of photo-reactivation was investigated on pilot-scale using MP equipment. This scenario is more representative of a UV disinfection system that would typically be used for surface water disinfection on larger scale. In this regard, some researchers have investigated photo-repair following MP UV irradiation of *E. coli* or coliform bacteria (Oguma *et al*., 2002; Quek & Hu, 2008a & B; Guo *et al*., 2009; Poepping *et al*., 2014; Bohrerova *et al*., 2015).

In this study regrowth after UV irradiation was investigated and recorded in terms of percentage log recovery. Investigating photo-repair of the TC population was credible since the majority of the group is representative of the faecal coliforms (*E. coli*) subgroup. This is seen in Table 4.6 where the difference in the initial concentrations of TC and FC detected in the river water (Trials 1 to 3) was in the range of 0.64 to 0.66 log cfu.100 mL^{-1}.

The data presented in Figure 4.6 show TC cell concentrations before (-1.0 h) and after (0.0 h) MP UV disinfection at doses of 13 and 24 mJ.cm^{-2}, respectively. The period 0.0 to 5.0 h represents exposure to photo-reactivating light at an intensity of 3.5 kLux.

Disinfection efficiency

Reductions of 3.30 and 3.36 log, respectively, were achieved when river water was irradiated with MP UV light at 13 and 24 mJ.cm^{-2} (Fig. 4.4). Although the conductivity was higher than the allowed limit set by DWAF (1996) (Tables 3.1 and 4.2), the quality of water in this study was generally better (i.e. higher UVT %; lower TSS, VSS, COD, Turbidity) than reported in Study 1 (*MP UV*).

Owing to improved water quality, the better disinfection observed in this study (compared to the previous report) was expected. While the same UV dose was always delivered, lower levels of interfering substances probably resulted in a lesser degree of light scattering and blocking, consequently resulting in greater inactivation. Also, a larger proportion of the microbial population might have been free-floating, rather than being associated with particles in a biofilm. Suspended microorganisms would be more susceptible to the harmful effects of UV irradiation. It is also possible that the TC population in this study was inherently more sensitive to UV irradiation in comparison to that encountered in Study 1 (*MP UV*). River water characteristics, in terms of the type and levels of contaminants being present, could also have influenced the UV sensitivity of the population due to its effect on microbial growth and the expression of the RpoS (s^{38}) stress response factor.

Photo-reactivation following UV irradiation

The plots presented in Figure 4.4 clearly show that upon exposure to visible light, UV inactivated coliforms were able to regenerate and repopulate the water in which they were initially active. Following 5 h of exposure to 3.5 kLux light, the TC population size reached 4.41 and 3.93 log cfu.100 mL^{-1} in water irradiated with UV doses of 13 and 24 mJ.cm^{-2}, respectively (Fig. 4.4). This equated to significant increases ($p<0.05$) of 1.62 and 1.19 log. Clearly, the level of photo-repair was significantly greater ($p<0.05$) following irradiation at the lower UV dose (13 mJ.cm^{-2}) (Fig. 4.4). The control in this experiment was untreated (non-irradiated) river water which was exposed to identical conditions used to evaluate photo-repair, i.e. 5 h exposure to 3.5 kLux fluorescent light. A statistically insignificant increase ($p=0.40$) in population size (0.05 log cfu.100 mL^{-1}) was observed over the 5 h period, signifying that growth in the two irradiated samples principally resulted from light-induced DNA repair.

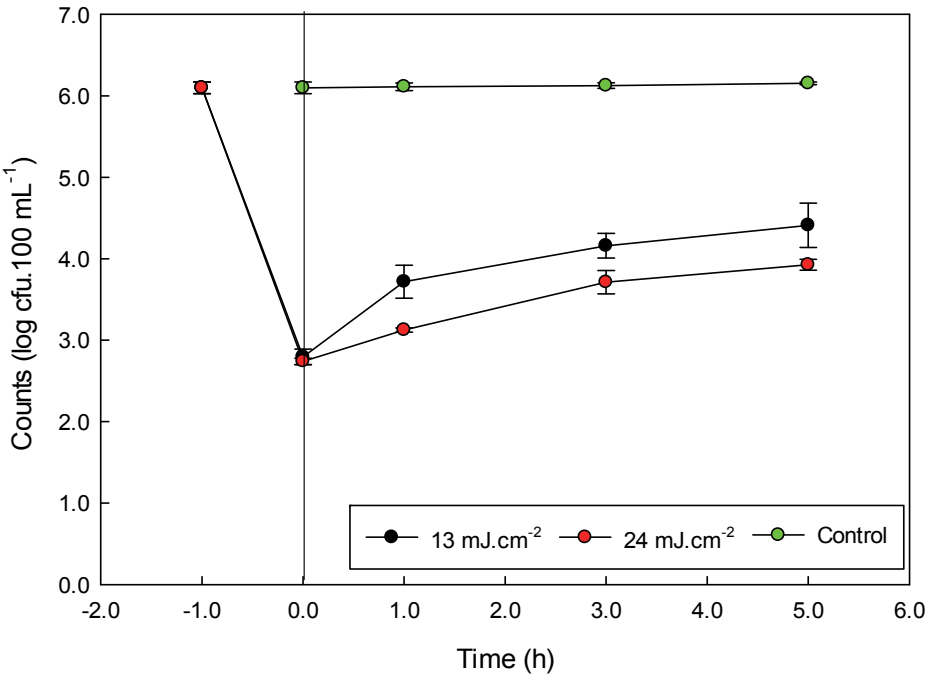


Figure 4.4 Total coliform (TC) population size before and 1, 3 and 5 h after exposure to photo-reactivating light following MP UV irradiation. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

The data presented in Figure 4.5 show the percentage log recovery recorded for TC in two test samples following 1, 3 and 5 h of exposure to photo-reactivating light. In water exposed to a MP UV dose of 13 mJ.cm^{-2} recovery reached 49.18% after 5 h. The corresponding value for water irradiated at 24 mJ.cm^{-2} was 35.37% (Fig. 4.5). For the first sample (13 mJ.cm^{-2}) it was seen that the greater part of the total reactivation occurred within the first hour of exposure to visible light. After 1 h, 28.08% of the inactivated coliforms were again active. After 3h and 5 h additional recovery of 13.39% and 7.72%, respectively, were observed. For the 24 mJ.cm^{-2} sample, however, photo-reactivation was slightly delayed with only 11.45% of the microorganisms being revived within the first hour. This is also clear in Figure 4.5 which show a slighter gradient for the 24 mJ.cm^{-2} sample in the time interval 0 to 1 h compared to that represented by the 13 mJ.cm^{-2} sample. Additional regrowth of 17.62 and 6.30% was measured between hours 1 to 3 and 3 to 5, respectively. Note that the bars denoted 1, 3 and 5 h in Figure 4.5 show total reactivation at each point in time and not the individual contributions during the different time intervals mentioned above. The results discussed here show that the higher UV dose constantly (following each time interval) represented significantly lower ($p<0.05$) levels of total microbial regrowth (Fig. 4.5).

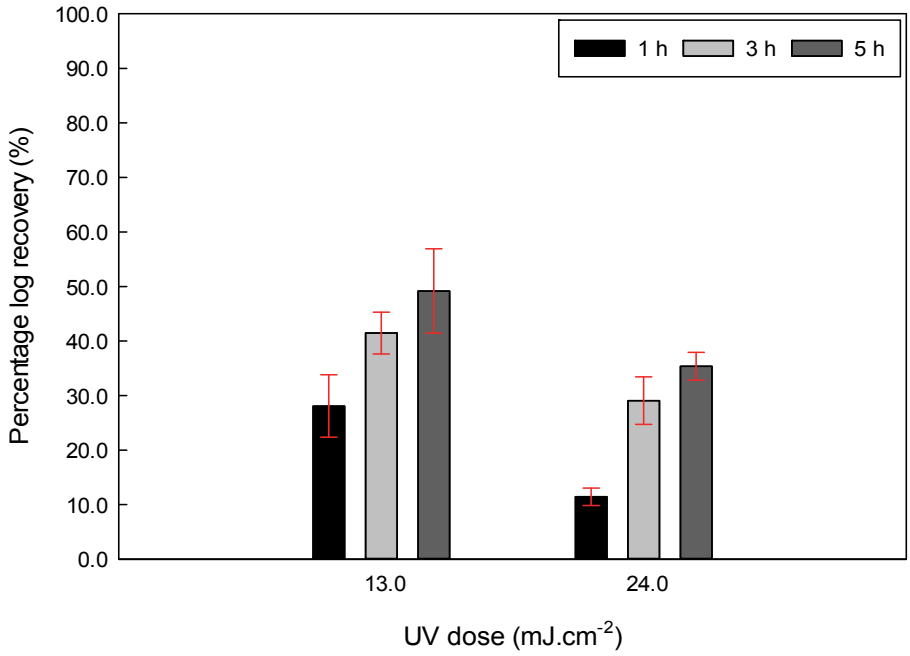


Figure 4.5 Percentage log recovery of TC after 1, 3 and 5 h of exposure to photo-reactivating light following two MP UV treatments. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

As is evident in Figure 4.5, significantly lower percentage log recovery was observed when the UV dose was increased from 13 to 24 mJ.cm^{-2} ($p<0.05$). This was in agreement with Guo *et al*. (2009) who also reported that higher UV doses resulted in a lesser degree of photo-reactivation. They suggested that the increased formation of pyrimidine dimers (at higher doses) may lower the extent of repair that could occur within a specific period of time. Furthermore, Quek *et al*. (2006) evaluated photo-repair of *E. coli* following MP UV irradiation at doses in the range of 1.6-19.7 mJ.cm^{-2}. Again, it was reported that lower degrees of photo-reactivation were observed when the UV dose was higher.

In addition to DNA damage, i.e. the formation of pyrimidine dimers, the extent of photo-reactivation in MP UV disinfection, specifically, may be related to the condition of the photolyase enzyme. It has been reported that the latter contains a cofactor (flavin adenine dinucleotide (FAD)) which shows meaningful absorbance at a wavelength of 280 nm (Harm, 1980). If FAD absorbed UV photons, potential damage to the photolyase enzyme could result in impaired photo-repair potential. In order to evaluate the effect of MP UV irradiation on the activity of endogenous *E. coli* photolyase Hu & Quek (2008) exposed the enzyme to doses ranging from 10 to 40 mJ.cm^{-2}. It was shown that with an increase in UV dose a consequent decrease in dimer repair rate could be observed. This implies that, within a given space of time, higher UV doses could result in lower degrees of total reactivation.

As discussed earlier, it was observed for the 13 mJ.cm^{-2} sample that the major part of photo-reactivation (28.08%) occurred within the first hour of exposure to fluorescent light (Fig. 4.5). In the following hours, levelling off of the regrowth curve was observed (Fig. 4.4). This effect was less pronounced for the 24 mJ.cm^{-2} treated sample with only 11.45% reactivation being observed in the first hour (Fig. 4.5). Also, the representative curve in Figure 4.6 only began to slightly level off in the 3 to 5 h time interval. This is consistent with other researchers who have also reported that most of the photo recovery occurs within the first hour (Quek & Hu; 2008b).

Any increase observed in microbial population size following UV irradiation could, however, also be attributed to elements other than photo-repair. As suggested by Guo *et al*. (2011) the

following three factors are of particular importance: normal growth of unharmed microorganisms; photo-repair of the damaged microorganisms; and normal growth of the rejuvenated microorganisms. Although the influence of the latter could not be quantified, normal growth of unharmed microorganisms served as control in this experiment. Since a very slight increase (0.05 log) was observed in this sample, the influence thereof on the total percentage recovery was very small. Thus, one can clearly observe the impact that photo-reactivation may have on the final concentrations of indicator bacteria in UV disinfected waters. The results of the current study serve as a very applicable example. Each of the two UV doses (13 & 24 $mJ.cm^{-2}$) were capable of reducing the initial TC population (6.10 log $cfu.100\ mL^{-1}$) to within the limit set for FC in water used for fresh produce irrigation (3 log $cfu.100\ mL^{-1}$) (Fig. 4.4) (Table 3.1). However, within the first hour of exposure to visible light the TC population, in both samples, exceeded the mentioned limit. This is worrisome since FC represent the major portion of the TC population and is expected to react in similar fashion since the two groups were similarly affected by UV irradiation in Study 1 (*MP UV)*.

Furthermore, Quek & Hu (2008b) found that with an increase in light intensity, the extent of photo-reactivation was enhanced. They also evaluated the effect of actual sunlight on photo-repair. Even at low light intensity (< 5 kLux) photo-reactivation of more than 60% was recorded. Again, when sunlight intensity was higher, even greater recovery was seen. This observation is important as it has been reported that the intensity of sunlight can reach 100 kLux in tropical regions (Neppolian *et al*., 2002). These results thus indicate that the phenomenon of photo-repair could present serious implications for UV disinfection of irrigation water in South Africa.

Considering this, it is clear that the number of variables influencing photo-reactivation can be vast and the comparison of data therefore becomes a tedious task. Factors such as light intensity, wavelength and temperature, for example, are well-known to influence the repair of DNA damage performed by the photolyase enzyme (Bohrerova & Linden, 2006; Hu & Quek, 2008). With regards to the literature, most researchers made use of pure *E. coli* strains and media such as buffered saline or sterilised water when investigating photo-repair. In the current study, nonetheless, river water and its actual TC population were utilised for this purpose. Additional variability, referring to the influence of water quality and the diversity of the TC population, consequently becomes important.

In this regard, Guo *et al*. (2009) evaluated inactivation and photo-repair of TC in wastewater from different origins using MP UV doses up to 40 $mJ.cm^{-2}$ and photo-reactivating light produced by a 20 W sunlight lamp. Although they did not report on the intensity of the light, it was expected to be comparable to that produced by the two 10 W fluorescent lamps used in the present study. It was found that a MP UV dose of 15 $mJ.cm^{-2}$ was sufficient to restrain photo-reactivation to < 10%, irrespective of water quality. The results in Figure 4.4, however, show that even at a dose of 24 $mJ.cm^{-2}$, greater reactivation of TC was observed in this investigation. It could be possible that the study conducted by Guo *et al*. (2009) showed a lower degree of photo-reactivation as a result of the better general quality of the treated water in comparison to that shown in Table 4.2. For instance, they recorded UV transmission up to 76% while the turbidity of the samples never exceeded 6.70 NTU. The difference in results may also be attributed to microbial factors as some coliform species may show greater UV resistance and/or greater levels of photolyase activity compared to others. It is thus clear that a variety of environmental factors can influence UV disinfection potential and subsequent photo-repair. These should be considered carefully when suggesting parameters.

Nevertheless, in this study photo-reactivation was investigated under optimal conditions of lighting. To obtain a more realistic understanding of photo-repair it was important to determine the impact thereof under less optimal conditions. Also, it was important to determine whether the release of nutrients from UV-damaged microbial cells could possibly promote the growth of living microorganisms which could incorrectly be attributed to photo-repair.

4.5 Study 2.2: Photo and dark-repair under varied conditions following pilot-scale MP UV disinfection (*UV photo + dark-repair*)

The effect of increased UV dosage and a varied treatment technique on the potential of photo-reactivation were investigated. River water was exposed to MP UV doses of 40 mJ.cm^{-2} and half of that in a sequential manner (2 x 20 mJ.cm^{2}), respectively. Irradiated samples were subjected to photo-reactivating light as in Study 2.1 (*UV photo-repair*)(3.5 kLux) and were also kept in the dark for the same period (5 h). Furthermore, photo-reactivation under less-optimal conditions (lower light intensity) was investigated by exposing the irradiated water samples to the ambient light found in the laboratory. The latter represented intensity in the range of 1.0 to 2.0 kLux.

Photo-reactivation under 3.5 kLux light: 40 mJ.cm^{-2} vs 2 x 20 mJ.cm^{-2}

The results presented in Figure 6 show that, regardless of more extreme UV irradiation (compared to Study 2.1(*UV photo-repair*), inactivated coliforms were again capable of recovering upon exposure to fluorescent light. The TC population reached concentrations of 3.49 and 3.30 log cfu.100 mL^{-1}, respectively, following the 40 and 2 x 20 mJ.cm^{-2} treatments and exposure to 3.5 kLux light. Log recovery equated to 0.81 and 0.52 units for the respective treatments. The sequential treatment regime was slightly more effective in suppressing photo-reactivation (Fig. 4.6).

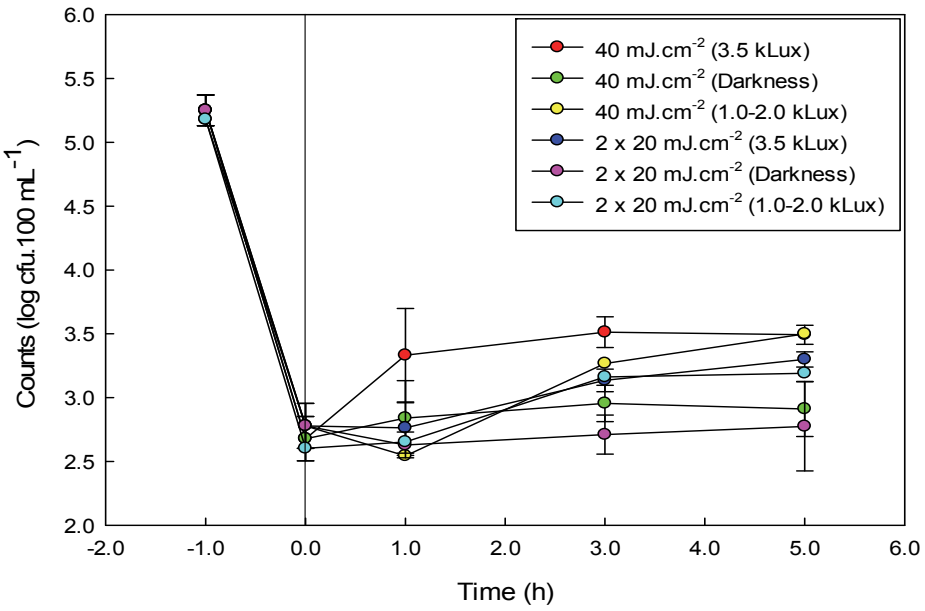


Figure 4.6 Total coliform (TC) population size before (Time = -1.0), directly after (Time=0.0) and 1, 3 and 5 h after exposure to different conditions of light and darkness following two respective MP UV treatments. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

Figures 4.7 & 4.8 show the percentage recovery achieved under the different test conditions. Upon exposure to 3.5 kLux light, recovery of 31.55 and 20.88% were achieved within 5 h in the 40 and 2 x 20 mJ.cm^{-2} irradiated samples, respectively. The latter thus repressed photo-repair to a

greater extent with a significant difference ($p<0.05$) of 10.67% being observed between the two treatments.

For the 40 mJ.cm^{-2} treatment, rapid reactivation within the first hour was observed and resulted in recovery of 25.81%. Thereafter, the recovery rate stabilised with additional recovery of 6.67% occurring in the 1 to 3 h period (Fig. 4.7). During the 3 to 5 h period no significant recovery ($p=0.78$) occurred.

For the 2 x 20 mJ.cm^{-2} treatment, reactivation of TC was delayed and was only observed after 1 h (Fig. 4.8). Recovery of 14.39% was observed between 1 and 3 h, with an additional 6.49% recorded during the 3 to 5 h interval. These results indicate that the rate of dimer repair was much lower following the 2 x 20 mJ.cm^{-2} treatment compared to the 40 mJ.cm^{-2}dose. The sequential treatment technique therefore showed disinfection efficiency equivalent to a dose exceeding 40 mJ.cm^{-2} as Hu & Quek (2008) reported that increased UV doses resulted in decreased dimer repair rates. However, it was observed that the two treatments resulted in very similar initial reductions of the TC population (Fig.4.8). Values of 2.57 and 2.47 log were recorded for the 40 and 2 x 20 mJ.cm^{-2} treatments, respectively, which were not significantly different ($p>0.05$). The lower degree of photo-reactivation observed following the latter treatment (2 x 20 mJ.cm^{-2}) could thus be related to the effect of UV irradiation on photolyase as the difference in initial reductions were statistically insignificant. In other words, the similar reductions imply that the two treatments (40 and 2 x 20 mJ.cm^{-2}) resulted in comparable levels of DNA damage, while the difference in their effects on photolyase was more significant.

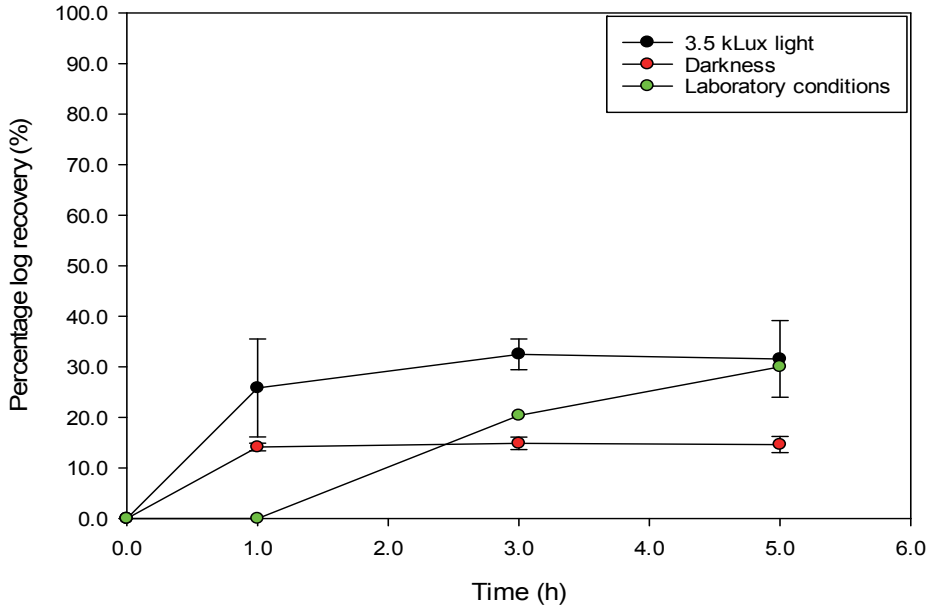


Figure 4.7 Percentage log recovery of TC with time of exposure to different conditions of light and darkness following MP UV treatment at a dose of 40 mJ.cm^{-2}. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

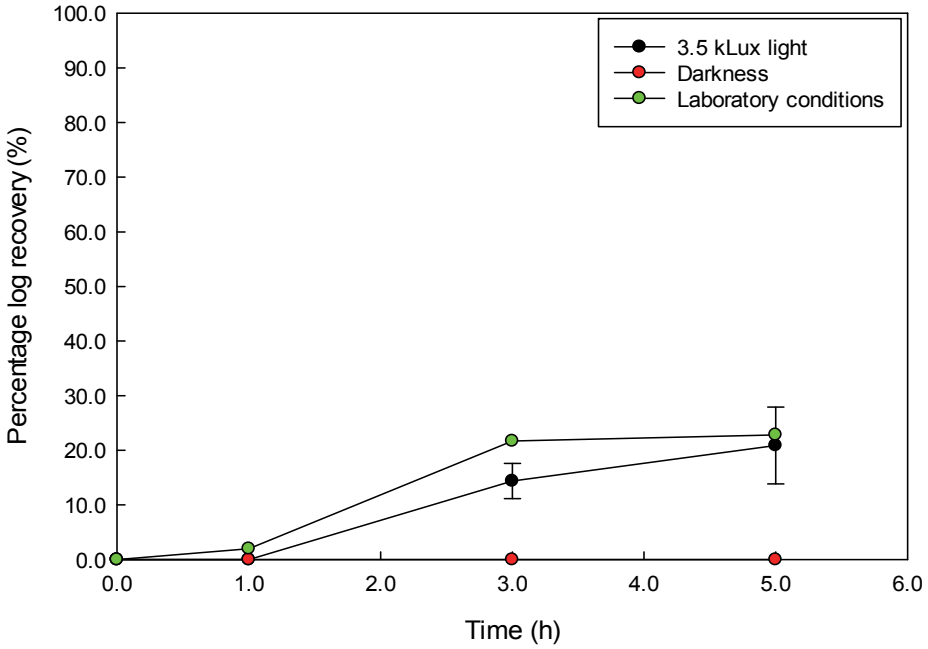


Figure 4.8 Percentage log recovery of TC with time of exposure to different conditions of light and darkness following MP UV treatment at two successive doses of 20 mJ.cm^{-2}. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

Photolyase in known to show high absorption of UV light at wavelengths of 280 and 384 nm (Hu & Quek, 2008). This could lead to reversible (structural) damage and irreversible damage (by means of oxidation) which would both result in decreased dimer repair ability (Hu & Quek, 2008). It is possible that the 2 x 20 mJ.cm^{-2} treatment resulted in greater photolyase damage (compared to the 40 mJ.cm^{-2} treatment), consequently explaining the lower rate of repair. Taking the effect of photo-repair into account, effective reductions of 1.76 and 1.95 log for the same treatments were, respectively, achieved. In this sense, the 2 x 20 mJ.cm^{-2} treatment was slightly more effective.

Effect of increased UV dose on photo-reactivation potential

In comparison to the 24 mJ.cm^{-2} treatment (Study 2.1 (*UV photo-repair*)), lower degrees of photo-repair was observed following both treatments (40 and 2 x 20 mJ.cm^{-2}) and exposure to 3.5 kLux light in the present study. Photo-reactivation was 3.82% and 14.49% lower following 5 h of incubation for the 40 and 2 x 20 mJ.cm^{-2} samples, respectively, compared to the 24 mJ.cm^{-2} sample. Only the 2 x 20 mJ.cm^{-2} sample had significantly lower repair ($p<0.05$) compared to the 24 mJ.cm^{-2} treatment after 5 h. These results were in agreement with that found previously as greater repair was observed after the 13 mJ.cm^{-2} treatment than after 24 mJ.cm^{-2} in Study 2.1 (*UV photo-repair)*. Interesting, however, is the fact that the significantly higher UV doses utilised in Study 2.2 (*UV photo + dark-repair*) produced initial log reductions that were only 0.79 (40 mJ.cm^{-2}) and 0.89 (2 x 20 mJ.cm^{-2}) log lower compared to that reached at 24 mJ.cm^{-2} in Study 2.1 (*UV photo-repair*) (3.36 log). This was not expected as intensified UV irradiation was utilised while water quality in Study 2.2 (*UV photo + dark-repair*) generally compared well (and in terms of COD, was better) to that reported for Study 2.1 (*UV photo-repair*) (Table 4.2).

In terms of UV lethality then, it was observed that within the TC population present in the Plankenburg River, a small fraction of the group always showed extreme resistance to MP UV irradiation. Irrespective of the UV dose, or the quality of the treated water, a magnitude of 5 to 15 viable cfu's were always detected in the undiluted sample following UV irradiation. Seeing that the

initial TC population size in Study 2.2) *(UV photo + dark-repair)* (5.25 log cfu.100 mL^{-1}) (Table 4.2) was lower than that in Study 2.1 (*UV photo-repair*) (6.10 log cfu.100 mL^{-1}) (Table 4.2) the lower reductions were expected. Again, these results suggest that the restraining effect of higher UV doses on photo-recovery could be related to its influence on the photolyase enzyme, rather than on the genetic material of the irradiated microorganisms. In this regard, taking photo-reactivation into account, the effective reductions achieved at 40 and 2 x 20 $mJ.cm^{-2}$ were 0.41 and 0.22 log lower, respectively, compared to the 24 $mJ.cm^{-2}$ treatment used earlier. Nevertheless, the important fact is that increased UV doses resulted in lower percentages of repair under the same experimental conditions. This was true for both the 40 and 2 x 20 $mJ.cm^{-2}$ treatments in comparison to the 24 $mJ.cm^{-2}$ treatment used in Study 2.1 (*UV photo-repair)*. However, from these results it can be concluded that the sequential treatment technique, in particular, offers increased effectiveness, possibly due to increased damage of the photolyase enzyme.

Looking at literature, results of previous studies are often contradictory to that observed here. Guo *et al*. (2009) reported that a MP UV dose of 40 $mJ.cm^{-2}$ was sufficient to restrain photo-reactivation of TC in wastewater to below 1%. They suggested that such a dose resulted in the formation of dimers to an extent that could prevent repair within a specific time. Also, Hoyer (1998) found that the same dose (40 $mJ.cm^{-2}$) was sufficient to entirely prevent photo-repair. Other researchers have also reported that minimal photo-repair was observed when MP UV disinfection was employed (Oguma *et al*., 2001; Zimmer & Slawson, 2002). Nonetheless, Bohrerova *et al*. (2015) investigated photo-reactivation of two *E. coli* strains in sterilised drinking water and treated wastewater effluent. The authors reported that maximum recovery (0.7 log) was recorded in the wastewater following MP UV treatment at 40 $mJ.cm^{-2}$. This result was more in line with that observed in the current study as recovery of 0.81 log was observed following treatment at the same dose. Nonetheless, although it is often suggested in literature, it is evident that even high MP UV doses may not be entirely capable of preventing the phenomenon of photo-reactivation.

Effect of light intensity on photo-reactivation

According to Guo *et al*. (2013) limited work has been done to investigate the effect of light intensity on the extent of photo-repair. Nevertheless, the degree of photo-reactivation induced by lowering the light intensity (laboratory conditions) in the current study again resulted in meaningful regrowth (Fig. 4.8). In fact, following the 5 h incubation period, the TC population reached concentrations of 3.49 and 3.19 log cfu.100 mL^{-1} for the 40 and 2 x 20 $mJ.cm^{-2}$ treated samples, respectively. Evidently, it was only for the 2 x 20 $mJ.cm^{-2}$ sample that light of higher intensity (3.5 kLux) resulted in slightly greater (0.11 log) regrowth. Nevertheless, the differences in the percentage log recovery following 5 h incubation under 3.5 and 1.0 to 2.0 kLux light were statistically insignificant ($p=0.55$ and $p=0.40$) for both of the respective treatments (40 and 2 x 20 $mJ.cm^{-2}$). As was observed for samples exposed to 3.5 kLux light, the 2 x 20 $mJ.cm^{-2}$ treatment again prevented photo-reactivation (at 1.0-2.0 kLux) to a greater extent than observed for the 40 $mJ.cm^{-2}$ treatment (Fig. 4.6).

As indicated in Figures 4.7 & 4.8, photo-reactivation under laboratory lighting showed somewhat different trends over a 5 h period in comparison to that observed under 3.5 kLux light. This was expected as it was suggested by Hallmich & Gehr (2010) that factors such as light source, the type of UV lamp used and the species of microorganisms present, for instance, may influence the initiation of photo-repair. It is therefore quite clear that the phenomenon of photo-reactivation cannot be solely reported on a time-basis as several factors will influence the progression of the process.

For the sample treated at 40 $mJ.cm^{-2}$, repair at ambient light was slower with no reactivation observed in the first hour (Fig. 4.7). After 3h and 5h, recovery of 20.36% and 9.62%, respectively, were observed and a maximum of 29.98% was reached (Fig. 4.7). Thus, even though it was

slightly delayed total photo-reactivation was very close to the 31.55% reached under 3.5 kLux light. A similar observation was made by Quek & Hu (2008b) who evaluated the effect of light intensity on the reactivation of *Escherichia coli*. The authors reported that photo-reactivation occurred at a faster rate when fluorescent light intensity was increased. Since photo-repair is a light-mediated, enzymatic process it was expected that increased light intensities would result in greater reactivation (Quek & Hu, 2008b). Also, it was reported that this effect was only observed up until a certain intensity, indicating that MP UV irradiation additionally induced irreversible damage in microbial DNA (Quek & Hu, 2008b). Since the 31.55% reactivation (under 3.5 kLux light) was referred to as the maximum achievable recovery, it was expected that further increases in intensity would not result in greater reactivation in the present study. Thus, while the lower intensity tested here resulted in slower photo-reactivation, the observed maximum was very nearly reached.

For the 2 x 20 mJ.cm^{-2} treated sample, photo-reactivation under laboratory light (1.0 to 2.0 kLux) was more rapid than that observed under 3.5 kLux light. The major part of reactivation (19.71%) occurred during the 1 to 3 h period where after recovery stabilized. In the 3 to 5 h period additional reactivation of only 1.13% was recorded and a total of 22.82% was reached (Fig. 4.8). Although this was slightly higher than the 20.88% reached in the same sample exposed to 3.5 kLux light, the difference in percentage log recovery reached after 5 h under the different conditions of light, however, was not statistically significant ($p=0.40$). The result reported here was not expected and contradicts the work of previously mentioned researchers. It is thus possible that the effects of the 40 and 2 x 20 mJ.cm^{-2} treatments on photolyase differed so that the importance of light intensity on reactivation rate was more profound after the 40 mJ.cm^{-2} treatment. The degree of photo-reactivation can be influenced by many factors, including temperature, the time and intensity of light exposure, UV dose and UV lamp type (Kashimada *et al*., 1996; Oguma *et al*., 2002; Zimmer & Slawson, 2002; Salcedo *et al*., 2007; Hu & Quek, 2008).

Dark-repair following UV irradiation

In addition to the effect of photo-reactivation, it has also been reported that UV induced damage in microorganisms may be reversed by means of dark-repair mechanisms (Jungfer *et al*., 2007; Locas *et al*., 2008; Guo *et al*., 2011). As opposed to photo-reactivation, dark-repair is described as a light-independent process in which numerous enzymes are coordinated to remove DNA damage (Friedberg *et al*., 1995; Locas *et al*., 2008; Guo *et al*., 2013). The influence thereof, however, is generally regarded as being less important compared to that of photo-reactivation over short term (Guo *et al*., 2011).

According to Bohrerova *et al*. (2015) dark-repair is a complex process which is difficult to investigate as a result of its delayed occurrence post UV irradiation. This implies that it might be challenging to distinguish between normal regrowth and actual dark-repair. Nevertheless, the most frequently described and encountered dark-repair mechanism is termed nucleotide excision repair (NER) (Zimmer-Thomas *et al*., 2007; Gáyan *et al*., 2014; Bohrerova *et al*., 2015). This process is capable of removing a variety of DNA lesions through the action of the UvrABC exinuclease, which initiates a range of cascade reactions. In the literature, however, the phenomenon of dark-repair is less extensively studied compared to photo-reactivation, yet it may occur in distribution systems following disinfection. The investigation of dark-repair potential in the present study was therefore largely appropriate.

As observed in Figure 4.6, exposure of the UV irradiated samples to darkness had a significantly lower effect on microbial growth (in terms of an increase in log value) ($p<0.05$) compared to exposure to the different intensities of light following the 40 and 2 x 20 mJ.cm^{-2} treatments, respectively. After 5 h, dark-repair resulted in a final TC population reaching 2.91 log cfu.100 mL^{-1} following the 40 mJ.cm^{-2} treatment. This equated to a significant increase ($p<0.05$) of 0.23 log over the 0 to 5 h period. For the 2 x 20 mJ.cm^{-2} treatment no significant increase in cell

concentration (p=1.00) was observed in darkness over the same period and the final TC concentration was recorded as 2.77 log cfu.100 mL^{-1} (Fig. 4.10). Thus, as was reported for photo-reactivation, the sequential treatment technique was shown to better inhibit dark-repair.

The percentage recovery recorded for the dark-repair investigations following MP UV treatment are presented in Figures 4.7 & 4.9. For the 40 $mJ.cm^{-2}$ treatment, dark-repair was clearly observed and maximum recovery of 14.62% was recorded (Fig. 4.6). No increase in population size was detected for the 2 x 20 $mJ.cm^{-2}$ treated sample (Fig. 4.6). In Figure 4.9 it can be seen that recovery following the 40 $mJ.cm^{-2}$ treatment was initially rapid with a significant increase ($p<0.05$) of 14.13% in the number of culturable coliforms being recorded for the first hour of exposure to darkness. From 1 h onwards, no significant increase in the percentage recovery was seen ($p=0.86$ and $p=0.91$) and total recovery was recorded as 14.86% and 14.62% after 3 and 5 h, respectively. Thus, after 5 h of exposure to darkness recovery was 2.16 and 2.05 times lower than that achieved following exposure to visible light at intensities of 3.5 and 1.0 to 2.0 kLux, respectively, for the 40 $mJ.cm^{-2}$ treatment.

Locas *et al*. (2008) evaluated dark and light-repair of *E. coli* and enterococci in MP UV disinfected wastewater. Visible light at intensity of 5.6 kLux prompted the *E. coli* cell concentration to increase by seven times within 6 h, whereas no significant increase in the population size was seen following exposure to darkness. Likewise, Kollu & Örmeci (2015) reported that dark-repair was not detected for *E. coli*, nor faecal coliforms, in enriched phosphate-buffered saline (PBS), sterilised wastewater or natural wastewater following UV irradiation at a dose of 40 $mJ.cm^{-2}$.

Considering all of these results, dark-recovery observed following the 40 $mJ.cm^{-2}$ treatment was somewhat unexpected as most studies could not detect repair under comparable experimental conditions. However, in addition to dark-reactivation, increments in population size may also be attributed to normal growth of viable cells resulting from the increased availability of nutrients following UV disinfection. The UV inactivated cells remaining in the water may serve as a source of biodegradable carbon which could be utilised by other microorganisms (Bohrerova *et al*., 2015). In this regard, the same authors reported that the problem of regrowth of UV survivors was more influential than that of repair in the presence of UV inactivated cells. the increase in population size may also have resulted from actual dark-repair as Jungfer *et al*. (2007) have previously reported that UV doses of 40 $mJ.cm^{-2}$ (and higher) induced such mechanisms. They, however, only indicated the expression of some important repair genes (recA) but did not detect actual repair following cultivation experiments. Considering this, and the fact that repair was observed for the first hour only in this study, it is concluded that the percentage increase most probably resulted from regrowth due to a temporary increase in the availability of nutrients.

4.6 Study 3. The disinfection efficacy of UV light, chemical treatment and the combination thereof (*UV/Chemicals*)

The results obtained in studies 1 and 2 demonstrated that UV treatment alone might not be sufficient to disinfect highly polluted river water to such an extent that the faecal coliform levels are below the 1000 CFU.100 mL^{-1} guideline for safe irrigation of fresh produce (DWAF 1996). Following UV disinfection of highly contaminated river water, pathogens might thus still be present in fairly high numbers. In this regard, growth of microorganisms, as well as the phenomenon of photo-reactivation, may significantly threaten the microbiological quality of the treated water. These are likely events as irradiated river water may still provide nutrients for growth, while exposure to light following disinfection is often difficult to evade.

The aims of this study were, firstly, to compare MP UV irradiation efficacy to chemical disinfection at pilot-scale, and secondly, to investigate the efficacy of UV irradiation in combination

with chemical disinfection (and the phenomenon of damage-repair). This is important so that adequate control measures and suggestions regarding river water disinfection can be made.

As part of the larger Scoping study laboratory-scale investigations were conducted into the efficacy of LP UV irradiation against various *Escherichia coli* strains in saline and sterilised river water. This was also done for chemicals such as Peracetic acid (PAA), Chlorine (Cl) and hydrogen peroxide (H_2O_2). The efficacy of these chemicals against river water was also evaluated. These results are presented in Appendices A3, A4, and A5. In short, the following important conclusions were drawn:

- The *E. coli* strains investigated differed in their resistances to the various disinfection methods (indicating that combination treatments would be advantageous to get rid of highly resistant strains) – Appendix Figures A3.2; A4.3.8; A4.2.2; Giddey *et al.* (A4.1.1)
- Generally, environmental *E. coli* strains are more resistant to treatments than standard reference *E. coli* strains (indicating that if single strains are used for treatment method optimisation, careful consideration should be given to the choice of the test strain to be used). – Appendix Figures A3.2; A4.3.8; A4.2.2; Giddey *et al.* (A4.1.1)
- *E. coli* strains have a specific maximum tolerance threshold to chemical disinfectants. If concentrations of the disinfectants rise above these limits, resistant cells become increasingly sensitive. – Appendix A4.2.3
- Certain chemicals are more sensitive to poor water quality than others. For instance, chlorine was less affected than low concentrations of PAA, provided that the water pH remain neutral. – Appendix Figures A4.2.4; A4.2.5; A4.4.2-A4.4.5.
- The ever-changing microbial population composition of river water influences disinfection efficacy – Appendix Figures A3.9-A3.11; A4.1.2.1-A4.1.2.3; A4.4.2-A4.4.5.

Each disinfection method has its drawbacks, and because the microbial content of certain dynamic water sources (such as the Plankenburg River in this study) changes continually, a robust and adaptable approach might be necessary to ensure safe irrigation water. Combination treatments might be advantageous in this regard, especially in the case of highly (microbiologically) contaminated water sources.

In **Study 3** *(UV + UV/Chemicals)* the efficacy of UV light, chemical treatment and the combination thereof for the disinfection of microbiologically polluted river water at a pilot-scale level were investigated. During Trials 1-9, river water was exposed to the minimal UV dose that could be achieved at the given day due to variations in the water quality: UV doses of 18, 19 and 30 $mJ.cm^{-2}$. The efficacy of Peracetic acid (PAA) (Study 3.1 (*PAA + PAA/UV*) and Chlorine (Cl) (Study 3.2 (*Cl + Cl/UV*) was investigated to determine the most effective strategy. A contact time of 25 min was allowed for each of the respective chemicals to react with the river water in the 2 500 L holding tanks before UV treatment and sampling took place. (Details of treatments investigated in each trial are included in Table 4.3).

In Trials 10-15, the potential of photo-reactivation after chemical/UV combination treatments was also investigated for PAA, Cl and hydrogen peroxide (H_2O_2). For this purpose a UV dose of 30 $mJ.cm^{-2}$ was used in all instances, while a contact time of 25 min was allowed for each of the respective chemicals. A standard recovery period of 3 h under fluorescent light was included after treatment to allow photo-recovery to occur. (Details of treatments investigated in each trial are included in Table 4.4)

4.6.1 Study 3.1: PAA and PAA/UV treatments (*PAA + PAA/UV*)

The three combination treatment Trials (Trials 1-3) that involved the use of PAA (4 $mg.L^{-1}$ for 25 min) before UV (18 $mJ.cm^{-2}$), are presented in Figure 4.9. Initial (control) microbial loads and

population compositions were comparable (Table 4.5), but disinfection efficacy (in terms of log reductions) of the single treatments (UV alone and PAA alone) differed.

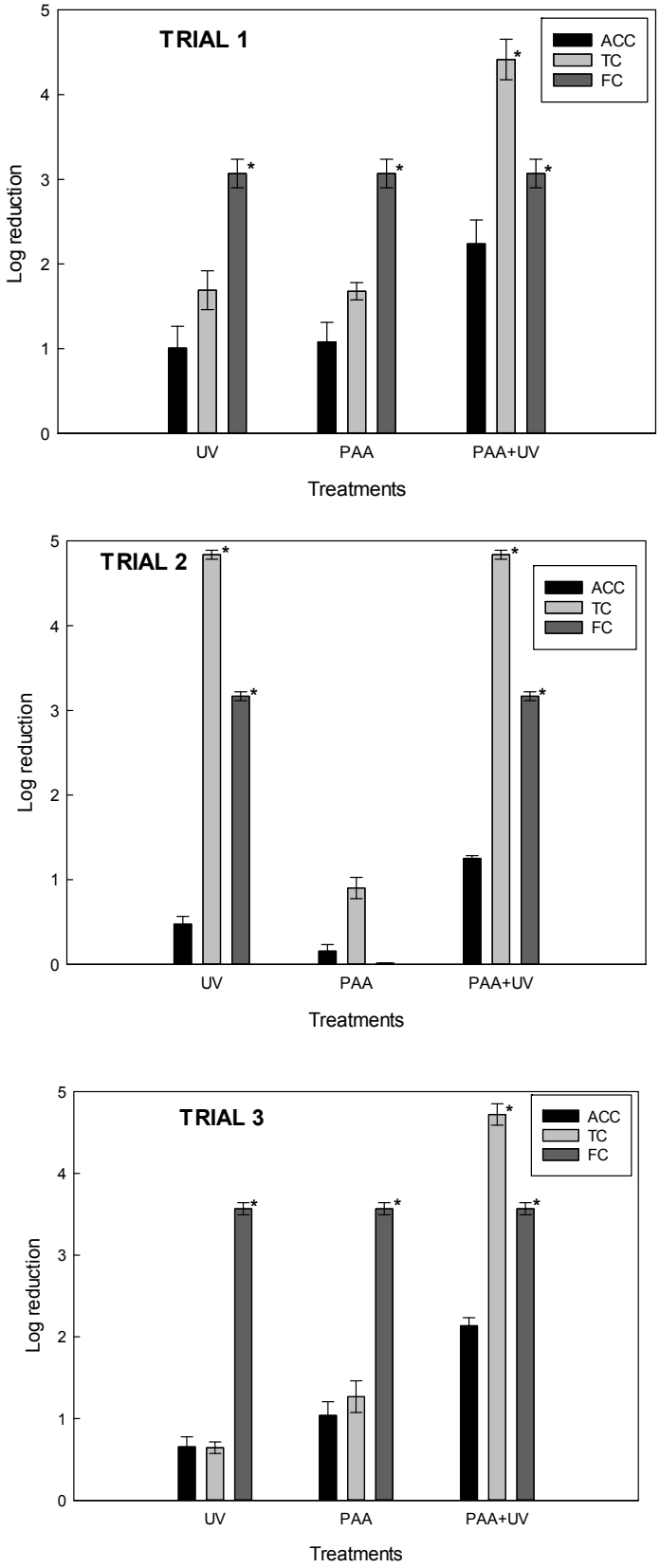


Figure 4.9 Log reduction values determined for ACC (aerobic colony count), TC (total coliforms) and FC (faecal coliforms) during a pilot plant Study 3.1 (*PAA + PAA/UV*), Trials 1, 2 & 3 where river water was treated with UV dose of 18 mJ.cm^2; or 4 ppm PAA for 25 min.; or a combination of PAA and UV (PAA+UV). Error bars are calculated from standard deviation at a 95% confidence level. * - No growth at lowest dilution (10^{-1}).

PAA treatments was, for instance very effective during Trial 1 in reducing coliforms (faecal and total), and no significant differences were observed when compared to log reductions obtained for UV treatment alone. Significant differences were, however observed between UV and PAA efficacy in Trial 2. Water quality indicators were highly comparable for Trials 1 and 2 (Table 4.3, except that the UVT% was better and the alkalinity of the water was higher in Trial 2. A higher UVT% could have improved UV disinfection, while higher alkalinity might have a neutralising effect on low PAA concentrations (as also indicated in A4.2). Both these reasons might explain why UV treatment was significantly better than PAA treatment in reducing coliforms (total and faecal) in Trial 2, while no differences were observed in efficacy during Trial 1 (Fig. 4.9).

The complex influence that a variety of water quality factors can have on treatment efficacy is again illustrated in Trial 3, where the UV treatment was only as effective as PAA in reducing the faecal coliform content, but not for ACC and TC populations (where the PAA treatment was significantly better) (Fig. 4.9). This was in spite of the fact that the UVT% and turbidity and alkalinity values (all of which could influence both UV and PAA treatments negatively) during Trial 3 were worse than those measured for Trials 1 and 2 (Table 4.3). The only water quality indicator that was significantly better than those observed for Trials 1 and 2, was the COD content (Table 4.3).

The combination treatment tested in Trials 1-3 was the only treatment that gave consistent results in terms of coliform (total as well as faecal) inactivation, with no detectable coliforms observed after treatment. It was also the only treatment that could reduce the heterotrophic plate count significantly (1-2 log) in all three trials.

When considering the 1 000 cfu.100 mL^{-1} limit set for faecal coliforms in water intended for the irrigation of fresh produce (Table 3.1), it was clear from the results that the initial microbial levels in the water before treatment did not meet this guideline in Trials 1, 2 and 3. After treatment it was only in Trial 2 that PAA treatment alone did not result in water with acceptable faecal coliform levels. Both the UV treatment alone as well as the combination treatment resulted in acceptable faecal coliform reduction in all three trials in spite of varying water quality characteristics.

The results for the PAA/UV combination/reactivation trials are presented in Figure 4.10. In these trials, water was exposed to fluorescent light for 3 h after treatment with either UV or a combination of PAA and UV. The effect of the water quality variance within the same season are apparent when comparing both physico-chemical properties (Table 4.4) and microbiological content (Table 4.5). These trials were both conducted in winter, where the occasional rainy spell could have contributed to increases in factors such as turbidity and COD as a result of increased run-off (as discussed earlier), which would result in lower UVT%. From these results it can be concluded that the overall water quality of the water in Trial 14 was lower than in Trial 15. This difference was also evident in the log reduction values (Fig. 4.10). In Trial 15 complete inactivation of FC after all treatments as well as after reactivation resulted in water that complied with the DWA guideline for faecal coliforms (Table 3.1). In Trial 14 only the combined treatment (before and after reactivation) resulted in complete inactivation of the FC load. The UV treatment just reached the two log reduction required (based on initial levels detected – Table 4.5) to decrease FC to acceptable levels, although photo-reactivation resulted in log recoveries in both the FC and TC populations.

Overall the total aerobic population (ACC) was the most resistant population to treatment, and was never completely inactivated in any of the Trials (1-3; 14 & 15). The treatment that resulted in the highest ACC log reductions were the combination treatments. Photorecovery also did not have any significant effect on the combination treatments (Fig. 4.10). The efficacy of PAA was severely influenced by low water quality. In Trial14 the combination of PAA with UV did however result in a significantly higher log reduction in FC than what was obtained with combined reductions of UV alone and PAA alone, indicating the possible role of PAA in advanced oxidation during UV irradation.

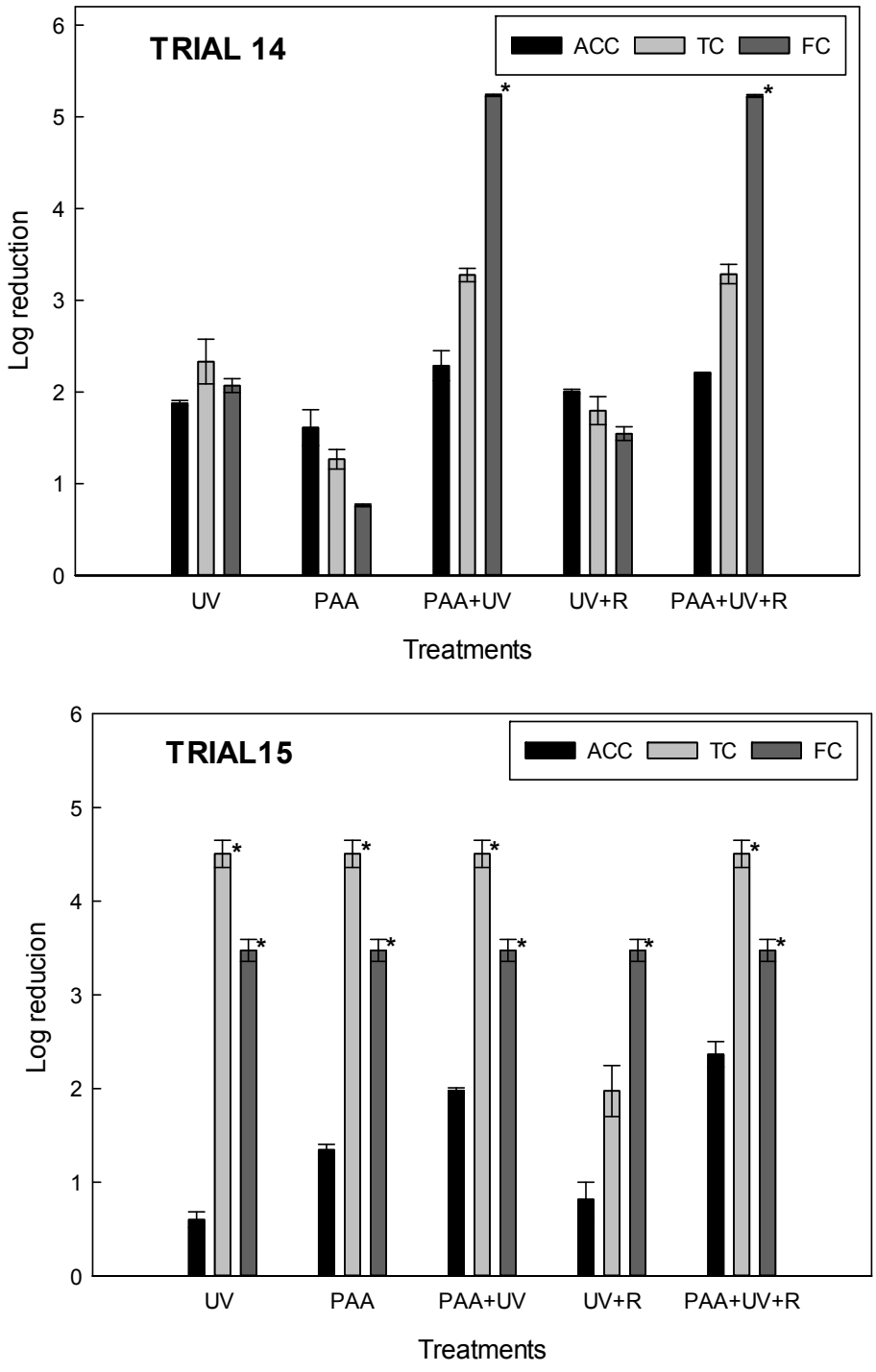


Figure 4.10 Log reduction values determined for ACC (aerobic colony count), TC (total coliforms) and FC (faecal coliforms) during a pilot plant Study 3.1 (*PAA + PAA/UV*), Trials 14 & 15 where river water was treated with UV dose of 30 mJ.cm^2; or 4 ppm PAA for 25 min.; or a combination of PAA and UV (PAA+UV). Photo-reactivations was tested by exposing both the UV sample (UV+R) and the combination treatment sample (PAA+UV+R) to fluorescent light for three hours. Error bars are calculated from standard deviation at a 95% confidence level. * - No growth at lowest dilution (10^{-1}).

4.6.2 Study 3.2: Cl and Cl/UV treatments (*Cl + Cl/UV*)

The disinfection efficacy of UV was compared to Cl disinfection as well as to the combined effect of Cl and UV in Trials 4-9. In Trials 4 and 5 the effect of 3 ppm Cl was investigated in combination with UV, while 6 ppm Cl was tested in Trials 6-9. Different UV doses were applied, based on the minimum dose possible for a specific trial given the water quality on the day. The effect that these treatments had on the microbial load in river water are represented in Figures 4.11 (Trials 4 & 5), 12 (Trials 6 & 7) and 13 (Trials 8 & 9). The results for the Cl/UV combination/reactivation Trials 10 & 11 are presented in Figure 4.14. In these trials (Trials 10 & 11), a constant UV dose of 30 $mJ.cm^{-2}$ was used in both trials, after which the water (treated with either UV or a combination of Cl and UV) was exposed to fluorescent light for 3 h after treatment.

In terms of water quality indicators (Tables 4.3 & 4.4), it was concluded that the water quality of the river water treated in these trials was better overall than that observed during Trials 1-3. The average COD and turbidity values were lower, while the UVT % was generally higher. The average pH was also higher than that observed during the first three trials. All these factors could have had a positive influence on UV as well as chlorine treatment efficacy.

Once again, all the control water samples tested during Trials 4-11 indicated that the water in general did not meet the 1 000 cfu.100 mL^{-1} limit set for faecal coliforms in water intended for the irrigation of fresh produce (Tables 3.1 and 4.5). All the treatments UV, Cl and Cl/UV combination) resulted in water in which no faecal coliforms could be detected, which can be considered as acceptable for irrigating fresh produce except for the chlorine disinfection results in Trial 4 (Fig. 4.11). These are in sharp contrast with what was obtained for 3 ppm chlorine treatments at pilot-scale in Trials 5 (Fig. 4.11) and 10 & 11 (Fig. 4.14). The physico-chemical as well as microbiological properties of the water treated in Trial 4, (which included factors important to Cl disinfection like pH and COD) were better overall that those of Trials 5, 10, and 11. Given this fact, it was concluded that

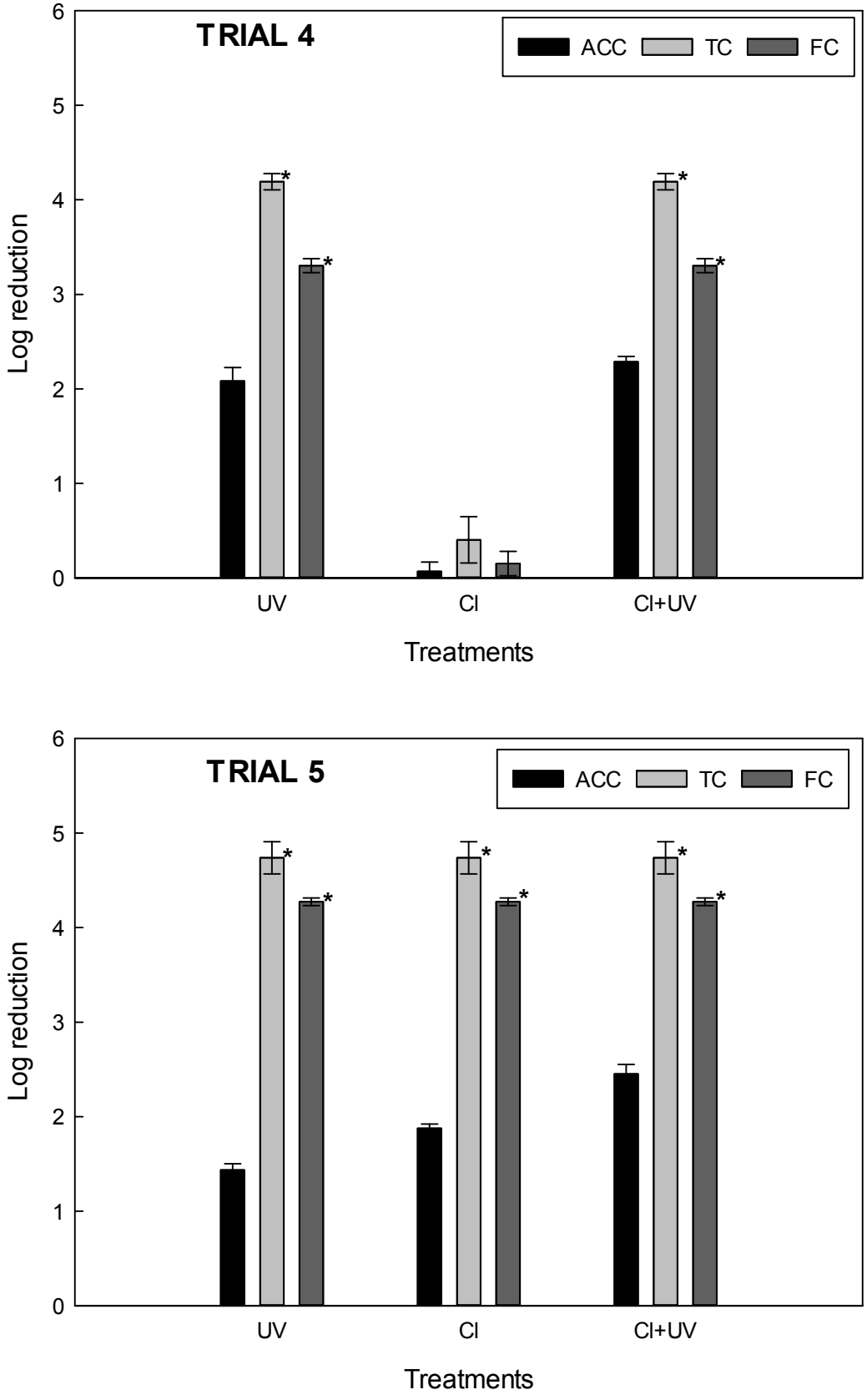


Figure 4.11 Log reduction values determined for ACC (aerobic colony count), TC (total coliforms) and FC (faecal coliforms) during a pilot plant Study 3.2 (*Cl + Cl/UV*), Trials 4 & 5 where river water was treated with UV doses of 30 mJ.cm^{-2} (Trial 4) and19 mJ.cm^{-2} (Trial 5); or 3 ppm chlorine (Cl) for 25 min; or a combination of Cl and UV (Cl+UV). Error bars are calculated from standard deviation at a 95% confidence level. * - No growth at lowest dilution (10^{-1}).

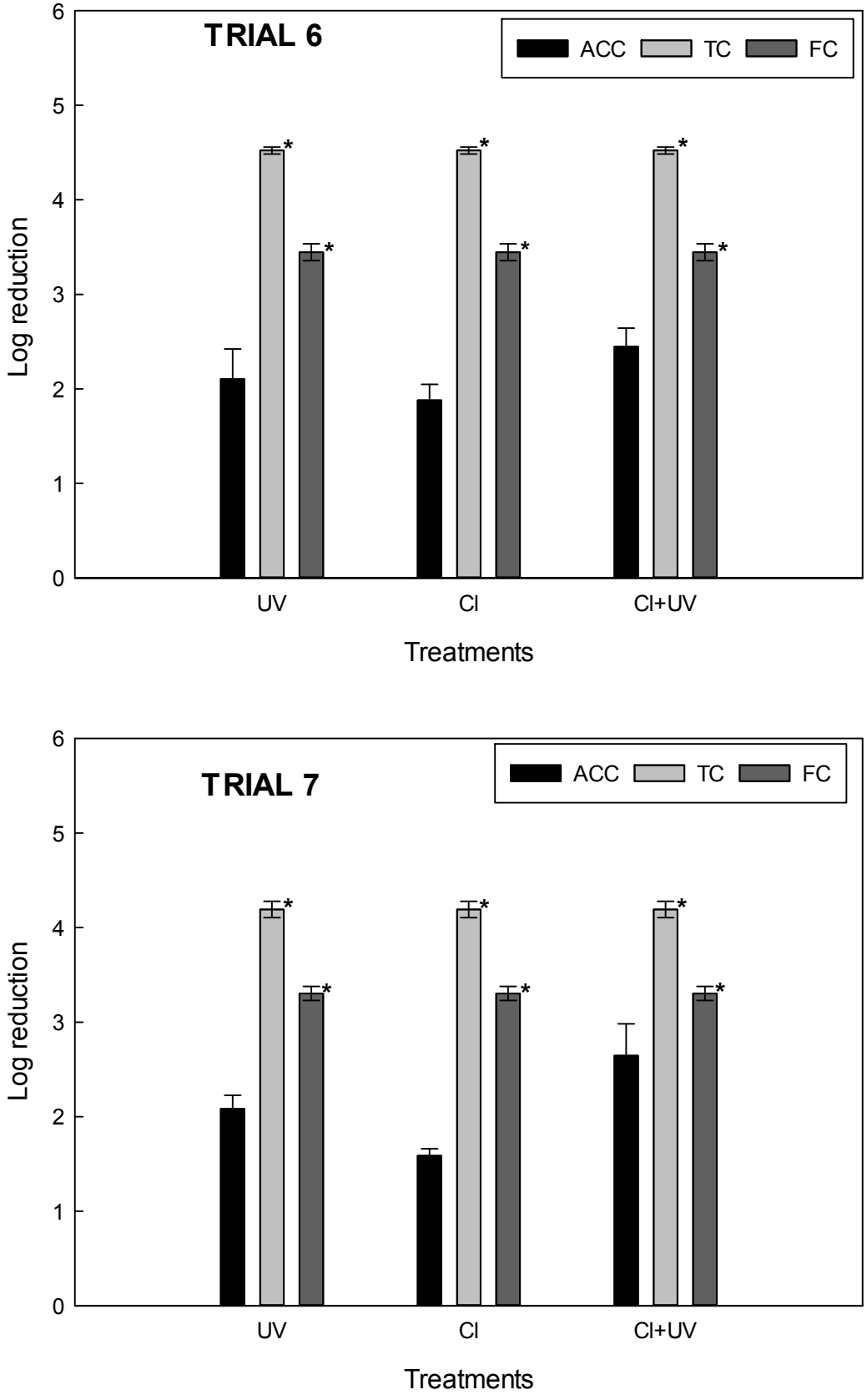


Figure 4.12 Log reduction values determined for ACC (aerobic colony count), TC (total coliforms) and FC (faecal coliforms) during a pilot plant Study 3.2 (*Cl + Cl/UV*), Trials 6 & 7 where river water was treated with UV dose of 30 mJ.cm^{-2}; or 6 ppm chlorine (Cl) for 25 min; or a combination of Cl and UV (Cl+UV). Error bars are calculated from standard deviation at a 95% confidence level. * - No growth at lowest dilution (10^{-1}).

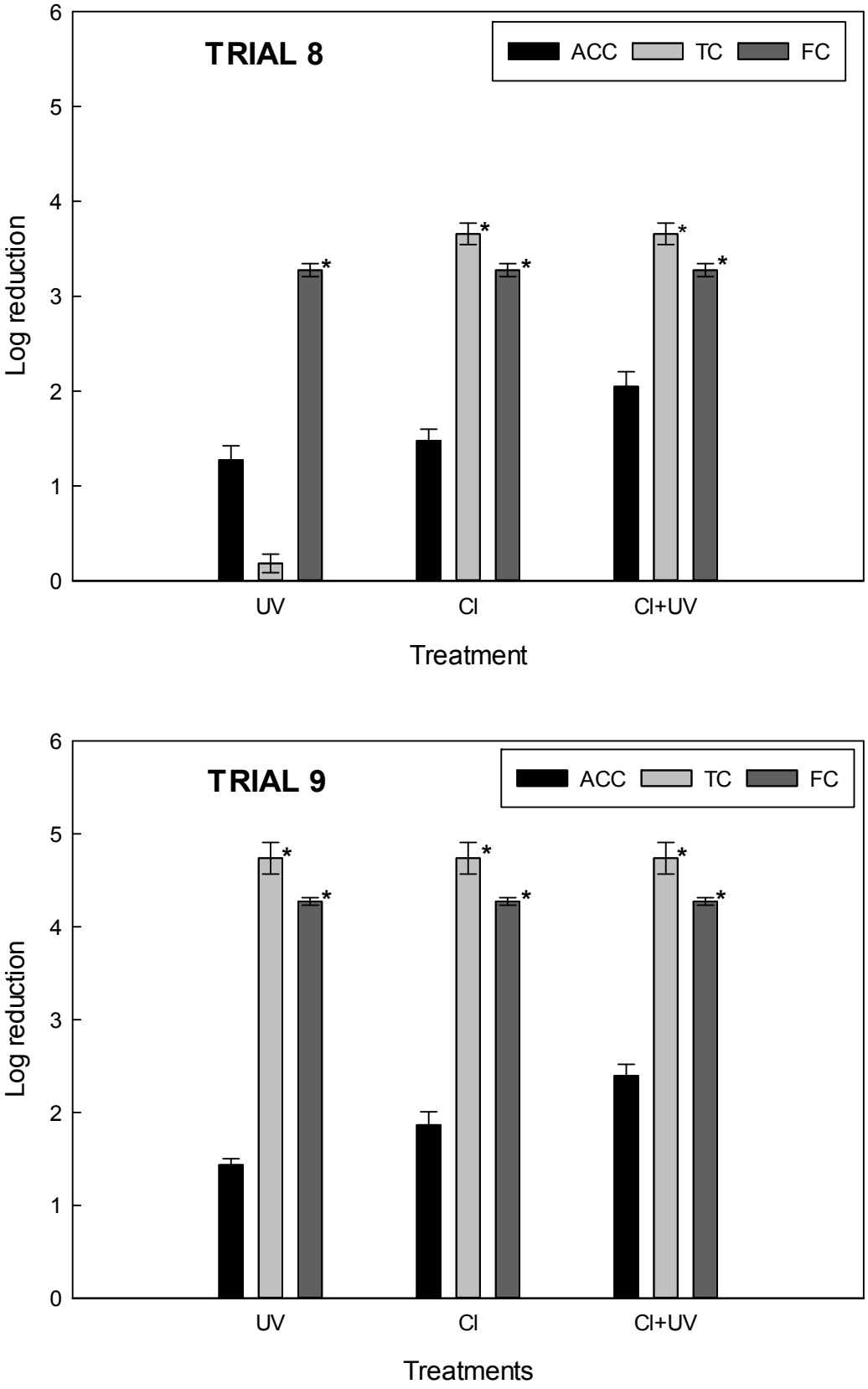


Figure 4.13 Log reduction values determined for ACC (aerobic colony count), TC (total coliforms) and FC (faecal coliforms) during a pilot plant Study 3.2 (*Cl + Cl/UV*), Trials 8 & 9 where river water was treated with UV dose of 19 mJ.cm^2; or 6 ppm chlorine (Cl) for 25 min; or a combination of Cl and UV (Cl+UV). Error bars are calculated from standard deviation at a 95% confidence level. * - No growth at lowest dilution (10^{-1}).

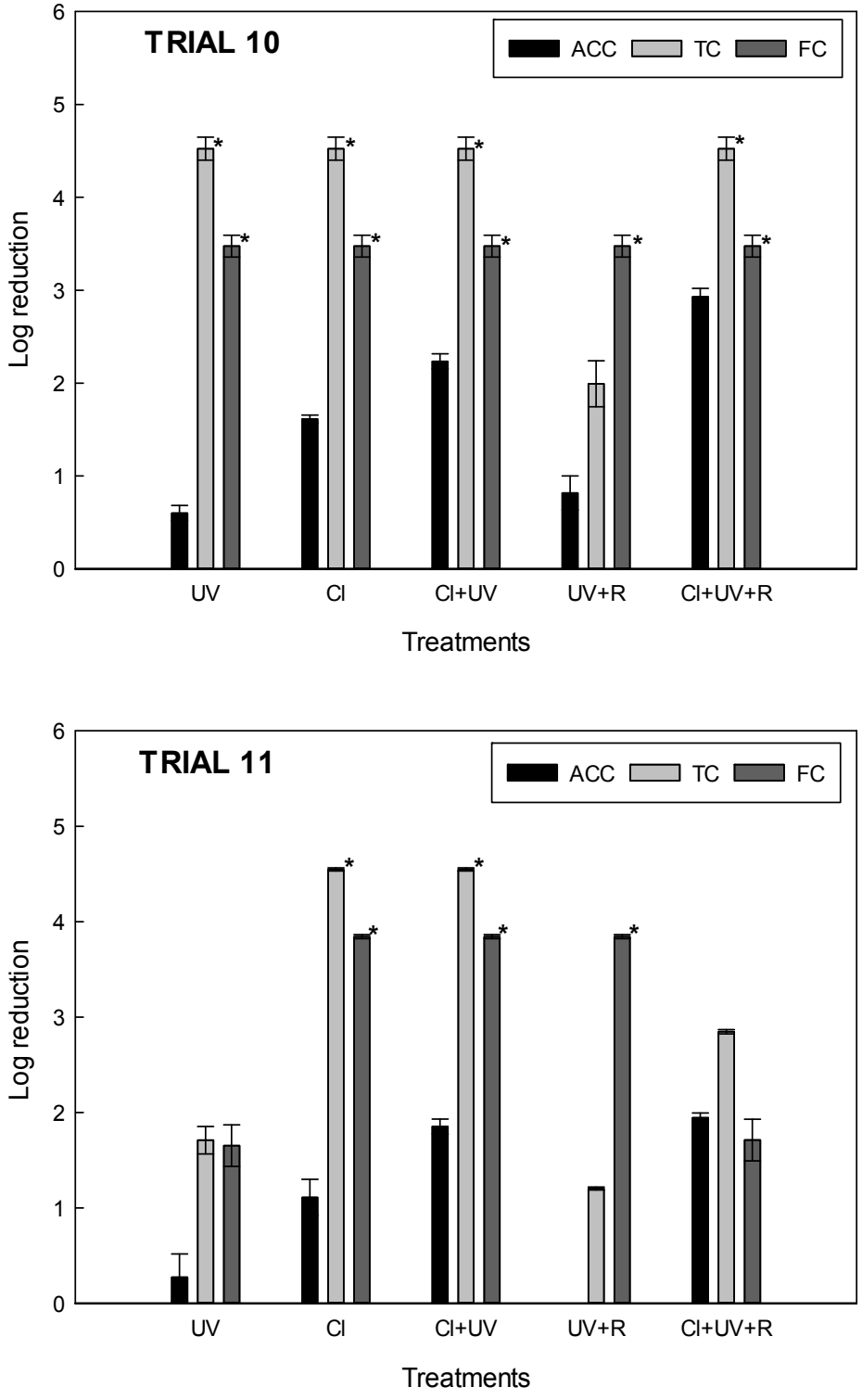


Figure 4.14 Log reduction values determined for ACC (aerobic colony count), TC (total coliforms) and FC (faecal coliforms) during a pilot plant Study 3.2 (Cl + Cl/UV), Trials 10 & 11 where river water was treated with UV dose of 30 mJ.cm^2; or 3 ppm Cl for 25 min.; or a combination of Cl and UV (Cl+UV). Photo-reactivation was tested by exposing both the UV sample (UV+R) and the combination treatment sample (Cl+UV+R) to fluorescent light for three hours. Error bars are calculated from standard deviation at a 95% confidence level. * - No growth at lowest dilution (10^{-1}).

an experimental error occurred in the determination of the Cl disinfection results for Trial 4 and therefore these results are excluded from further discussion.

The efficacy of the Cl/UV combination treatment in comparison to the UV treatment alone and the Cl treatment alone are, therefore, only considered in terms of the effect it had on the ACC population (ACC). It was observed that the maximum reduction in the hetrotrophic plate counts of Trials 4-9 were always < 3 log, irrespective of whether 3 ppm or 6 ppm Cl was used. This implies that a 2 log ACC population (of unknown identity) remained after disinfection. For the UV treatments alone, the highest log reductions were observed in the trials where the highest dose (30 $mJ.cm^2$) was applied (Trials 4, 6, 7) (Figures 4.11, 4.12), which was expected. The combination treatments tested, consistently resulted in the highest log reductions in ACC numbers in all the trials, compared to the single UV and single Cl treatments.

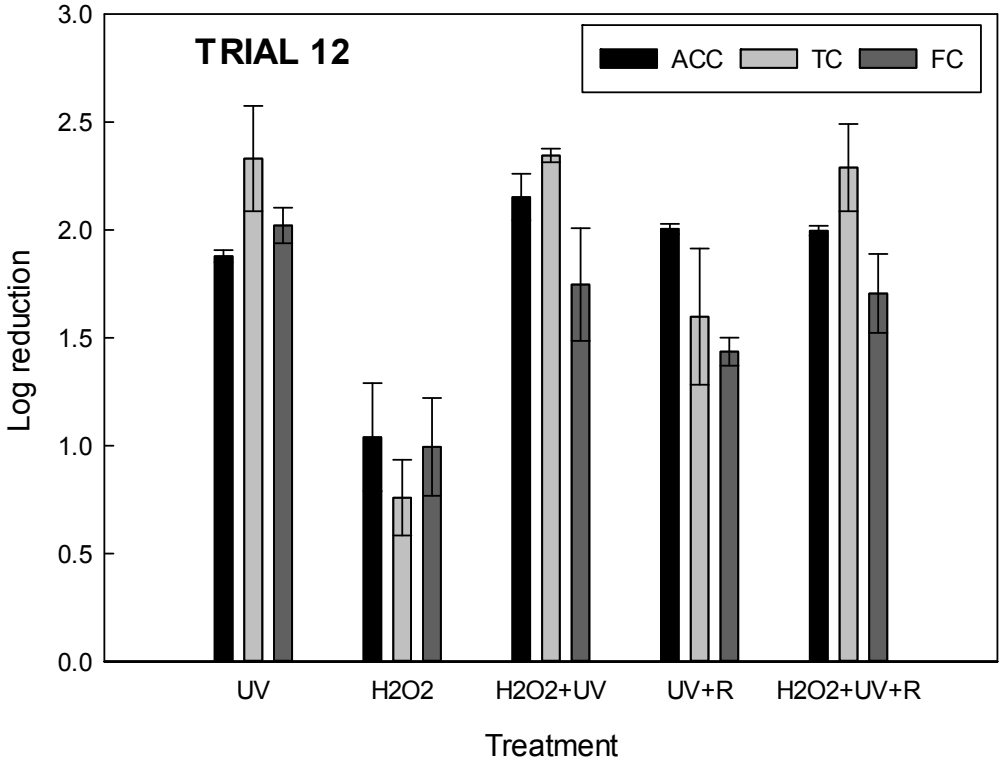
From these results (Trials 4-9) it was concluded that, under the conditions tested, UV irradiation can be improved by simply raising the irradiation dose. This can be seen by comparing results in Trial 4 and 5 and also between results in Trials 6 & 7 and Trial 8 &9. In Trials 4 and 5, the increase in UV dose from 19 to 30 $mJ.cm^{-2}$ resulted in an increase in the log reductions of the ACC from *ca*. 1. to 2 log (Fig. 4.11), while the increase in UV dose between Trials 6 & 7 and 8 & 9 (Fig. 4.12 and Fig 4.13) resulted in ACC log reductions increasing from 1.4-1.5 log to ca. 2 log. Chlorine has certain limitations regarding the residual levels after treatment, which would require constant monitoring of the chlorine demand of the river water prior to treatment. Although the combination treatment caused the highest reduction in ACC counts, the extra cost involved in dosing and mixing should be carefully considered. Under optimum conditions for UV irradiation, like those reported for Trials 4-9, UV treatment alone might be sufficient, depending on the degree of photo-reactivation. The ability to increase the UV dose might result in better log reductions (which was not possible due to equipment constraints).

The combination treatments tested as part of the Cl/UV combination/reactivation Trials 10 and 11 (Fig. 4.14) also resulted in the highest log reductions in ACC numbers, with even increased log reductions observed in Trial 10 after a reactivation period of 3 h. This was probably as a result of the residual effect of chlorine present. All the treatments investigated in Trial 10 yielded water of acceptable quality for irrigation purposes after complete inactivation of the FC content. Trial 11 yielded conflicting results after the reactivation period. Increased log reduction (cell death) was observed for the FC content of the UV sample, possibly indicating damaged cells that died off during the 3 h reactivation period as a result of the lack of nutrients (See low COD value for Trial 11 – Table 4.4). Decreased log reductions (cell increases) were observed for the FC and TC content after combination treatment (Cl+UV+R), in spite of a chlorine residual being present after treatment (Table 4.4). It could be that these increases could be the result of a portion of the chlorine-damaged cells (some of the more chlorine-resistant strains) entering a viable-but-not-culturable (VBNC) state during treatment (resulting in a “no growth” result directly after treatment). These cells might then have recovered sufficiently in the presence of nutrients from killed cells after the three hour recovery period to be enumerated on agar. The original study design did, however, not allow for any of these speculations to be verified. The variation in log reductions obtained during Trials 10 and 11 do, however, once again demonstrate how the ever-changing microbial content present in a flowing river can influence treatment efficiency.

It can be concluded when evaluating the results from Trials 4-11 that a free chlorine dose of 3 $mg.L^{-1}$ was effective enough to inactivate the TC and FC, resulting in chlorine residuals below the 1 $mg.L^{-1}$ chosen for this study (There is no chlorine limit set for fresh produce irrigation, but the USEPA, (2004) guideline for chlorine residual left after treatment of reclaimed water used for land irrigation is <1 $mg.L^{-1}$.

4.6.3 Study 3.3: H_2O_2 and H_2O_2/UV combination treatments (*H_2O_2 + H_2O_2/UV*)

The combined effect of H_2O_2 and UV was tested on river water in Trials 12 and 13 and the results are presented in Fig. 4.15. The effect that poor water quality can have on treatment efficiency is once again reflected in the log reductions as these two trials collectively had the poorest water quality of all the trials (Table 4.3 and 4.4) in terms of UVT%, COD content, turbidity values, TSS and alkalinity. None of the treatments in Trials 12 and 13 resulted in total deactivation of FC. Even the maximum two log reduction obtained for FC (during the UV treatment in trial 12) was still not enough to reduce FC to acceptable levels within the guideline of 1 000 cfu.100 mL^{-1}. Trial 12 was conducted on the same day as Trial 14, and it is clear when the results are compared that PAA combined with UV (Study 3.1, Trials 1, 2, 3, 14 and 15; Figs. 4.9 and 4.10) had a much more positive effect on FC log reduction than H_2O_2 and UV (Fig. 4.15) – > 5 log reduction of FC for PAA/UV (Figs. 4.9 and 4.10) compared to < 2 log reduction with H_2O_2/UV. It can be seen from Trial 12 and 13 that UV is more effective than H_2O_2 (even in water of poor quality). This was also observed in the laboratory-scale investigations done previously (Appendix A3). The poor, and varying water quality in Trials 12 and 13 also resulted in differences in efficiency. In Trial 12, UV was slightly more effective against TC and FC, while in Trial 13, it was more effective against the ACC (Fig. 4.15). A combination of H_2O_2 and UV was only slightly more effective than UV alone (Fig. 4.15). The poor water quality and subsequent low log reductions also resulted in more regrowth/photo reactivation in ACC, TC and FC, as opposed to mainly regrowth in TC and FC in previous photo-reactivation trials (Trials 10, 11, 14 and 15; Figs. 4.10 and 4.14). The poor log reductions observed during Trial 13 can ultimately be linked to the poor water quality. Although the UVT% was not the lowest recorded for all the trials, the COD and Turbidity as well as the TSS content were the highest – all of which could influence both disinfection efficiency.



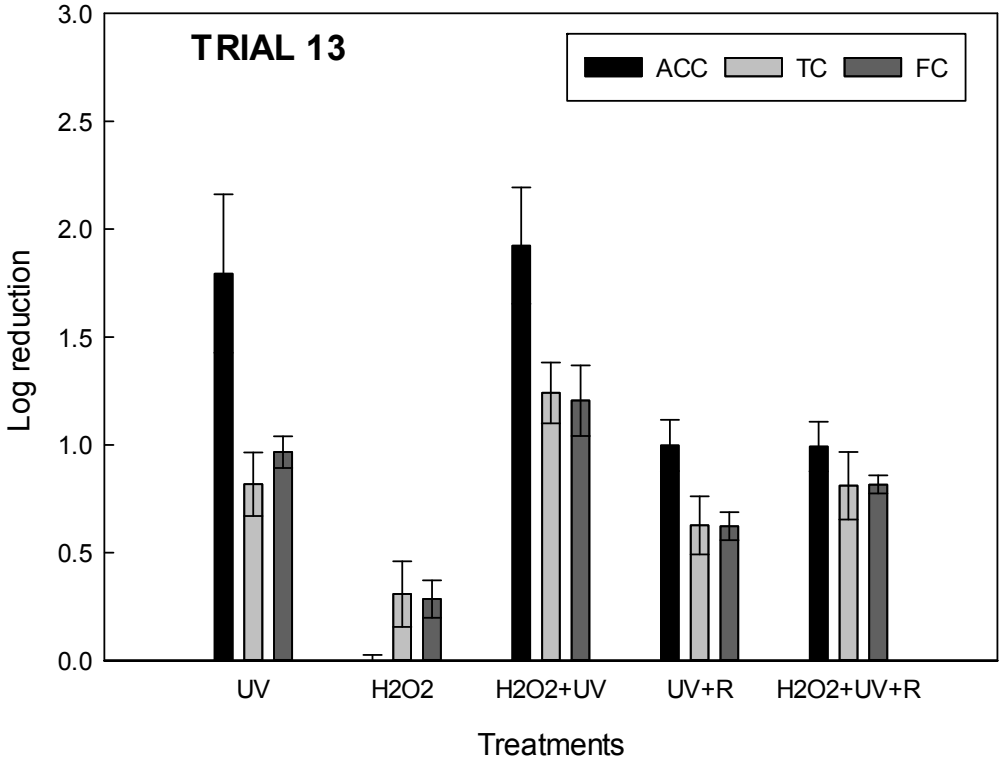


Figure 4.15 Log reduction values determined for ACC (aerobic colony count), TC (total coliforms) and FC (faecal coliforms) during a pilot plant Study 3.3 (*H_2O_2 + H_2O_2/UV*), Trials 12 & 13 where river water was treated with UV dose of 30 mJ.cm^2; or 2.5 ppm of H_2O_2 for 25 min.; or a combination of H_2O_2 and UV (H_2O_2+UV). Photo-reactivation was tested by exposing both the UV sample (UV+R) and the combination treatment sample (H_2O_2+UV+R) to fluorescent light for three hours. Error bars are calculated from standard deviation at a 95% confidence level. * - No growth at lowest dilution (10^{-1}).

5. SCOPING STUDY FINDINGS

5.1 Findings from the Scoping Study

1) *Media/sand filtration* should be an integral part of any irrigation water treatment method that is affected by water with high TSS, COD and organic matter as this technology is proven and readily available;
2) Some *chemical treatments*, such as bromine and ozone, are not feasible for irrigation water disinfection. *Bromine* is pH and COD dependent, requires longish contact times, high concentrations, is corrosive, forms disinfectant by-products and is not effective against *Cryptosporidium* and *Giardia*. Ozone is very effective against a wide variety of micro-organisms, but is expensive, unstable, corrosive, and forms disinfectant by-products. *Ozone* is also dependent on pH, temperature, COD and contact times (minutes rather than seconds). Direct in-line dosing of ozone gas also complicates irrigation efficiency;
3) *Ultrasound* has the ability to remove all pathogens during disinfection, but is dependent on various water quality parameters especially turbidity and also requires high ultrasonic intensities that will lead to further increasing the costs (high capital and operating cost also) and extended contact times, consequently limiting its use for large-scale disinfection of irrigation water;
4) During the *exploratory on-farm treatment* investigation, comparing the efficacy of UV to H_2O_2, it was found that log reductions achieved by both treatments were lower than expected, most likely due to the fact that dosages used had been calculated from recommendations in literature for reference strains. It was, however, noted that UV displayed better efficacy than the H_2O_2. Monitoring changes in microbial levels throughout the irrigation cycle were, however, not possible and this resulted in the change of the project method to laboratory-scale investigations followed by pilot-scale investigations, which would allow more detailed comparisons to be investigated;
5) *Laboratory-scale studies into the use of UV and combinations of UV and H_2O_2 and in H_2O_2 alone saline* solutions, showed again that reference *E. coli* strains are more sensitive to treatment than environmental strains. In some cases UV was more effective than UV/H_2O_2 combination against single *E. coli* strains tested. For *UV, H_2O_2 or combination treatments at laboratory-scale in river water*, differences in sensitivity to treatment were again observed with the reference strains being more sensitive. It was seen that long contact times (up to 120 min) and high concentrations (up to 350 $mg.L^{-1}$) were required for H_2O_2 alone, which still were not sufficient to achieve > 3 log reductions. It was also observed that higher UV doses would need to be investigated than the maximum of 10 $mJ.cm^{-2}$ used in these studies;
6) *Laboratory-scale studies into the use of peracetic acid as an alternative treatment in saline solutions and river water against selected E. coli isolates*, indicated that the *E. coli* strains investigated responded differently to PAA disinfection and the variability of strains within the same species was clearly evident. *E. coli* isolates that served as reference strains were in all cases more sensitive to PAA disinfection than the environmental *E. coli* strains. The optimum PAA dosage and contact time suggested as a treatment option for contaminated river water at pilot-scale would be 4.5-6.0 $mg.L^{-1}$ for a contact period of 25 min. It was also observed that the efficacy of PAA was negatively affected by high amounts of organic material in the water, Therefore, further investigation (at pilot-scale) into how effective PAA is against an unknown, mixed microbial population in river water, taking into account varying water quality properties should be investigated to give an indication of concentrations and contact times needed for irrigation water disinfection;

7) *Laboratory-scale studies into the use of chlorine as treatment in saline and river water against selected E. coli isolates*, indicated the reference strains were always more sensitive than the environmental strains. Of the two chlorine sources investigated during saline studies, the $Ca(OCl)_2$ was much more effective than the NaOCl, but is preferred for large-scale commercial applications due to the solubility issues with $Ca(OCl)_2$. Chlorine disinfection is pH dependent. The addition of chlorine to well-buffered systems is of utmost importance since the optimum pH range for chlorine disinfection is between 7.2 and 7.4. Hence, chlorine was more effective in river water studies than in saline, and was also not negatively affected by the low level of COD in the river water. A treatment of 12 $mg.L^{-1}$ (for 120 min) resulted in > 5 log reductions of the *E. coli* strains investigated. The long contact time and residual chlorine levels (> 2 $mg.L^{-1}$) could be a problem. Therefore, further research investigating the efficacy of NaOCl on river water disinfection is necessary;
8) *Laboratory-scale studies into the use of chlorine and peracetic acid as treatment in river water with an unknown mixed microbial population*, indicated that water quality played an important role during chemical disinfection. The ACC population was more resistant to disinfection by either chemical than the TC and FC's. The disinfection efficiency of PAA was greatly influenced at high COD levels, and his was not the case for chlorine. A chlorine residual level of ≤ 1 $mg.L^{-1}$ was seldom achieved, even at dosages of 3 $mg.L^{-1}$ chlorine. A chlorine concentration of ≤ 3.0 $mg.L^{-1}$ for a contact time of at least 30 min is suggested for river water disinfection, depending on the water quality on the particular day. Together with its low cost and high availability, chlorine will be a feasible option for irrigation water disinfection at commercial-scale (in terms of microbiological quality), although the environmental effects, formation of DBP's and increasing chlorine resistance of micro-organisms are possible concerns. On the other hand, PAA doses exceeding 4.5 $mg.L^{-1}$ are recommended for river water disinfection. The efficiency of this chemical is influenced by high COD levels in water. Increased PAA dosages negate these inferences during microbial disinfection. Higher PAA dosages imply higher costs, however, its use poses a lower risk to the environment than chlorine. In conclusion, water quality is ever changing, therefore required chemical dosages would also be subject to change. River water resources displaying a different water quality characteristics than the Plankenburg River could also react differently to chemicals and this should be considered in future studies;
9) *Laboratory-scale studies into the use of UV, chlorine, H_2O_2 and peracetic acid and combinations (PAA/UV, Cl/UV and H_2O_2/UV) as treatment in river water with an unknown mixed microbial population,* indicated that water quality played an important role during chemical disinfection. The ACC population (< 2 log reductions) was more resistant to disinfection by either chemical than the TC and FC's (mostly > 4 log reductions). The UV doses applied were only slightly higher (13 $mJ.cm^{-2}$) than in previous laboratory-scale studies (up to 10 $mJ.cm^{-2}$). The efficacy of UV was generally higher than that of PAA (4 $mg.L^{-1}$) and H_2O_2 (2.5 $mg.L^{-1}$) and similar to that of chlorine (6 $mg.L^{-1}$). Chlorine was less dependent on the water quality, however. The combination treatments of chemical disinfectants with UV were only slightly more effective than UV alone – raising the question of what efficacy would be possible at higher UV doses?
10) *Study 1: Pilot-scale studies on Medium Pressure UV (MP UV) of river water* indicated that generally, the ACC population was more resistant to treatment than the TC and FC's, but that the sensitivity also varied between the different sampling days. At UV doses of 24 $mJ.cm^{-2}$, log reductions were only close to 3 log, but it was evident that water quality parameters were shown to influence the efficiency of UV. Even when water quality was better, differences were observed in the efficacy of the UV treatment, most likely due to

microbial population differences. Higher UV doses would have to be investigated to achieve higher log reductions;

11) *Pilot-scale Study 2.1 (UV photo-repair) and Study 2.2 (UV photo + dark-repair)* showed that the occurrence of photo-repair decreases as the UV dose is increased. UV doses applied in these investigations (up to 40 $mJ.cm^{-2}$) did achieve > 3 log reductions in TC's. It was also evident that less photo-repair occurred at a dose of 2 x 20 $mJ.cm^{-2}$ than at one dose of 40 $mJ.cm^{-2}$. Dark-repair was minimal, but also less at 2 x 20 $mJ.cm^{-2}$ than at one dose of 40 $mJ.cm^{-2}$. Results thus indicated that photo-reactivation (post-disinfection) deserves attention as its influence could be significant) and that dark-repair does not show potential to significantly influence the disinfection efficiency of UV disinfection of irrigation water;
12) *Pilot-scale Study 3.1 (PAA + PAA/UV)* which investigated the efficacy of PAA (4 $mg.L^{-1}$ and 25 min) and UV (18 $mJ.cm^{-2}$) and a combination thereof in river water, showed that the efficacy of UV and PAA were similar in river water (except when UVT% was high – favouring UV or when alkalinity was high – detrimental to PAA efficiency). When water quality was of a better standard (UVT% > 50%; low turbidity; low COD) complete reduction of TC and FC's was achieved by UV, PAA and PAA/UV (i.e. > 4 log and > 3 log reductions, respectively). In terms of re-activation, only limited re-activation was observed for TC;
13) *Pilot-scale Study 3.2 (Cl + Cl/UV)* which investigated the efficacy of chlorine (3 and 6 $mg.L^{-1}$ and 25 min) and UV (19 and 30 $mJ.cm^{-2}$) and a combination thereof in river water, showed that chlorine (3 and 6 $mg.L^{-1}$) was slightly more effective than UV in river water. The combination treatment (Cl/UV) was only slightly more effective than either chlorine or UV alone. It was also evident that chlorine, UV or the combination (Cl/UV) was effective in reducing the TC and FC's (> 3 log reduction for FC's and > 4 log reduction for TC's), but not the ACC population (ACC's)(< 3 log reductions) – higher doses of either chlorine or UV would be required. Re-activation was mainly seen only for the TC and FC's, with very minimal regrowth for the ACC's;
14) *Pilot-scale Study 3.3 (H_2O_2 + H_2O_2/UV)* which investigated the efficacy of H_2O_2 (2.5 $mg.L^{-1}$) and UV (30 $mJ.cm^{-2}$) and a combination thereof in river water, showed that generally, UV was more effective than H_2O_2 (as seen in laboratory-scale studies). Some differences were observed for the efficacy of UV, most likely due to population differences in water samples (sensitivity of the ACC compared to TC and FC differed slightly in two different trials). The combination treatment (H_2O_2/UV) was only slightly more effective than either chlorine or UV alone. Re-activation was seen for the ACC, TC and FC's.

5.2 Cost comparison of treatments achieving ≥ 3 log reductions of faecal coliforms

A preliminary cost comparison was undertaken (with specific limitations) to gauge the difference in treatment costs, for treatments that achieved at least a 3 log reduction in faecal coliforms. For the comparative cost calculations at pilot-scale, only the re-circulation (in tank) and pumping through the UV unit were considered to limit the number of variables.

Description of pilot-scale treatment unit

The pilot scale UV Unit (Fig. 3.1a) unit allowed for water to be pumped from the river (submersible pump), through a sand filter, to any of three 2 500 L holding tanks. Once filled, the water within each tank was continuously recirculated (circulating pumps). During chemical treatments the respective chemicals were added directly to the holding tanks, after which proper recirculation was allowed. For UV treatments water was pumped (UV pump) directly from the holding tanks through the in-line UV system. The pilot-scale system was designed to allow flow rates in the range of 30 to 200 litres per minute (LPM).

Parameters
Only treatments that achieved ≥ 3 log reductions of faecal coliforms where chosen for comparison.
Cost calculation/comparison based on treatment of 1 kL (1 000 L) of water in pilot-scale unit.
Electricity cost = R1.90.kWh^{-1}
UV unit (power consumption) = 0.85 kW
UV pump (power consumption) = 0.75 kW
Circulating pump (power consumption) = 0.32 kW
Lamp replacement (€224.50/10 000 h, at €1 = R17.2: R0.38 per hour and thus R0.06 per 10 min).
Average Flow rate for calculation purposes = 100 LPM (thus a 10 min treatment time for the 1 kL).
Chemical treatments only involved the circulating pump (0.32 kW) for 25 min.
UV treatments involved the circulating pump (0.32 kW) for 10 min and UV pump (0.75 kW) for 10 min.
Combination treatments involved the circulating pump (0.32 kW) for 25 min and UV pump (0.75 kW) for 10 min

The following points need to be kept in mind when considering the cost differences:

- Only the re-circulation (in tank) and pumping through the UV unit were considered to limit the number of variables. Thus, only the electricity costs for re-circulation, pumping through UV, the UV itself and the cost of chemicals were compared
- Only chemical costs as incurred during the project were used – buying chemicals in bulk at commercial/full-scale operations might be cheaper, but in full-scale operations different UV lamps and larger pumps would also be used, so different costs would also apply to those.
- Warm-up times for the UV system were also not included in the calculations.
- Required UV dose and chemical dose for a large scale situation will vary from the pilot test.
- Chemical cost vs. UV cost is not linear (i.e. double the UV dose is not double the cost, while double the chemical dose is double the cost.
- Operator involvement; simple maintenance once every 10 000 h or constant monitoring of chemical dosing is not reflected.
- UV lamp replacement cost every 10 000 h is also not included – this would amount to R0.06 for a 10 min treatment.
- Life cycle cost is much more complicated than cost for chemicals vs Bulb and energy on a pilot scale (In depth studies, in the literature, of lifecycle cost for UV vs. chlorine has been done and is not part of this study).
- CAPEX is not part of this calculation (UV equipment, contact tanks, dosing equipment, additional booster pumps, etc.)

Table 5.1 Cost comparison of treatments achieving ≥ 3 log reductions of faecal coliforms in trials at pilot-scale level.

Treatment	Study	Chemicals ($R.kl^{-1}$)	Pumps electrical ($R.kl^{-1}$)	UV electrical ($R.kl^{-1}$)	Total ($R.kl^{-1}$)
Cl (3 $mg.L^{-1}$) @ 25 min	Study 3.2 (Trials 5, 10, 11)	0.60	0.25		0.85
Cl (6 $mg.L^{-1}$) @ 25 min	Study 3.2 (Trials 6, 7, 8, 9)	1.20	0.25		1.45
PAA (4 $mg.L^{-1}$) @ 25 min	Study 3.1 (Trial 1, 2, 3, 14,15)	0.90	0.25		1.15
UV (18-19 $mJ.cm^{-2}$)	Study 3.1 (Trials 1, 2, 3) Study 3.2 (Trials 4, 8, 9)		0.34	0.27	0.61
UV (24 $mJ.cm^{-2}$)	Study 1 (Trial 1)		0.34	0.27	0.61
UV (30 $mJ.cm^{-2}$)	Study 3.1 (Trials 14, 15) Study 3.2 (Trials 4, 6, 7, 10)		0.34	0.27	0.61
Cl (3 $mg.L^{-1}$) @ 25 min + UV (19 $mJ.cm^{-2}$)	Study 3.2 (Trials 5)	0.60	0.49	0.27	1.36
Cl (3 $mg.L^{-1}$) @ 25 min + UV (30 $mJ.cm^{-2}$)	Study 3.2 (Trials 4, 10, 11)	0.60	0.49	0.27	1.36
Cl (6 $mg.L^{-1}$) @ 25 min + UV (19 $mJ.cm^{-2}$)	Study 3.2 (Trials 8, 9)	1.20	0.49	0.27	1.96
Cl (6 $mg.L^{-1}$) @ 25 min + UV (30 $mJ.cm^{-2}$)	Study 3.2 (Trials 6, 7)	1.20	0.49	0.27	1.96
PAA (4 $mg.L^{-1}$) @ 25 min + UV (18 $mJ.cm^{-2}$)	Study 3.1 (Trials 1, 2, 3)	0.90	0.49	0.27	1.66
PAA (4 $mg.L^{-1}$) @ 25 min + UV (30 $mJ.cm^{-2}$)	Study 3.1. (Trials 14, 15)	0.90	0.49	0.27	1.66

Remarks

The only conclusions that can be drawn from the cost comparison in Table 5.1 is that it seems that at a basic level there are differences. Although UV seems to be cheaper the capital cost and lamp replacement costs need to be considered. For the chemical treatments, the need for storage tanks to achieve the contact times and dosing equipment need to be considered. The differences in costs are, however, significant enough to warrant a proper, detailed financial feasibility study that takes into account actual capital costs, operational cost (electricity usage and chemicals), and maintenance costs. Furthermore, the environmental and full life-cycle analysis costs should also be included (i.e. financial cost of production of chemicals or UV lamps and systems) and the health and environmental hazards of each treatment process should also be factored in. Only then will a more accurate assessment of the financial feasibility and sustainability of on-farm treatment processes be possible.

6. GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

General Conclusions

Overall, it was concluded that all disinfection treatments, whether chemical or UV or combinations, were significantly influenced by changes in water quality. Some of these changes could predictably be linked to seasonal factors (i.e. dryer summer months with a more concentrated level of pollutants, or wetter winter months resulting in either lower pollution levels as result of dilution, or sporadic increases in pollution and turbidity as result of increased run-off during rainy spells). Other differences in water quality could not be linked to seasonal variance, and were probably the result of random pollution from either point or non-point sources. Differences in treatment efficiency were also seen where the physico-chemical characteristics were very similar – these were most likely as a result of differences in the microbial population present in the water.

It was evident from the laboratory-scale studies that environmental *E. coli* strains are more resistant to disinfection than reference strains. This implies that the use of commonly encountered indicator microorganisms (such as *E. coli*) in laboratory-scale investigations may not be the most accurate method for establishing/suggesting parameters for larger scale water disinfection. Microbial analysis in all the laboratory and pilot-scale studies also revealed that FC were the most sensitive of the populations monitored, and a variety of different strategies resulted in water that conformed to the water guideline for fresh produce irrigation (<1000 faecal coliforms.100 mL^{-1}) (DWAF, 1996). It was however evident that resistant members of the ACC population always survived, and as a result of their high initial levels, carry-over to fresh produce would be highly probable. This population can still contain important food pathogens which could have a negative effect on food safety of such produce.

The phenomenon of photo-reactivation was shown to occur (more so in TC and FC's than in the ACC population, which also exhibited lower log reductions) and deserves further attention as its influence on disinfection efficiency could be significant. It was also seen that sequential UV doses, rather than one exposure to a higher dose, could be beneficial to disinfection efficiency. It was concluded that dark-repair was not likely to significantly influence the efficiency of UV disinfection of irrigation water, but could warrant further investigation.

The chemical and UV treatment trials (UV, chlorine, PAA, H_2O_2, Cl/UV, PAA/UV and H_2O_2/UV) done as part of the Pilot-scale investigations once again illustrated that each disinfection method has its advantages and disadvantages, and that the unpredictable and ever-changing microbial properties of a flowing river can have a negative influence on the reliability of treatment efficacy. It was concluded that the treatment options that should be further investigated are chlorine, PAA and UV, and possibly combinations of chlorine and PAA with UV, based on the following conclusions:

Chlorine as an irrigation water disinfectant:

- Very effective against TC and FC's, less so against the heterotrophic group (ACC). Research has also shown limited efficacy against *Cryptosporidium* and *Giardia*;
- Disinfection efficiency is not overly affected by poor water quality;
- Concerns do exist that micro-organisms will continue to build up resistance against chlorine;
- Concentrations required could result in residual levels in water which could be detrimental to the environment (residual levels of chlorine during storage of the water before irrigation could be beneficial, but the residual at time or irrigation is the determining factor);
- Disinfectant by-products (DBP's) are formed in water containing organic material;
- Long contact times are required;
- Safety issues arise during transport, storage and handling of chlorine based disinfectants;
- The overall carbon footprint of chlorine production also needs to be taken into account;
- Increasing the chlorine dose results in a linear increase in cost.

Peracetic acid (PAA) as an irrigation water disinfectant:

- Effective against TC and FC's, less so against the heterotrophic group (ACC). Research has also shown efficacy against *Cryptosporidium* and *Giardia;*
- Disinfection efficiency is affected negatively by poor water quality (especially COD, TSS, alkalinity and poor UVT%);
- Although peracetic acid decomposes in to harmless by-products such as acetic acid, hydrogen peroxide and water, concerns do exist that micro-organisms will build up resistance, resulting in higher dosages being necessary;
- The harmless decomposition by-products also results in much less formation of DBP's, but reduction of organic material in water to be treated is recommended;
- Long contact times are required;
- Safety issues arise during transport, storage and handling of peracetic acid based disinfectants;
- The overall carbon footprint of peracetic acid production also needs to be taken into account;
- Increasing the peracetic acid dose results in a linear increase in cost.

UV irradiation as an irrigation water disinfectant:

- Effective against TC and FC's, less so against the ACC population. Research has also shown better efficacy against *Cryptosporidium* and *Giardia* than chlorine and peracetic acid;
- Disinfection efficiency is affected negatively by poor water quality (especially COD, TSS, organic matter that affect the UVT%);
- Higher doses might be necessary to achieve higher log reductions;
- No disinfection by-products are formed in water during UV treatment and no chemicals are added to the water;
- Very short contact times are necessary and sequential doses are possible, although the issue of photo-reactivation (especially in highly polluted water) requires attention;
- No concerns arise due to transport, storage and handling as no corrosive chemicals need to be stored (concerns are sometimes expressed about the Mercury content of UV lamps, but these are usually contained within the protective quartz sleeve);
- The overall carbon footprint of UV lamp production also needs to be taken into account. It has been shown that although the on-site energy requirement of a UV installation is higher

than that for chlorine, the full life cycle energy requirement and greenhouse gas emissions for a UV installation are much lower those for chlorine (Hu, 2007);
- Increasing the UV dose can be achieved by pre-treatment of the water to improve the water quality in terms of UVT% (mainly by reducing COD, TSS and organic material in the water) or by increasing the lamp size (where the increase in output is not linear – i.e. doubling the dose will not necessarily involve doubling the cost).

Combinations of chlorine or peracetic acid with UV irradiation as an irrigation water disinfectant:
- Efficacy was in all cases only improved slightly (in terms of log reductions of ACC. TC and FC's);
- Similar advantages and disadvantages apply to combination treatments, such as water quality, contact time, DBP's, transport, storage and handling, as well as increased costs and the fact that the use of chlorine specifically is also being phased out in the EU;
- Optimising the pre-treatment (sand/media filtration) would most likely be more beneficial in all treatment options;
- The linear vs non-linear cost differences between increased chemical dosing and increased UV doses warrants further investigation.

Recommendations for future research

The results from the Scoping Study indicated that resistance variation between strains were evident for all the treatments (Chemical and UV). It was also observed that environmental strains (isolated from rivers and fresh produce) were in general more resistant than reference strains. This once again illustrates the ability of bacteria to adapt to environmental stress.

Treatments tested on River water samples did also indicate that disinfectant efficacy for all treatments was greatly influenced by river water quality. Water quality, measured in terms of physicochemical parameters such as COD, UVT%, TSS, pH, etc., had a direct influence on the available chlorine, and peracetic acid levels during disinfection, as well as on the degree of photo reactivation that can occur after UV irradiation. The chemical treatments (chlorine and peracetic acid) also had disadvantages in terms of their range of efficacy, DBP's, concerns about the safety and effect on the environment, microbial resistance, cost, long contact times and overall carbon footprint. UV was, however, shown to have potential as an environmentally friendly and safer disinfection treatment for polluted irrigation water.

Certain factors still need to be considered, based on the limitations of this Scoping Study. One of the most important issues to be addressed is how effective UV disinfection of water from other rivers with other physicochemical properties (than the Plankenburg River) would be. Another important question is what would the maximum tolerated limits be for quality parameters such as COD, UVT%, TSS within which optimum UV disinfection (with minimum photo recovery) can be achieved. The use of specific pre-treatment technologies to achieve water with quality parameters below these limits should also be considered for severely polluted rivers.

This Scoping Study focussed only on the microbial standards (*E. coli* < 1000 cfu.100 mL^{-1}) established for water intended for irrigation of fresh produce by the WHO and DWA (WHO, 1989; DWAF, 1996). From a food safety perspective the effect of disinfection on other important food pathogens linked to fresh produce, such as *Salmonella*, *Listeria*, entero-haemorragic *E. coli*, protozoan pathogens (i.e. *Cryptosporidium* and *Giardia*) and viruses also needs to be considered. Included in these considerations is the effect of photo reactivation and dark repair, and how it is minimised by pre-treatment technologies and increased UV dosages. From a practical point of view, it is also important to be able to better collate UV disinfection trials done at laboratory-scale on a collimated beam with what dosages are required in pilot and full-scale systems.

Therefore, the use of ultra-violet (UV) treatment of irrigation water to ensure food safety should be further researched by conducting a study on the technical and financial requirements for an on-farm irrigation water UV treatment system to ensure food safety; by

- Choosing and optimising a pre-treatment step (sand/media filtration and/or flocculation/sedimentation process) to optimise the water quality (in terms of physico-chemical characteristics such as COD, TSS, turbidity, UVT%, pH, alkalinity) for different river waters used for irrigation of fresh produce;
- Determining the microbial loads (specifically the ACC population) of different river waters used for irrigation of fresh produce and, by using a collimated beam set-up, making recommendations as to the UV dose required to sufficiently reduce the most resistant micro-organisms;
- Investigating the effect of UV (higher doses and residuals) on a wider range of indicator organisms (total and faecal coliforms), the ACC population, *Enterobacteriaceae* and specific pathogens (incl. *Salmonella*, *Listeria*, *E. coli*, Enterococci, Protozoa (*Cryptosporidium* and *Giardia*) and viruses (in terms of disinfection efficiency, photo and/or dark repair) so as to make recommendations as to the required dosages to achieve sufficient reductions of the most resistant micro-organisms implicated in food safety;
- Correlating collimated beam dosage determinations to actual (pilot or full-scale) required dosages;
- Make recommendations as to expanding current guidelines pertaining to the microbiological quality of irrigation water for fresh produce, over and above the faecal coliform guideline levels;
- To perform a comprehensive analysis of costs (capital and operational) of the selected full-scale pre-treatment technologies and UV treatment of river water of differing qualities.

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LIST OF APPENDICES:

A1. A PRELIMINARY INVESTIGATION INTO THE EFFICACY OF MEDIUM PRESSURE UV AND HYDROGEN PEROXIDE AS ON-FARM TREATMENT METHODS TO REDUCE THE MICROBIAL LOAD OF IRRIGATION WATER – MONITORING OVER THE ENTIRE IRRIGATION CYCLE

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A7. KNOWLEDGE DISSEMINATION

ARCHIVING OF DATA GENERATED DURING THE PROJECT

Large volumes of data were generated during this WRC Project. The raw data generated during this project is thus being archived at the Department of Food Science, Stellenbosch University, c/o Hammanshand and Helshoogte Roads, Stellenbosch, 7600, Western Cape, South Africa, (33˚55'45"S 18˚52'13"E)

A1. A PRELIMINARY INVESTIGATION INTO THE EFFICACY OF MEDIUM PRESSURE UV AND HYDROGEN PEROXIDE AS ON-FARM TREATMENT METHODS TO REDUCE THE MICROBIAL LOAD OF IRRIGATION WATER – MONITORING OVER THE ENTIRE IRRIGATION CYCLE

Summary

A baseline study was performed of the water at Limberlost Farms, located just outside Stellenbosch. The farm irrigates fresh produce with water obtained from the Eerste River. The study was done over a five month period, at six preselected sampling points, to determine the microbial and chemical parameters of the water so a baseline could be established to compare the results to when the ultraviolet (UV) apparatus was installed. Aerobic colony count (ACC), total coliforms (TC) and *Escherichia* coli (*E. coli*) were tested for during the microbial study, while the chemical analysis comprised of temperature, pH, conductivity, chemical oxygen demand (COD), alkalinity and total soluble solids (TSS). The UV was installed and functioning by the end of February 2013. The UV study was performed over a three month timeline, at eight different sampling points. During this study, the original six sampling points were sampled again. The additional sampling points included before and after UV. The same microbial tests were performed during the UV study, but turbidity and percentage ultraviolet transmittance (% UVT) were performed additionally during chemical analysis. During the baseline study ACC, TC and *E. coli* counts as high as 9 600 cfu.mL^{-1}, 13 799 MPN.100 mL^{-1} and 2 098 MPN.100 mL^{-1} were isolated at the river, respectively. While performing the UV treatment study ACC, TC and *E. coli* counts as high as 142 000 cfu.mL^{-1}, 241 960 MPN.100 mL^{-1} and 6 867 MPN.100 mL^{-1} were isolated at the river, respectively. As a result it was concluded that the Eerste River was mostly unsuitable for irrigation of fresh produce that are consumed raw on its own. The higher counts in the river, during the UV treatment study might be attributed to the increase in rainfall that occurred in the sampling months (March to May 2013). The counts as measured at the point of irrigation are of greater importance, since the counts present in the river might still decrease to below the guideline levels after passing through sand filters and the addition of hydrogen peroxide (the farm's current mode of treatment) or after passing through the UV in the UV treatment study. The ACC, TC and *E. coli* counts during the baseline study were as high as 8 800 cfu.mL^{-1}, 24 196 MPN.100 mL^{-1} and 85 MPN.100 mL^{-1} at the point of irrigation, respectively. After hydrogen peroxide addition log-reductions ranging between 0.0 and 2.0 were seen, but reduction was never constant. The counts at the point of irrigation remained more or less constant as at the river due to contamination that occurred at the sand filters, making the water unsuitable for irrigation of fresh produce. In the UV treatment study ACC, TC and *E. coli* counts were as high as 35 000 cfu.mL^{-1}, 10 462 MPN.100 mL^{-1} and 63 MPN.100 mL^{-1} at the point of irrigation, respectively. Log-reductions in the range of 0.0 to 1.5 were achieved, but it was inconsistent. After treatment with chlorine and re-sanding of the sand filters, no further contamination occurred and the counts decreased to below guideline limits, making the water was safe for irrigational use in terms of all of the microbial parameters, but this was not necessarily due to the effect UV had on the water. It was of great importance to find a treatment that would bring the counts in the water too below the limits required for safe irrigation since pathogens can be carried over from water onto fresh produce.

Introduction

According to numerous studies performed in the last decade it was found that the water quality of many South African rivers has been declining dramatically due to an increase in pollution levels (Paulse *et al*., 2009; Ackermann, 2010; Ijabadeniyi, 2010; Lötter, 2010; Kikine, 2011; Gemmell & Schmidt, 2012; Huisamen, 2012). Several factors are known to contribute to the condition of South

Africa's rivers. These include pollution with improperly treated human, industrial and municipal wastes due to improperly functioning or damaged sewage treatment plants, storm water overflows and agricultural effluent run-off (Schultz-Fademrecht *et al.*, 2008; Lötter, 2010). Informal settlements are yet another major source of source water contamination in South Africa, since they are mostly located upstream from areas of a river used for irrigation, thus all the waste and effluents produced wind up in the natural water sources and contribute to crop contamination (PDC, 2005; Lötter, 2010).

Many South African farmers are forced to use water from nearby rivers for crop irrigation, since it is the most affordable and sometimes only source of water available to them. It is thus of utmost importance that the quality of the water used to irrigate crops is known, since pathogens can be carried over from water onto fresh produce (EC, 2002; Ijabadeniyi *et al.*, 2011).

In the past few years consumers have started consuming more fruits and vegetables as they became increasingly aware of their health and as a result there has been a visible increase in produce-associated foodborne outbreaks. (Brackett, 1999; Pollack, 2001, Buck *et al*., 2003; Lynch *et al*., 2009; Panigrahy *et al*., 2011).

Once river water has been contaminated, little can be done to improve the quality of the water. Therefore if possible, contaminated water should not be used to irrigate fresh produce (Ackermann, 2010). Good quality water for irrigation purposes is becoming harder and more expensive to obtain (Newman, 2004; Yiasoumi *et al*., 2005). Disinfection of water is of great importance since it controls growth of microbiological pathogens in the irrigation system and reduces the risk of introducing disease to the farm and crops through irrigation water (Yiasoumi *et al*., 2005; Pehlivanoglu-Mantas *et al*., 2006).

Thus, the objective of this study was firstly to investigate the change in water quality (in terms of microbial and chemical parameters) over the entire irrigation system and secondly to investigate the efficacy of a UV treatment system in the study irrigation system.

Materials and Methods

Site selection

For the purpose of this study an appropriate site had to be selected. As part of the site selection, certain aspects had to be taken into consideration to find the most desirable site. These aspects included the irrigation water sources available on the farm, the type of contamination occurring, also referred to as the microbial loads present in the water, the type of farming, the type of vegetable or fruit crop being irrigated, the type of irrigation system used, the irrigation usage periods as well as the availability and access of the site over an extended period of time.

After visiting several potential sites, Limberlost Farms, was chosen (Fig. A1.1 & A1.2). The farm is situated approximately nine kilometres south-west of Stellenbosch on the Annandale road. Limberlost Farms is situated approximately eight kilometres downstream from where the Plankenburg and Jonkershoek Rivers merge into the Eerste River (Fig. A1.1). Water samples were obtained from several preselected sampling points along the irrigation system on Limberlost Farms, Stellenbosch. Water drawn from the Eerste River is currently used to irrigate strawberries, bell peppers and tomatoes, but passes through a series of filters and dams and is also dosed with chemicals before irrigation.

Sampling Points

Water is pumped from the Eerste River (Fig. A1.2), through sand filters (Conn 40 Manual sand filter 120 microns, South Africa) to a first holding dam (7 000-8000 m^3 in size, lined with low-density polyethylene) at a flow rate of 90 $m^3.h^{-1}$. When the first holding dam is full, it overflows into a second holding dam. After the second holding dam (7 000-8000 m^3 in size, lined with low-density polyethylene), the water passes through several sand filters (Conn 40 Manual sand filter 120 microns, South Africa) and hydrogen peroxide (H_2O_2) is added to the water as a disinfection measure. The farmer adds approximately 5-20 parts per million (ppm) H_2O_2 to achieve an end concentration of at least 1 ppm at the point of irrigation. According to NETAFIM (2009) when H_2O_2 is continuously injected at a low dosage into the irrigation cycle, the injected concentration (in the pump house at Sampling Point 4) should be between 10-50 ppm so a residual concentration (point of irrigation at Sampling Point 6) of at least 0.5 ppm could be reached for disinfection to be effective. Thereafter, the water is pumped to a holding tank (400 m^3, to facilitate contact time for the H_2O_2). Water from the holding tank is pumped, via a pump room, to the point of irrigation. An acid-mixture (sulphuric and phosphoric) dosing was regularly applied to the water leaving the holding tank to lower the pH to within the SAWQG for irrigation water, if necessary (DWAF, 1996b; Gregory, 2001). The study was divided into a baseline study (river water through on-farm irrigation system) and a UV treatment study (river water through irrigation system and UV system) (Fig. A1.3).

The sampling points for the baseline river and irrigation system water study were as follows (in sampling order) (Fig. A1.3): 1) river and sand filters; 2) inlet into first holding dam; 3) overflow from first holding dam into second holding dam; 4) after second holding dam and sand filters (H_2O_2 added); 5) in pump house after holding tank and 6) point of irrigation. The UV apparatus (Berson Inline$^+$ 100 WW, Lamp type: B810H berson Multiwave®) was installed in a closed off room after Sampling Point 4 and before Sampling Point 5. After the installation of the UV apparatus into the current irrigation system, at a point after the sand filters and before the pump room, an additional two sampling points were created.

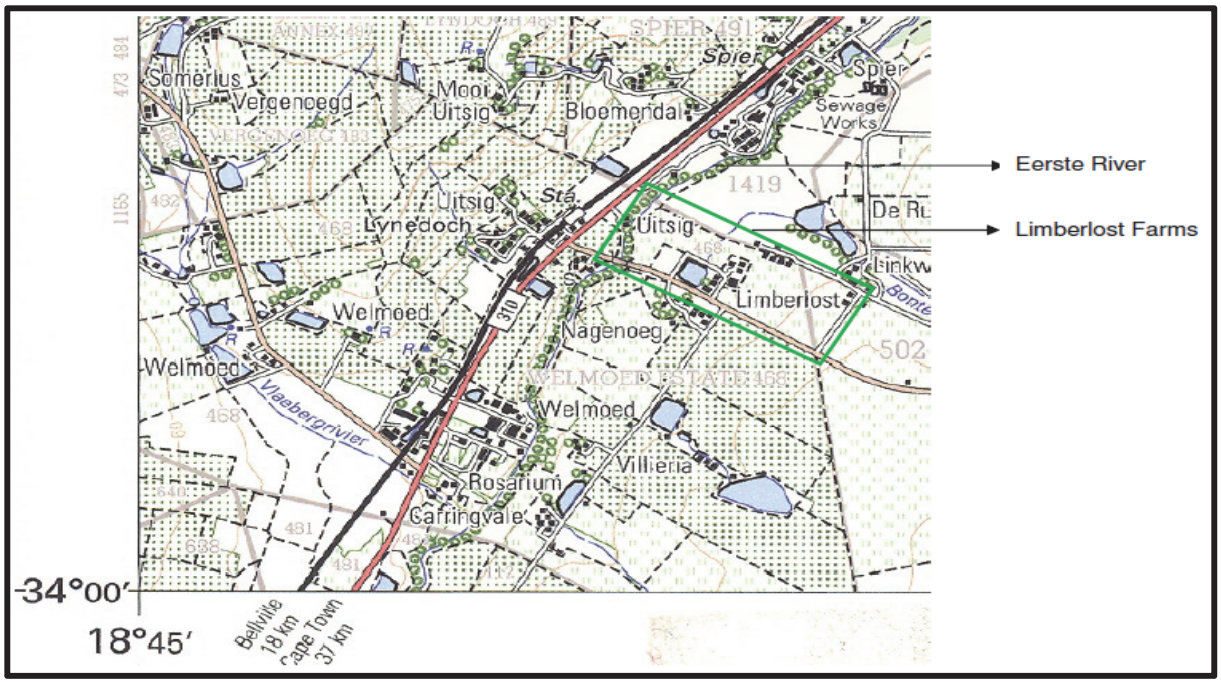


Figure A1.1 Section of a topographical map showing Limberlost Farm (Stellenbosch is to the north-east).

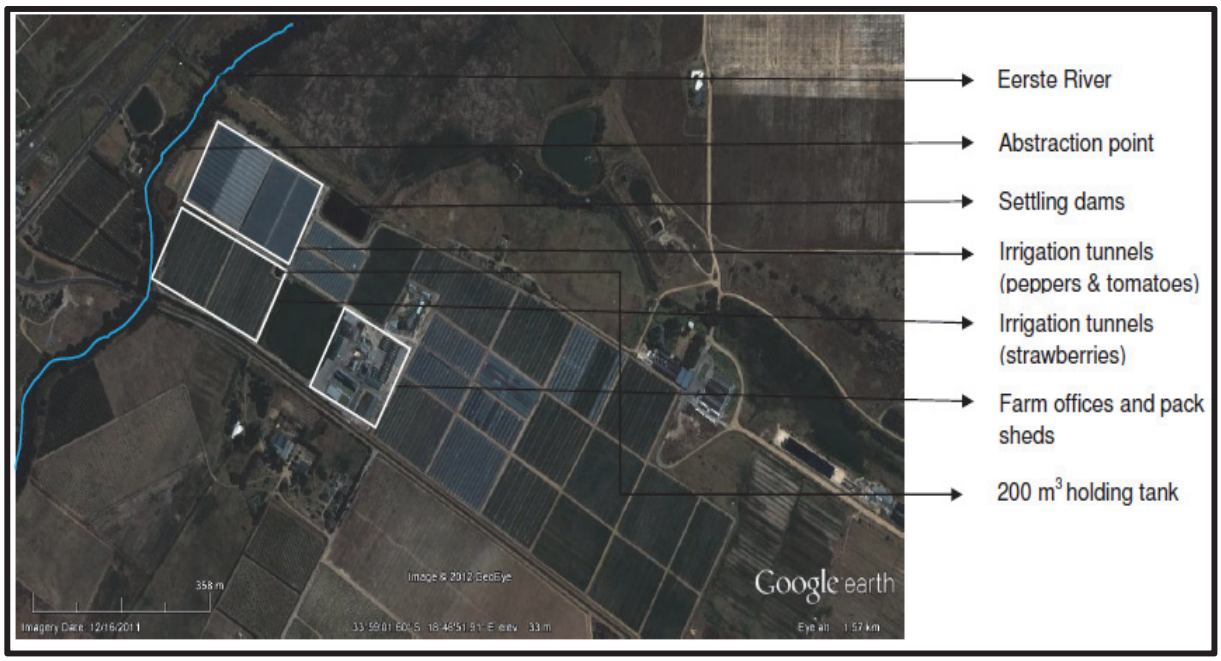


Figure A1.2 Map of Limberlost Farms and layout of farming operations and the Eerste River were samples are taken from.

a) b)

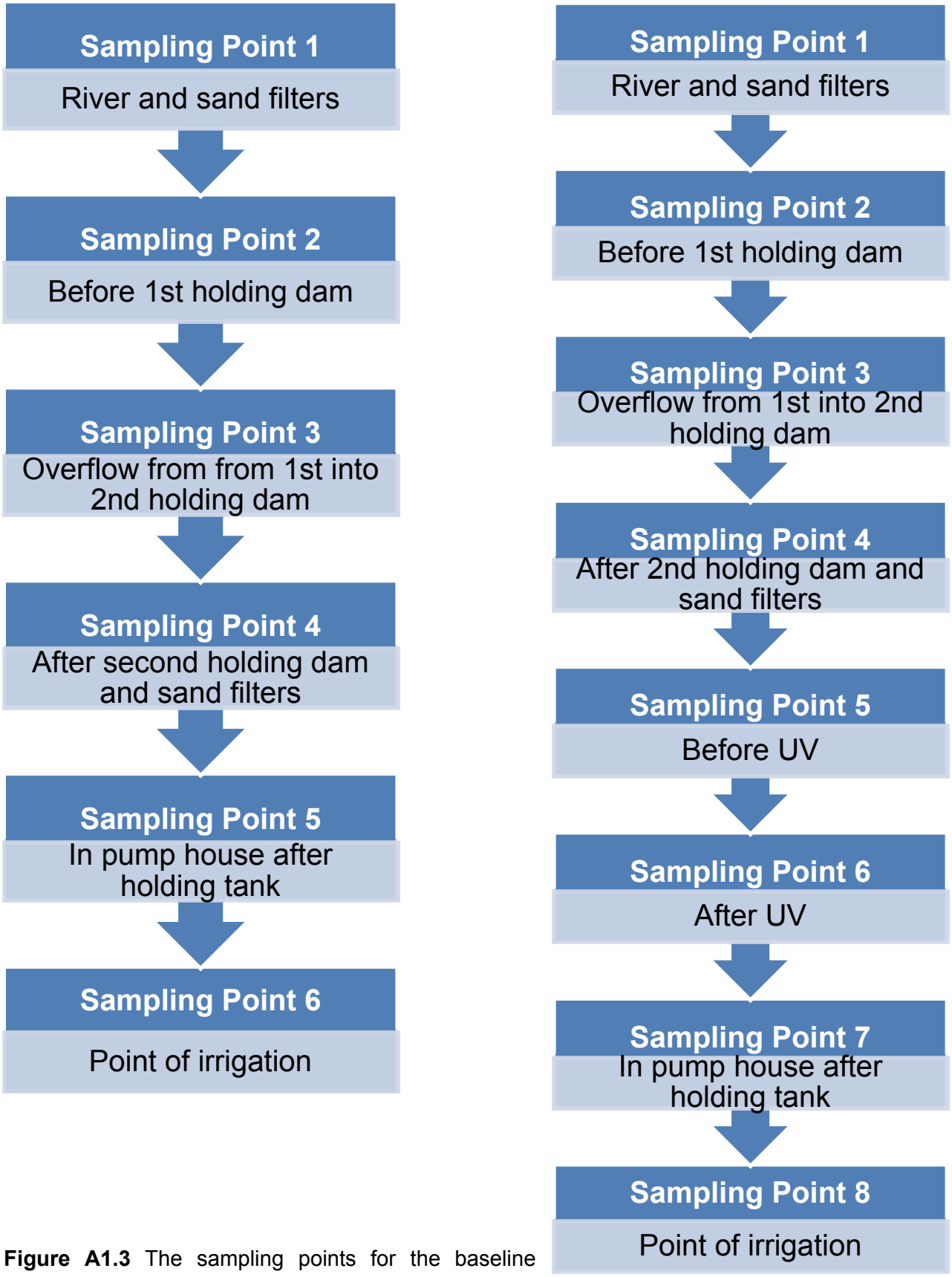


Figure A1.3 The sampling points for the baseline study (a) and UV treatment study (b).

The sampling points for the river study after the installation of UV (also referred to as the UV treatment study) are referred to as follows (in sampling order): 1) river and sand filters; 2) before first holding dam; 3) overflow from first holding dam into second holding dam; 4) after second

holding dam and sand filters; 5) before UV; 6) after UV; 7) in pump house after holding tank and 8) point of irrigation. The flow rate of the water during the UV treatment study was set at 30 $m^3.h^{-1}$.

Initially some microbiological tests were performed at different flow rates to determine whether it affects the success of the treatment. After initial testing it was decided to take samples at a flow rate of approximately 500 $L.min^{-1}$. Sampling took place over a three month period to gather information for the UV treatment study. Sampling was done from March till the end of May 2013 to determine the microbiological and chemical effects of UV on the water. For the purpose of this study, no H_2O_2 was added to the water, to investigate the efficacy of UV in reducing the microbial counts present in the water, to below the allowed limits for irrigation water.

Sampling Frequency

Nine sample sets were collected over a five and three month period during the baseline (each set consisted of samples from Sampling Points 1-6) and UV treatment study (Sampling Points 1-8), respectively. Samples were collected every one to two weeks for the duration of the microbial and chemical baseline and UV treatment study performed from October 2012 up to February 2013 and March till May 2013, respectively. Samples were collected on a Tuesday morning usually between 08h30 and 09h30, after which samples were transported back to the laboratory in cooler boxes for chemical and microbiological analysis.

Sampling method

The sampling of river and irrigation water was carried out according to the SANS 5667-6 (2006) guidelines. Safety precautions were taken, to not only ensure the safety of the sampler, but also to improve the accuracy of the results achieved. Safety measures included wearing surgical gloves and protective waterproof footgear when sampling. Sterile, Schott bottles (1L) were used to collect the water at the different sampling points (six in the baseline study and eight in the UV treatment study). The Schott bottles were sterilised and marked beforehand at the laboratory and transported in cooler boxes containing ice bricks. Samples were transported back to the lab in the cooler boxes (as close to 4°C as possible) and analysed within six hours of sampling.

For the collection of the river samples, care was taken to not disturb any sediments and the sample was taken as far away from the river bank as possible. A sterile Schott bottle (1L) was opened under the water surface and submerged to a depth of approximately 30 cm (if permitted), pointing in the direction of the water flow. The bottle was filled to the top and the cap replaced before removing it from the water. If there was a noticeable difference in the flow of the river, appearance and any accompanying odours, these were also recorded. Collection of samples from Sampling Points 2 and 3 (inlet into first holding dam and overflow into second holding dam) was performed as follows: Sterile Schott bottles (1L) were opened while water was flowing over the bottles. The bottles were held facing the flow and caps were replaced once the bottles were full, while still being held in the water flow. Sampling at all the other Sampling Points (4-8) was done at taps. The caps of the Schott bottles were removed after opening the taps and only replaced once the bottles were full. Samples at the point of irrigation were taken from the drip irrigation system (October 2012-February 2013) and from a sprinkler irrigation system (March-May 2013). All of the samples were placed upright in cooler boxes for transportation and were analysed four to six hours after sampling.

Chemical and environmental parameters

Temperature and pH
The temperature and pH of the water was measured simultaneously at each of the Sampling Points with the probe of a WTW pH320 digital pH-meter (Xylem Inc., Germany). The pH was determined according to Standard Methods (APHA, 1998).

Conductivity
The conductivity of the water was measured with a HI 8711 conductivity meter (Hanna Instruments, South Africa). The conductivity meter was calibrated once a month according to the instruction manual (Hanna Instruments, South Africa) using 12880 μS/CM @ 25°C Conductivity Calibration Solution (Hanna Instruments, South Africa). Once calibrated, the probe was placed into the sample. All air bubbles were removed before taking the reading. After calibration and between each sample the probe was cleaned with distilled water and dabbed dry with a piece of tissue paper. A reading was taken only once the display had stabilised. The units of measurement ($mS.m^{-1}$) were adjusted according to the instruction manual (Hanna Instruments, South Africa).

Turbidity
The turbidity of the water was measured with a Thermo Scientific ORION AQUAfast AQ3010 turbidity meter (Thermo Fisher Scientific Inc., United States). The meter was calibrated with Standard Calibration Solutions according to the instruction manual every time the standards no longer read within 10% of the nominal NTU value for the standard (Thermo Scientific ORION AQUAfast AQ3010 turbidity meter User Guide, United States). Once calibrated, samples were poured into glass vials up to the line and the cap replaced before being measured in the turbidity meter. The unit of measurement used is nephelometric turbidity units (NTU).

Chemical oxygen demand
A DR2000 spectrophotometer (Hach Co. Loveland, CO) and Standard Methods were used to colorimetrically determine chemical oxygen demand (COD) (APHA, 1998).

Alkalinity
Alkalinity was determined by means of a titration method as described according to Standard Methods (APHA, 1998). The unit of measurement used is mg $CaCO_3.mL^{-1}$.

Total suspended solids
Total suspended solids (TSS) was determined according to Standards Methods (APHA, 1998).

Ultraviolet transmittance (%UVT)
Ultraviolet transmittance percentage was measured with a UVT meter (Berson, Netherlands). The meter was calibrated with de-ionised water to a reading of 100% UVT. There after the de-ionised water was removed from the cuvette. The cuvette was rinsed with distilled water after which the cuvette was filled with the sample. A cap was used to cover the sample cuvette to prevent any light from penetrating and influencing the results. The results were expressed in terms of percentage.

Microbiological parameters

Aerobic colony count

The aerobic colony count (ACC) technique was performed according to the methods described in SABS ISO 4833 (2007) and Standard Methods (APHA, 1998). The Schott bottles, containing the water samples (kept as close to 4°C in the cooler boxes), were shaken vigorously before 1 mL was withdrawn to prepare a dilution series of 10^{0}-10^{-6}, for each sample. The dilutions were done in McCartney's containing 9 mL sterile saline solution. A high dilution series was prepared since it was anticipated that the water might carry a high microbial load. Using a sterile pipette, 1 mL of each dilution was carried over into correspondingly marked Petri dishes. This was performed in duplicate for each of the water samples. Approximately 10-12 mL liquefied Plate Count Agar (PCA) (Merck) was aseptically added to each of the plates to create pour plates. After the addition of PCA, each of the Petri dishes was carefully moved in a figure eight motion to ensure that the samples were evenly distributed in the agar. Once the agar had fully set, the plates were inverted and incubated at 35°C for 48 hours. Only plates containing 30 to 300 colonies were counted. The total number of coli-forming units (CFU) per millilitre was determined by taking the dilution factor of each pour plate into consideration (APHA, 1998).

Total coliforms and *Escherichia coli*

The Schott bottles, kept at refrigerator temperatures in the cooler boxes, were shaken vigorously before 10 mL was used to aseptically prepare a dilution series of 10^{-1}-10^{-5}, for each sample. The dilutions were done in 100 mL Schott bottles containing 90 mL sterile saline solution. Duplicates of each dilution to be tested were made in additional 100 mL Schott bottles, originally containing 90 mL sterile saline solution to ensure an end-sample volume of 100 mL in each. Colilert-18 (IDEXX Laboratories, South Africa) nutrient-indicator also referred to as 4-methylumbelliferyl-β-D-glucuronide (MUG) was added to each of the duplicates. After the MUG was completely dissolved, the samples were poured into Quanti-Tray's, after which they were sealed (Quanti-Tray® Sealer Model 2X) and incubated at 37°C for 18 hours. After incubation, total coliforms were determined by counting all the wells that turned yellow. *E. coli* was determined by counting all the wells that fluoresced under UV light (Spectroline® Model CM-10 Fluorescence Analysis Cabinet) in a dark environment. After the positive counts were determined, the actual total coliforms and *E. coli* counts were established by reading the values of an IDEXX Quanti-Tray®/2000 most probable number (MPN) table. Both counts were presented as MPN.100mL^{-1}.

Results and Discussion

Baseline study

Environmental and chemical results

The averages of the environmental and chemical parameters obtained from samples taken at the various sampling points on the farm, between October 2012 and February 2013, are summarised in Table A1.1. As expected the temperature of the river water increased as the ambient temperature increased (October to December 2012) and stayed relatively constant till the end of February 2013.

The river water temperature at Sampling Point 1 varied between 15.3°C in October 2012 and 19.8°C in February 2013, whereas the pH varied between 7.17 and 7.41 for the same time period. The Department of Water Affairs and Forestry (DWAF, 1996b) stipulates that there is a relationship between water temperature and the corresponding pH value (since pH can be influenced by water temperature). This might be a possible explanation for the slightly higher pH's during months with

a higher ambient temperature. According to the South African Water Quality Guidelines (SAWQG) water of an acceptable quality for irrigational purposes should have a pH value ranging between 6.5 and 8.5 (DWAF, 1996b). The pH values of the water at Sampling Point 1 always fell within the aforementioned guideline ranges, thus making the water "generally safe to use for irrigation and recreational purposes where chemical parameters are concerned" (DWAF 1996a; DWAF, 1996b). No relationship was seen between pH and temperature and the other chemical parameters such as alkalinity, conductivity, COD and TSS. The alkalinity of the river water ranged between 25.0 and 125.0 mg $CaCO_3.L^{-1}$. According to Spellman (2008), a solution's alkalinity value should be above 80.0 mg $CaCO_3.L^{-1}$, for it to have an effective buffering capacity against the environment. The alkalinity of the water was only below 80.0 mg $CaCO_3.L^{-1}$, in October 2012. In November 2012 till February 2013 the alkalinity was above 80.0 mg $CaCO_3.L^{-1}$. Thus it can be concluded that the water has a high buffering capacity and that it offers a great amount of resistance against the effect of environmental changes on the pH. Salinity is a measure of the dissolved salts that are

Table A1.1 Chemical analysis of the water during the baseline study.

Sampling date	Temperature (°C)	pH	Alkalinity (mg $CaCO_3.L^{-1}$)	Conductivity ($mS.m^{-1}$)	COD ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
Sampling Point 1						
Oct 2012	15.3	7.17	25.0	0.30	37	28
Nov 2012	17.4	7.38	100.0	0.37	23	13
Dec 2012	20.0	7.49	100.0	0.71	29	5
Jan 2013	19.9	7.53	117.0	0.71	21	4
Feb 2013	19.8	7.41	125.0	0.45	22	10
Sampling Point 2						
Oct 2012	15.9	7.07	62.5	0.29	12	37
Nov 2012	18.6	7.15	112.5	0.35	29	2
Dec 2012	21.5	7.32	143.8	0.51	23	2
Jan 2013	21.6	7.56	125.0	0.56	15	21
Feb 2013	21.6	7.37	149.8	0.53	20	33
Sampling Point 3						
Oct 2012	16.8	7.35	62.5	0.26	36	39
Nov 2012	20.0	7.00	112.5	0.34	14	6
Dec 2012	22.1	7.25	137.5	0.47	21	2
Jan 2013	22.1	7.41	125.0	0.51	22	9
Feb 2013	24.4	7.92	143.8	0.53	19	43
Sampling Point 4						
Oct 2012	17.9	7.03	100.0	0.26	14	21
Nov 2012	20.5	6.81	125.0	0.33	31	4
Dec 2012	23.1	6.80	137.5	0.46	28	3
Jan 2013	22.4	7.50	120.7	0.50	26	23
Feb 2013	23.3	8.08	143.8	0.53	19	19

Sampling date	Temperature (°C)	pH	Alkalinity (mg $CaCO_3.L^{-1}$)	Conductivity ($mS.m^{-1}$)	COD ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)
Sampling Point 5						
Oct 2012	18.2	7.00	87.5	0.25	8	17
Nov 2012	20.8	6.98	187.5	0.32	19	2
Dec 2012	21.8	7.47	125.0	0.45	46	7
Jan 2013	21.8	7.42	116.7	0.49	25	17
Feb 2013	20.6	7.64	131.3	0.53	24	8
Sampling Point 6						
Oct 2012	19.6	4.39	0.00	1.12	25	13
Nov 2012	21.7	5.91	37.5	1.24	18	6
Dec 2012	25.0	6.98	100.0	0.76	22	11
Jan 2013	25.4	7.12	95.8	0.78	15	15
Feb 2013	24.7	7.27	125.0	0.66	14	17

present in water and is measured as electrical conductivity (McCaffrey, 2011). It is important that the salt content of water used to irrigate crops is not too high, since it might damage crops or in some cases even cause soil permeability problems (McCaffrey, 2011). According to the SAWQG the Target Water Quality Range (TWQR) for electrical conductivity is 40.00 $mS.m^{-1}$ (DWAF, 1996b). The conductivity of the water samples ranged between 0.30 and 0.45 $mS.m^{-1}$ and thus never exceeded 40.00 $mS.m^{-1}$ throughout all of the sampling months, thus indicating that the water contains low salt levels ensuring that salt sensitive crops can be grown without yield decreases (DWAF, 1996b). According to the SAWQG (DWAF, 1996d), the TWQR for COD in water used for agricultural irrigation is not available; therefore it was decided to use the DWAF guidelines as set out for industrial use (DWAF, 1996c). According to DWAF (1996c), COD levels may not exceed 30.0 $mg.L^{-1}$ in water used for industrial use. The COD levels ranged between 21 and 37 $mg.L^{-1}$ during the sampling months. The COD levels for the sampling months November 2012 throughout February 2013 were all below 30 $mg.L^{-1}$. The COD levels which were below the limits were indicative of a low demand for oxygen from chemical pollution present in the water. As a result it can be concluded that the levels of chemical pollution in the water are low enough for the water to be considered acceptable for industrial use and indirectly, irrigational use (DWAF, 1996c). The COD levels of the sample taken in October 2012 were slightly higher than the limit, indicating that the water from that sample would not have been suitable for irrigation purposes (DWAF, 1996c). A limit of 0.050 $mg.L^{-1}$ is recommended by Capra & Scicolone (2007) for TSS to prevent clogging of the irrigation system. The SAWQG of TSS for irrigational use is 0-50 $mg.L^{-1}$ (DWAF, 1996b). This is also the limit referred to as the point after which uniform irrigation will be affected. The TSS values ranged between 5 and 28 $mg.L^{-1}$, thus never exceeding the SAWQG (DWAF, 1996b).

At Sampling Point 2 a small correlation could again be seen between the temperature and pH values, in that the pH increased slightly with increasing temperatures. The water temperature ranged from 15.9 to 21.6°C, whereas the pH's ranged from 7.07 to 7.56 for the same time period. The pH values all fell within the SAWQG thus making it safe for irrigational and recreational use (DWAF, 1996a; DWAF, 1996b). Again no relationship could be seen between temperature, pH and the other chemical parameters. The alkalinity of the water ranged between 62.5 and 149.8 mg $CaCO_3.L^{-1}$. The alkalinity of the water was only below the level recommended by Spellman (2008) of 80 mg $CaCO_3.L^{-1}$ in October 2012, indicating that the water had a relatively high buffering capacity in the other sampling months. The conductivity of the water ranged between 0.29 and

0.53 $mS.m^{-1}$ and never exceeded the SAWQG of 40.00 $mS.m^{-1}$ thus making the water safe for irrigational use (DWAF, 1996b). The COD levels ranged between 12 and 29 $mg.L^{-1}$ and never exceeded the SAWQG for industrial use, thus making the water suitable for use (DWAF, 1996c). The TSS values ranged between 2 and 37 $mg.L^{-1}$ and never exceeded the SAWQG of 50 $mg.L^{-1}$, thus making the water suitable for irrigational use (DWAF, 1996b; Capra & Scicolone, 2007).

At Sampling Point 3 the water temperature varied between 15.9 and 21.6°C, whereas the pH values varied from 7.00 to 7.92 for the same time period. The pH values were always within the SAWQG values, thus making it safe for irrigational and recreational use (DWAF, 1996a; DWAF, 1996b). The alkalinity of the water ranged between 62.5 and 143.8 mg $CaCO_3.L^{-1}$, while the conductivity of the water ranged from 0.26 to 0.53 $mS.m^{-1}$. Alkalinity was above 80 mg $CaCO_3.L^{-1}$ on all sampling occasions (except October 2012), indicating water of a good buffering capacity (Spellman, 2008). Conductivity of the water was also under the SAWQG values of 40.00 $mS.m^{-1}$, making it suitable for irrigational use (DWAF, 1996b). The COD and TSS levels varied between 14 and 36 $mg.L^{-1}$ and 2 and 43 $mg.L^{-1}$, respectively. The SAWQG for COD was only exceeded in October 2012, the rest of the samples fell below the guideline of 30.0 $mg.L^{-1}$, making it safe for use (DWAF, 1996c). The TSS of the water was below the SAWQG of 50 $mg.L^{-1}$ on all sampling occasions, indicating the water is suitable for a drip irrigation system (DWAF, 1996b).

The water temperature at Sampling Point 4 varied between 17.9 and 23.3°C, while the pH values ranged between 6.80 and 8.08. The pH values were always within the SAWQG, thus it was safe for recreational and irrigational use (DWAF, 1996a; DWAF 1996b). The alkalinity of the water varied from 100.0 to 143.8 mg $CaCO_3.L^{-1}$, whereas the conductivity of the water ranged between 0.26 and 0.53 $mS.m^{-1}$. Alkalinity was above 80 mg $CaCO_3.L^{-1}$ on all sampling occasions, indicating water of a good buffering capacity (Spellman, 2008). Conductivity of the water was also under the SAWQG values of 40.00 $mS.m^{-1}$, making it suitable for irrigational use (DWAF, 1996b). The COD and TSS levels ranged between 14 to 31 $mg.L^{-1}$ and 3 and 23 $mg.L^{-1}$, respectively. The SAWQG for COD was only exceeded in November 2012, the rest of the samples fell below the guideline of 30.0 $mg.L^{-1}$, making it safe for use (DWAF, 1996c). The TSS of the water was below the SAWQG of 50 $mg.L^{-1}$ on all sampling occasions, indicating the water is suitable for a drip irrigation system (DWAF, 1996b).

At Sampling Point 5 the water temperature varied between 18.2°C and 21.8°C, while the pH values ranged from 6.98 to 7.64. The pH values all fell within the SAWQG thus it is safe for irrigational and recreational use (DWAF, 1996a; DWAF, 1996b). The alkalinity of the water varied from 87.5 to 187.5 mg $CaCO_3.L^{-1}$ and was always above the recommended value of 80 mg $CaCO_3.L^{-1}$, indicating that the water has good buffering capacity (Spellman, 2008). Conductivity of the water varied between 0.25 and 0.53 $mS.m^{-1}$, thus it was always below the SAWQG of 40.00 $mS.m^{-1}$, indicating that the water contained low salt levels and were safe for irrigational use (DWAF, 1996b). The COD and TSS levels ranged from 8 and 46 $mg.L^{-1}$ and 2 to 17 $mg.L^{-1}$, respectively. The SAWQG for COD was only exceeded in December 2012, the rest of the samples were below the guideline of 30.0 $mg.L^{-1}$, making it safe for use (DWAF, 1996c). The TSS of the water always fell within the SAWQG of 50 $mg.L^{-1}$ on all sampling occasions, indicating the water is suitable for a drip irrigation system (DWAF, 1996b).

The water temperature varied from 19.6 to 25.4°C at Sampling Point 6, while the pH values varied between 4.39 and 7.27. The pH values did not meet the SAWQG guidelines in October or November 2012, thus it was not safe for irrigational and recreational use (DWAF, 1996a; DWAF, 1996b). The low pH values could be attributed to the addition of phosphoric and sulphuric acid in the pump house. These acids were added as a means to lower the pH values to within the SAWQG guidelines, but the dosing was not always correct, leading to pH values below the guidelines. The alkalinity of the water ranged between 0.0 and 125.0 mg $CaCO_3.L^{-1}$. Alkalinity was below 80 mg $CaCO_3.L^{-1}$ in October and November 2012, during the rest of the sampling

months the water had a good buffering capacity (Spellman, 2008). The alkalinity value of zero was caused by the extremely low pH of 4.39 during October 2012. The conductivity of the water varied between 0.66 and 1.24 $mS.m^{-1}$, while the COD levels ranged between 14 and 26 $mg.L^{-1}$. Conductivity of the water was always below the SAWQG values of 40.00 $mS.m^{-1}$, making it suitable for irrigational use (DWAF, 1996b). The higher than usual conductivity level could possibly be attributed to the addition of the acids to the water just before the point of irrigation. The SAWQG for COD was never exceeded and all of the samples fell below the guideline of 30.0 $mg.L^{-1}$, making it safe for use (DWAF, 1996c). The TSS values ranged between 6 and 17 $mg.L^{-1}$. The TSS of the water was below the SAWQG of 50 $mg.L^{-1}$ on all sampling occasions, indicating the water is suitable for a drip irrigation system (DWAF, 1996b).

Microbiological results

All of the water samples taken from the various sampling points from October 2012 up to the end of February 2013 were subjected to certain microbiological tests. The results obtained for aerobic colony count (ACC), total coliforms (TC) and *Escherichia coli* (*E. coli*) are represented in Figure A1.4, A1.5 and A1.6, respectively.

At Sampling Point 1 the ACC ranged between 4 500 and 9 600 $cfu.mL^{-1}$ in the river water (Fig. A1.4). No SAWQG for ACC was available in either the Agricultural Water Use: Irrigation (DWAF, 1996b) or the Field Guide (DWAF, 1996d) guidelines, thus it was decided to use the SAWQG as set out for Domestic Use (DWAF, 1996a) as a reference. According to SAWQG for Domestic Use an increased risk of disease transmission is possible when counts are higher than 1 000 $cfu.mL^{-1}$ (DWAF, 1996a). Total coliforms varied between 3 469 and 61 600 $MPN.100\ mL^{-1}$ (Fig. A1.5). No South African guideline could be found for TC present in irrigation water for the consumption of fresh produce eaten raw. Canadian guidelines, however, state that the TC count present in water used to irrigate crops that are consumed raw, should not exceed 1 000 $cfu.100\ mL^{-1}$ since it is indicative of poor water quality and treatment (Monaghan & Hutchison, 2010). The counts were higher than the Canadian regulations during all of the sampling months which indicate that this water would not be considered as safe for the irrigation of crops which are consumed raw. In previous years, Kikine (2011) and Huisamen (2012) found coliform counts as high as 13 000 000 and 7 000 000 $MPN.100mL^{-1}$ in samples tested from the Eerste River, respectively. The *E. coli* counts at Sampling Point 1 ranged from 110 to 2 098 $MPN.100\ mL^{-1}$ (Fig. A1.6). Both the World Health Organisation (WHO) and the South African Department of Water Affairs and Forestry (DWAF) have guidelines for the quality of irrigation water. According to the WHO (1989) irrigation water containing more than 1 000 faecal coliforms per 100 mL water is seen as a serious risk for the spread of disease. As *E. coli* is seen as an indicator of faecal contamination, many studies only report the count of *E. coli* present in the water. This applies to all water being used for the

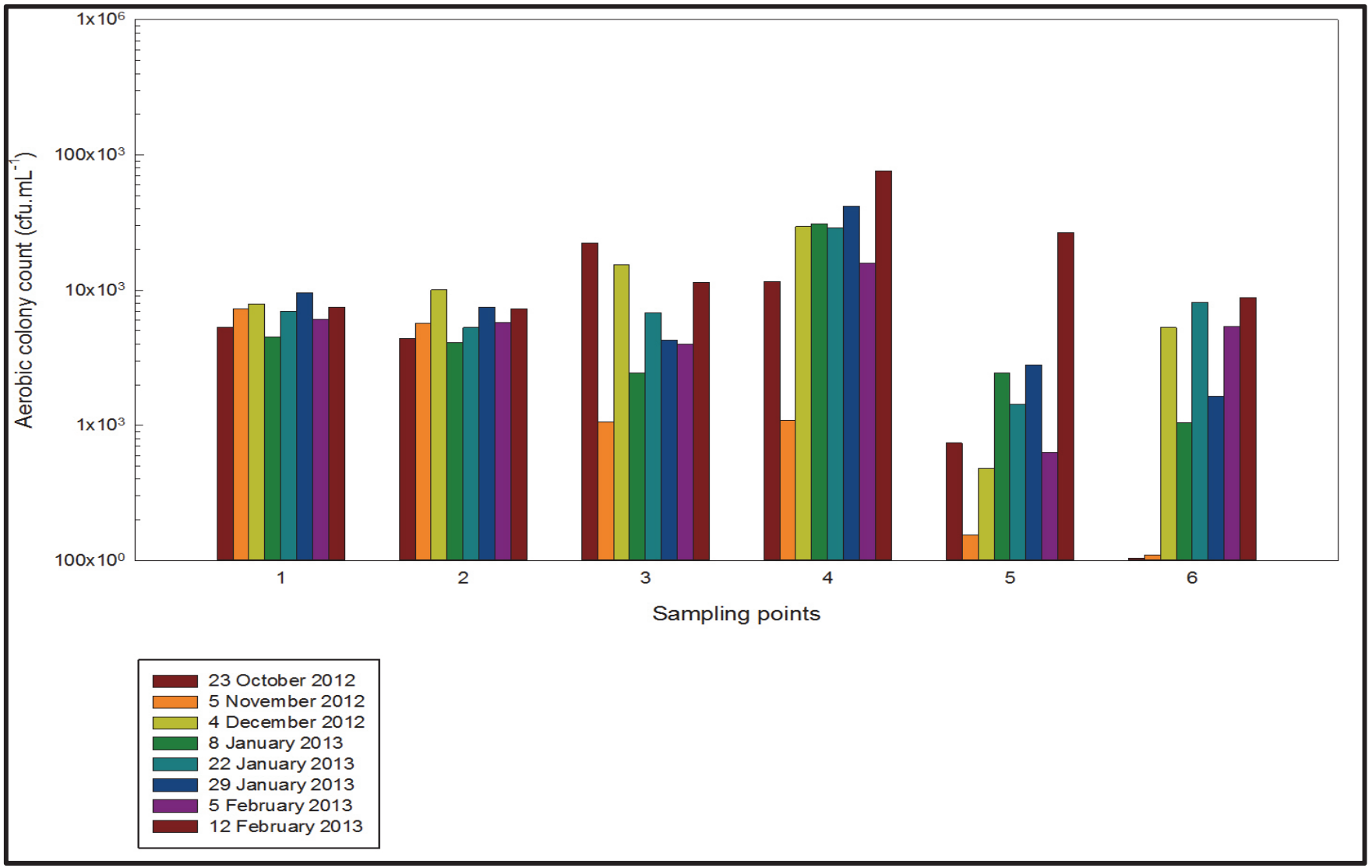


Figure A1.4 Aerobic colony counts at the six different sampling points as obtained during the baseline study, from October 2012 to February 2013.

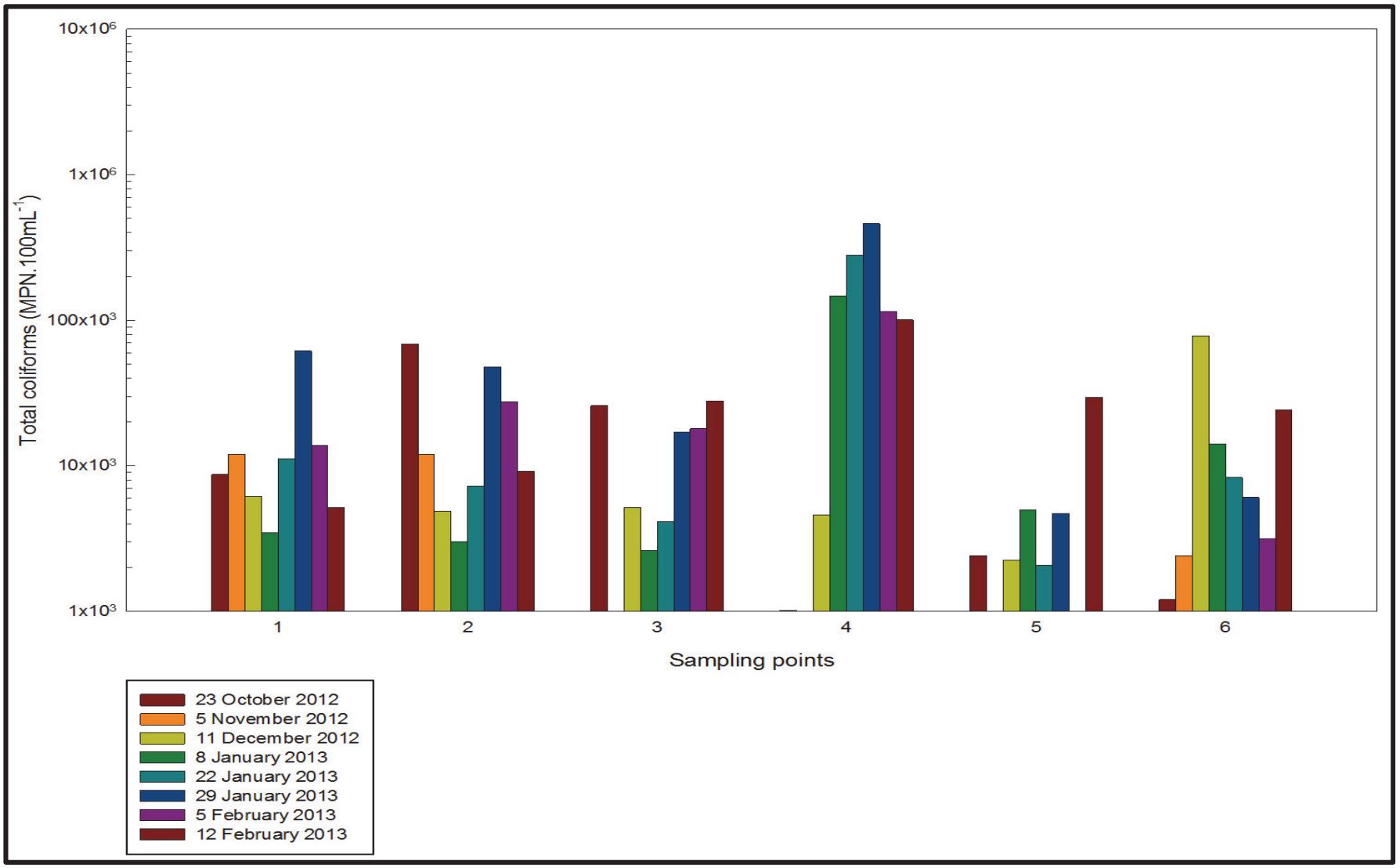

10x10⁶
1x10⁶
100x10³
10x10³
1x10³
Total coliforms (MPN.100mL⁻¹)
1
2
3
4
5
6
Sampling points
23 October 2012
5 November 2012
11 December 2012
8 January 2013
22 January 2013
29 January 2013
5 February 2013
12 February 2013

Figure A1.5 Total coliform counts at the six different sampling points as obtained during the baseline study, from October 2012 to February 2013.

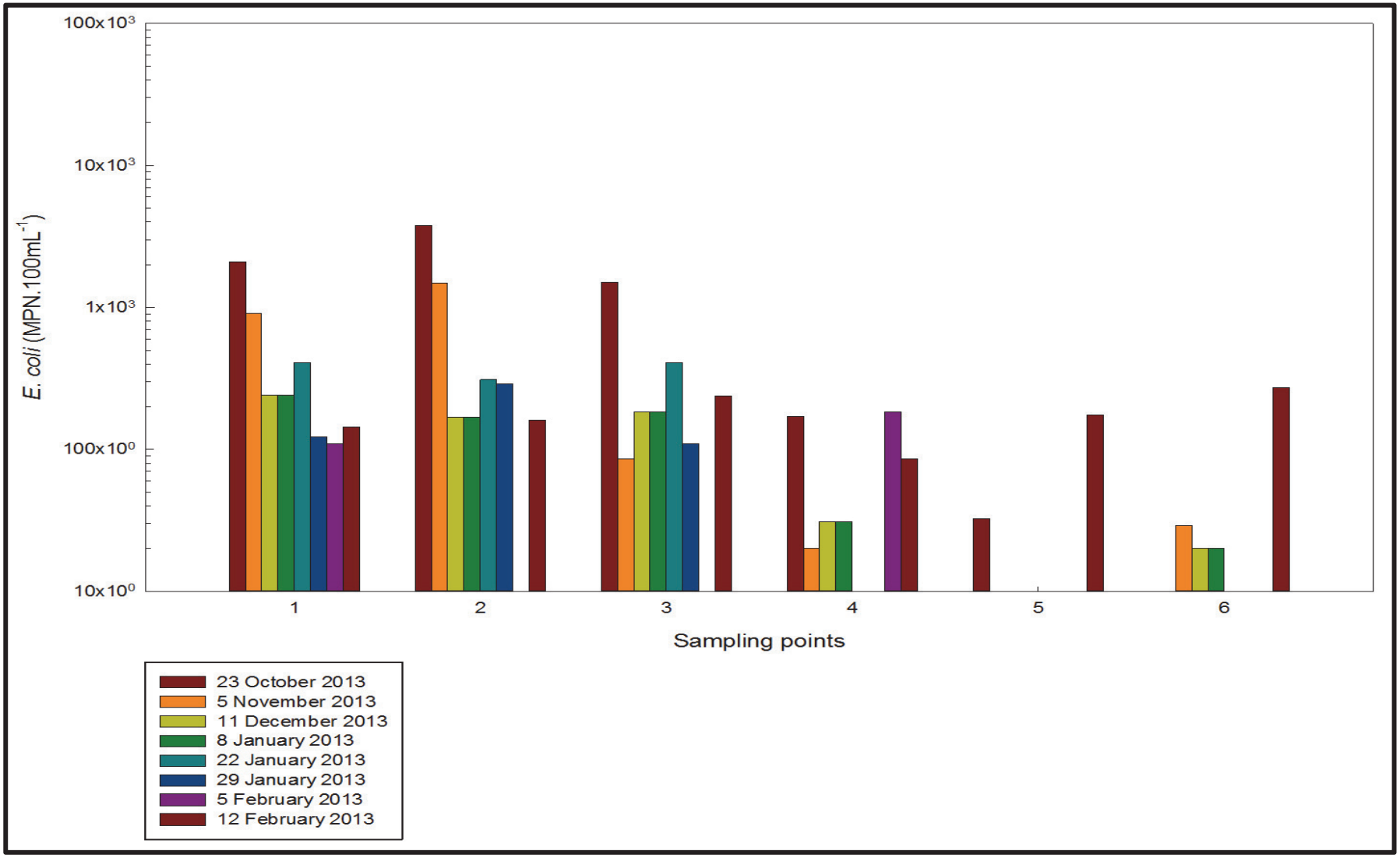


Figure A1.6 *Escherichia coli* counts at the six different sampling points as obtained during the baseline study, from October 2012 to February 2013.

irrigation of crops, irrespective of its source. According to SAWQG (DWAF, 1996b) and WHO (1989), water used for irrigation of crops may not exceed 1 000 organisms.100 mL^{-1}. The Canadian guidelines state that the *E. coli* count present in water used to irrigate crops that are consumed raw, should not exceed 100 cfu.100 mL^{-1} since it is indicative of poor water quality and treatment (Monaghan & Hutchison, 2010). Canadian guidelines for *E. coli* are a lot stricter than the SAWQG for the irrigation of fresh crops which are consumed raw. It is important to test for *E. coli* since they are almost exclusively of faecal origin and their presence is a definitive indicator of a food or water source being contaminated with faecal matter (Anon., 2011; Masters *et al*., 2011).

The *E. coli* limit for the SAWQG and WHO guideline was only exceeded in October 2012, making the water on those dates unsuitable for the irrigation of crops that is to be consumed raw (WHO, 1989; DWAF, 1996b). From November 2012 to February 2013 the SAWQG and WHO guideline was met, making the water from these sampling months suitable to use for the irrigation of fresh produce (WHO, 1989; DWAF, 1996b). Interestingly, the Canadian guidelines were only met in January and February 2013, which would have implied that the water sampled throughout all of the other sampling months were unsuitable to irrigate crops that are to be consumed raw (Monaghan & Hutchison). Although many of the river water samples exceeded the guidelines (WHO, 1989; DWAF 1996a; DWAF, 1996b; Monaghan & Hutchison, 2010) for irrigation of fresh produce, in many instances the levels were only slightly higher than the guidelines. This should be kept in mind, as water abstracted from rivers is usually not used directly for irrigation and often undergoes at least sand filtration before use.

The ACC at Sampling Point 2 ranged from 4 100 to 10 200 cfu.mL^{-1} (Fig. A1.4). The ACC were very similar to those of the river water (Sampling Point 1). It was expected that the sand filters between the river and the inlet to the first holding dam would result in a slight reduction in the microbial load (Hijnen *et al*., 2007). A possible reason for there being no decrease could be that the sand filters are used for extended periods without being re-sanded, leading to their inefficiency. An increased risk of disease transmission is possible due to ACC being higher than the SAWQG for domestic use in all of the water samples (DWAF, 1996a). The TC ranged between 3 005 and 68 670 MPN.100 mL^{-1} (Fig. A1.5). Similar to the ACC, the range for TC stayed more or less the same as at Sampling Point 1. The fact that the TC levels have not been reduced by the sand filtration means that the loads are still above the 1 000 cfu.mL^{-1} Canadian guideline (Monaghan & Hutchison, 2010). The *E. coli* counts varied between 10 and 3 790 MPN.100 mL^{-1} (Fig. A1.6). The SAWQG and WHO guideline for irrigation water (1 000 *E. coli*.100 mL^{-1}) were only exceeded in October and November 2012, thus the water could be regarded as suitable for irrigation of fresh produce in December 2012 till February 2013 in terms of the SAWQG and WHO guidelines for *E. coli* (WHO, 1989; DWAF, 1996b).

At Sampling Point 3 the ACC varied between 1 060 and 22 400 cfu.mL^{-1} (Fig. A1.4). Clear fluctuations in the ACC are visible in Figure A1.4 from Sampling Point 2 to Sampling Point 3. The ACC increased from Sampling Point 2 to Sampling Point 3 on 23 October 2012, 4 December 2012 and 12 February 2013 (Fig. A1.4). The ACC decreased from Sampling Point 2 to Sampling Point 3 on all the other sampling dates. The water was not safe for domestic use due to an increased risk of disease transmission in terms of ACC, since the SAWQG of 1 000 cfu.mL^{-1} were exceeded on all of the sampling occasions (DWAF, 1996a). The TC ranged between 581 and 27 900 MPN.100 mL^{-1} (Fig. A1.5). The TC levels at Sampling Point 3 were only once (November 2012) below the 1 000 cfu.mL^{-1} guideline for safe irrigation water as set out in the Canadian guideline (Monaghan & Hutchison, 2010). Thus on all other sampling dates, the water would still be considered unsuited for irrigational use (Monaghan & Hutchison, 2010). The TC loads remained more or less the same from Sampling Point 2 to Sampling Point 3 on most of the sampling occasions (Fig. A1.4), with increases on some occasions and decreases on others. The *E. coli* counts ranged between 10 and 1 505 MPN.100 mL^{-1} (Fig. A1.6). The SAWQG and WHO guideline of 1 000 *E. coli*.100 mL^{-1}

were only exceeded in October 2012. Thus, at this stage of the irrigation cycle, the water can mostly be regarded as safe for irrigation of crops that are consumed raw (DWAF, 1996b). It can clearly be seen in Figure A1.6 that the *E. coli* loads remained relatively constant from Sampling Point 2 to Sampling Point 3. The sample for Sampling Point 3 was taken at the overflow from the first holding dam to the second holding dam. The overflow channel was quite narrow and not very deep (2 to 15 centimetres depending on the flow rate). Since the dams are open to the environment, birds have access to them and as a result the overflow area was sometimes covered with bird faeces. This might be a possible explanation for the increased counts visible (on some of the sampling dates) between Sampling Point 2 and 3. Decreases might be attributed to higher flow at the overflow channel, thus less sediment and faeces might have been part of the sample, attributing to the lower loads on certain sampling occasions.

The ACC ranged between 1 090 and 76 000 cfu.mL^{-1} at Sampling Point 4 (Fig. A1.4). It can clearly be seen in Figure 4 that ACC remained more or less constant from Sampling Point 3 to Sampling Point 4 during October and November 2012, respectively. Thereafter, ACC at Sampling Point 4 were higher than the levels at Sampling Point 3. A log-reduction, of 0.5 to 1.0 was visible in ACC after passing through the sand filters. Although there is no guideline in the SAWQG for irrigation water, the guideline for domestic water of 1 000 cfu.mL^{-1} was exceeded (DWAF, 1996a). The TC counts ranged from 683 to 461 100 MPN.100 mL^{-1} (Fig. A1.5). Similar to the trend seen for ACC, it can clearly be seen in Figure A1.5 that TC loads decreased from Sampling Point 3 to Sampling Point 4 during October and November 2012, respectively, but from 8 January 2013 and onwards, the TC loads at Sampling Point 4 were higher than the levels at Sampling Point 3 (Fig. A1.5). The TC levels were only below 1 000 MPN.mL^{-1} in October to December 2012 (i.e. below the Canadian guidelines for TC) (Monaghan & Hutchison, 2010), but exceeded these levels thereafter, displaying large increases after passing through the sand filters. Increases as high as 2.0 logs were seen on certain sampling dates. The *E. coli* counts ranged between 10 and 272 MPN.100 mL^{-1}. In Figure A1.6 it can clearly be seen that the *E. coli* loads mostly decreased from Sampling Point 3 to Sampling Point 4, except on 5 February 2013 when it increased. The SAWQG was met on all sampling occasions, thus the water was presumed as safe for the irrigation of fresh produce in terms of *E. coli* counts (DWAF, 1996b). It was expected that microbial loads would be reduced between Sampling Point 3 and Sampling Point 4, due to sand filtration that takes place (Hijnen *et al*., 2007). This was, however, not the case for ACC and TC, which after initial decreases from Sampling Point 3 to Sampling Point 4, actually increased. The *E. coli* counts (except for one instance) decreased from Sampling Point 3 to Sampling Point 4. A possible explanation for the increased ACC and TC loads, from December 2012 onwards could be caused by the sand filters which are used for extended periods without being re-sanded. As a result the filters might be clogged and not filtering properly. A possible explanation for this anomaly could be that a biofilm had formed throughout the sand, consisting mainly of ACC and TC, thus explaining the increase in counts at Sampling Point 4. The biofilm could thus be "trapping" the *E. coli*, which would possibly be "out competed" and thus die off, resulting in lower *E. coli* levels.

The on-farm irrigation system included a H_2O_2 dosing step, which took place after the second holding dam and sand filters (Sampling Point 4) and the holding tank and pump house (Sampling Point 5). The function of the holding tank is to facilitate the necessary H_2O_2 contact time. Water is pumped from the holding tank, via the pump house (Sampling Point 5) to the point of irrigation (Sampling Point 6). At Sampling Point 5 the ACC ranged from 155 to 26 800 cfu.mL^{-1} (Fig. A1.4). It can be seen that in all sampling instances the ACC decreased from Sampling Point 4 to Sampling Point 5 (Fig. A1.4). The log-reductions, however, ranged from 0.5 to 1.5, but in most instances it was less than 1.0. The efficiency of the H_2O_2 dosing thus varies and can be ascribed to insufficient contact time or ineffective dosing of H_2O_2 (either the dosed amount varying or variations in the water flow rate. The TC counts varied between 282 and 29 500 MPN.100 mL^{-1}

(Fig. A1.5). In Figure A1.5 it can clearly be seen that the TC loads decreased from Sampling Point 4 to Sampling Point 5, except on 23 October 2012 when an increase occurred (Fig. A1.5). The log-reduction in TC after treatment with H_2O_2 ranged from 0.5 to 2.0, but in most instances was also less than 1.0. *E. coli* counts ranged between 10 and 175 MPN.100 mL^{-1} (Fig. A1.6). In Figure A1.6 it can be seen that the counts decreased or remained constant from Sampling Point 4 to Sampling Point 5, except on 12 February 2013 when an increase in counts occurred. The addition of H_2O_2 thus resulted in the *E. coli* counts decreasing by between 0.01 and 0.5 logs. Since the counts were so low (10 to 272 MPN.100 mL^{-1}) after the sand filters (Sampling Point 4), it was expected that the addition of H_2O_2 would be more effective in reducing the *E. coli* counts, but this was not the case. The *E. coli* levels were, however, below the SAWQG of 1 000 cfu.100 mL^{-1}, making it safe to use for the irrigation of crops that are consumed raw (DWAF, 1996b). Log-reductions ranging from 0.5 to 1.5, 0.5 to 2.0 and 0.01 to 0.5 were achieved for ACC, TC and *E. coli*, respectively, during the baseline study. The average overall log-reduction achieved during the baseline study (H_2O_2 dosing) for ACC, TC and *E. coli* was 1.07, 1.13 and 0.65, respectively.

Sampling Point 6, the point of irrigation, was the most critical point in terms of meeting the guidelines for crops being irrigated that are to be consumed raw without any further hurdles implemented to reduce possible microbial loads. The ACC varied between 104 and 8 800 cfu.mL^{-1} (Fig. 4). The SAWQG for domestic use was only met on the first two sampling dates (DWAF, 1996a). Total coliform counts ranged from 1 203 to 24 196 MPN.100 mL^{-1} (Fig. A1.5). The counts were always above the Canadian guidelines, thus indicating an increased risk of infective disease transmission (Monaghan & Hutchison, 2010). During some sampling weeks there was an increase in ACC's and TC visible in the water from the pump house (Sampling Point 5) to the point of irrigation (Sampling Point 6). This could possibly be attributed to a dirty pipe system or the presence of a biofilm in the pipes from the pump house to the point of irrigation. As the irrigation system (from pump house to point of irrigation) experiences times of non-use, this could provide the opportunity for biofilm formation within the system. This is also more plausible, considering that the H_2O_2 dosing was not very effective in reducing microbial loads. The *E. coli* counts ranged between 5.2 and 85 MPN.100 mL^{-1} (Fig. 6). The SAWQG was met on all of the sampling dates, thus the water could be considered safe for irrigation of fresh crops in terms of *E. coli* counts (DWAF, 1996b).

UV treatment study

Environmental and chemical results

The averages of the chemical parameters obtained from samples taken at the various sampling points on the farm, between March and May 2013 during the UV treatment study, are summarised in Table A1.2. During this part of the study the river water temperature decreased as the ambient temperatures decreased. This can be attributed to the change in seasons

Table A1.2 Chemical analysis of the water during the UV treatment study.

Sampling date	Temp. (°C)	pH	Alkalinity (mg $CaCO_3.L^{-1}$)	Conduct. ($mS.m^{-1}$)	COD ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)	Turbidity (NTU)
Sampling Point 1							
Mar 2013	19.3	7.16	154.2	0.56	17	9	4.44
Apr 2013	15.9	6.82	120.8	0.35	43	19	137.97
May 2013	13.4	6.18	116.7	0.38	32	11	12.60
Sampling Point 2							
Mar 2013	21.0	7.13	170.8	0.53	12	12	3.95
Apr 2013	17.4	6.91	104.2	0.42	22	25	5.48
May 2013	14.5	6.25	108.3	0.43	14	4	5.61
Sampling Point 3							
Mar 2013	21.6	7.21	158.3	0.50	23	14	3.69
Apr 2013	18.0	7.20	125.0	0.46	27	25	4.45
May 2013	14.5	6.43	120.8	0.44	15	12	4.17
Sampling Point 4							
Mar 2013	21.6	7.23	162.5	0.50	18	12	5.75
Apr 2013	17.8	6.96	116.7	0.45	21	21	4.88
May 2013	14.8	6.38	116.7	0.44	95	10	3.89
Sampling Point 5 + 6							
Mar 2013	22.2	7.25	150.0	0.49	20	6	3.79
Apr 2013	18.1	7.07	120.8	0.46	32	26	3.50
May 2013	14.9	6.44	108.3	0.44	25	7	3.09
Sampling Point 7							
Mar 2013	21.8	7.29	150.0	0.51	38	3	4.00
Apr 2013	18.5	7.11	108.3	0.47	35	25	3.72
May 2013	15.1	6.30	108.3	0.44	27	7	2.08
Sampling Point 8							
Mar 2013	24.2	7.14	145.8	0.50	26	5	4.40
Apr 2013	19.7	6.94	129.2	0.49	21	19	2.87
May 2013	15.5	6.30	100.0	0.46	19	1	1.94

The river water temperature at Sampling Point 1 ranged between 19.3°C in March and 13.4°C in May 2013, with river water temperature decreasing as ambient temperature decreased. The pH varied between 7.16 and 6.18 for the same time period. As seen in the baseline study,

there was a correlation between temperature and pH, with the temperatures decreasing from March to May 2013, while the corresponding pH values also decreased. According to the SAWQG (DWAF, 1996b) the water was not always considered of acceptable quality for irrigational purposes, as the pH during the month of May 2013 was below 6.5. During this study no visible link could be found between pH and temperature and the other chemical parameters. The alkalinity of the river water ranged between 116.7 and 154.2 mg $CaCO_3.L^{-1}$, well above the lower limit recommended by Spellman (2008), thus it could be concluded that the water always had an effective buffering capacity. The conductivity of the water varied from 0.35 and 0.56 $mS.m^{-1}$ and was always below the SAWQG value of 40.00 $mS.m^{-1}$, making it suitable for irrigational use (DWAF, 1996b). According to the SAWQG (DWAF, 1996d) the TWQR for COD in water used for irrigational purposes is not available, but water used in industry should have a COD value below 30 $mg.L^{-1}$ (DWAF, 1996c). The COD levels ranged between 17 and 43 $mg.L^{-1}$ and were only below 30 $mg.L^{-1}$ in March, and thus would not be regarded as suitable in terms of industrial use (DWAF, 1996c). The TSS values varied between 9 and 19 $mg.L^{-1}$, thus never exceeding the SAWQG of 50 $mg.L^{-1}$, making it suitable to use for drip irrigation (DWAF, 1996b). The turbidity ranged from 4.44 and 137.97 NTU. Measuring turbidity gives an estimate of suspended solids in the water (McCaffrey, 2011; Elqert, 2012). Though high turbidity is often a sign of poor water quality and land management – crystal clear water does not always guarantee healthy water. Extremely clear water can signify very acidic conditions or high levels of salinity (McCaffrey, 2011). According to the SAWQG Field Guide (DWAF, 1996d), there is no TWQR for turbidity for agricultural irrigation. The SAWQG for domestic use is set at 1.00 NTU (DWAF, 1996a) – a quality which was not met in any instances. During April 2013, when the turbidity of the river water was extremely high, the water was brown and had a murky appearance. According to Daphne *et al*. (2011) there is a positive correlation between TSS and turbidity taken from river water samples. After reviewing Table A1.2, a slight correlation was seen between TSS and turbidity within the first three sampling points, respectively, but this trend was not followed all the way through to Sampling Point 8.

A relationship was again visible between the temperature and pH values measured at Sampling Point 2. The water temperature ranged from 14.5°C to 21.0°C, whereas the pH values varied between 6.25 and 7.13 for the same time period. The pH values only fell within the SAWQG (6.5 to 8.5) during March and April 2013, while the samples taken in May 2013 did not adhere to the SAWQG (DWAF, 1996b). Again no relationship could be found between temperature, pH and the other chemical parameters. The alkalinity of the water ranged between 104.2 and 170.8 mg $CaCO_3.L^{-1}$ and was thus always above the 80 mg $CaCO_3.L^{-1}$ recommended by Spellman (2008). The conductivity of the water varied between 0.42 and 0.53 $mS.m^{-1}$ and never exceeded the SAWQG of 40.00 $mS.m^{-1}$, making the water suitable for irrigational use (DWAF, 1996b). The COD levels ranged between 12 and 22 $mg.L^{-1}$ and thus never exceeded the SAWQG of 30 $mg.L^{-1}$ set for industrial use (DWAF, 1996c). The TSS values ranged from 4 and 25 $mg.L^{-1}$ and never exceeded the set SAWQG of 50 $mg.L^{-1}$, making the water suitable for use in drip irrigation systems (DWAF, 1996b). The turbidity of the water ranged between 3.95 and 5.61 NTU, thus exceeding the SAWQG for domestic use during all of the sampling occasions (DWAF, 1996a).

The water temperature varied between 14.5 and 21.6°C at Sampling Point 3, while the pH values varied from 6.43 to 7.21 for the same time period. The pH values only fell within the SAWQG (6.5 to 8.5) for irrigation water during March and April 2013 (DWAF, 1996b). The alkalinity of the water varied from 120.8 to 158.3 mg $CaCO_3.L^{-1}$, whereas the conductivity ranged from 0.44 to 0.50 $mS.m^{-1}$. Alkalinity was above 80 mg $CaCO_3.L^{-1}$ on all sampling occasions, indicating water of a good buffering capacity (Spellman, 2008). Conductivity of the water was also under the SAWQG values of 40.00 $mS.m^{-1}$, making it suitable for irrigational use (DWAF, 1996b). The COD and TSS levels ranged between 15 and 27 $mg.L^{-1}$ and 12 to 25 $mg.L^{-1}$, respectively. The TSS of the water was below the SAWQG of 50 $mg.L^{-1}$ on all sampling occasions, indicating the

water to be suitable for a drip irrigation system (DWAF, 1996b). The turbidity varied from 3.69 to 4.45 NTU. Even though the values are relatively low, they were still above the SAWQG for domestic use (DWAF, 1996a).

The water temperature at Sampling Point 4 varied from 14.8 to 21.6°C, while the pH values ranged between 6.38 and 7.23. The pH values only fell within the SAWQG (6.5 to 8.5) during March and April 2013, making it unsuitable for irrigational use in May (DWAF, 1996b). The alkalinity of the water varied between 116.7 and 162.5 mg $CaCO_3.L^{-1}$, while the conductivity ranged from 0.44 to 0.50 $mS.m^{-1}$. Alkalinity was above 80 mg $CaCO_3.L^{-1}$ during all of the sampling months, indicating water of a good buffering capacity (Spellman, 2008). Conductivity of the water was also under the SAWQG values of 40.00 $mS.m^{-1}$, making it suitable for irrigational use (DWAF, 1996b). The COD and TSS levels varied between 18 and 95 $mg.L^{-1}$ and 10 to 21 $mg.L^{-1}$, respectively. The TSS of the water always fell within SAWQG of 50 $mg.L^{-1}$ on all sampling occasions, indicating the water is suitable for a drip irrigation system (DWAF, 1996b). The turbidity of the water varied between 3.89 and 5.75 NTU. The values exceeded the SAWQG for domestic use, making it unsuitable for use (DWAF, 1996a).

At Sampling Point 5+6, where the UV apparatus was installed, the water temperature ranged from 14.9 to 22.2°C, whereas the pH values ranged from 6.44 to 7.25. The pH values only fell within the SAWQG during March and April 2013, making it unsuitable for irrigational use in May (DWAF, 1996b). The alkalinity of the water varied between 108.3 and 150.0 mg $CaCO_3.L^{-1}$. The water has good buffering capacity since alkalinity was always above 80.0 mg $CaCO_3.L^{-1}$ (Spellman, 2008). The conductivity of the water varied from 0.44 to 0.49 $mS.m^{-1}$, while the COD levels varied between 20 and 32 $mg.L^{-1}$. Conductivity of the water always was suitable for irrigational use since it always fell within the SAWQG values of 40.00 $mS.m^{-1}$ (DWAF, 1996b). The SAWQG for COD was only exceeded in April 2013, the rest of the samples fell below the guideline of 30.0 $mg.L^{-1}$, making it safe for use (DWAF, 1996c). The TSS values varied from 6 to 26 $mg.L^{-1}$, whereas the turbidity values ranged between 3.09 and 3.79 NTU. The TSS of the water met the SAWQG on all sampling occasions, indicating the water is suitable for a drip irrigation system (DWAF, 1996b). The turbidity counts exceeded the SAWQG for domestic use, making it unsuitable for use (DWAF, 1996a).

At Sampling Point 7 the water temperature ranged between 15.1 and 21.8°C, while the pH values ranged from 6.30 to 7.29. The pH values only fell within the SAWQG (6.5 to 8.5) during March and April 2013, making it unsuitable for irrigational use in May (DWAF, 1996b). The alkalinity of the water ranged from 108.3 to 150.0 mg $CaCO_3.L^{-1}$, thus the water has a relatively good buffering capacity since the values was always above 80.0 mg $CaCO_3.L^{-1}$ (Spellman, 2008). The conductivity of the water varied between 0.44 and 0.51 $mS.m^{-1}$, whereas the COD levels ranged between 27 and 38 $mg.L^{-1}$. Conductivity of the water was below the SAWQG of 40.00 $mS.m^{-1}$ on all occasions, making it suitable for irrigational use (DWAF, 1996b). The SAWQG for COD was only met in May 2013, making the rest of the samples unsuitable for use (DWAF, 1996c). The TSS values varied between 3 and 25 $mg.L^{-1}$, while turbidity values ranging between 2.08 and 4.00 NTU was observed. The SAWQG for TSS was always met (DWAF, 1996b). Even though the turbidity loads are relatively low, it was still above the SAWQG for domestic use, making it unsuitable for use (DWAF, 1996a).

The water temperature varied between 15.5 and 24.2°C at Sampling Point 8, while the pH values ranged between 6.30 and 7.14. The SAWQG for pH was only met during March and April 2013, making it unsuitable for irrigational use in May (DWAF, 1996b). The alkalinity of the water varied between 100.0 and 145.8 mg $CaCO_3.L^{-1}$, thus according to Spellman (2008) the water has relatively good buffering capacity since alkalinity was always above 80.0 $mg.L^{-1}$. The conductivity of the water ranged from 0.46 to 0.50 $mS.m^{-1}$, making it suitable for irrigational use since it always fell within the SAWQG of 40.00 $mS.m^{-1}$ (DWAF, 1996b). The COD and TSS levels varied between

19 to 26 mg.L^{-1} and 1 to 19 mg.L^{-1}, respectively. The SAWQG for COD of 30.0 mg.L^{-1} was met during all of the sampling occasions, making it safe for use (DWAF, 1996c). The TSS is suitable for a drip irrigation system since the SAWQG was always met (DWAF, 1996b). Turbidity values varied from 1.94 to 4.40 NTU. The loads exceeded the SAWQG for domestic use, making it unsuitable for use (DWAF, 1996a).

Microbiological results

The results for ACC on water samples from Sampling Point 1-8 are given in Figure A1.7. The ACC in the river water ranged between 900 and 142 000 cfu.mL^{-1} at Sampling Point 1 (Fig. A1.7). Even though there are no guidelines for ACC in irrigation water in South Africa, it is worth noting that counts greater than 1 000 cfu.mL^{-1} in domestic water, are considered to increase the risk of transmitting disease (DWAF, 1996a). The TC counts ranged between 2 723 and 241 960 MPN.100 mL^{-1} (Fig. 8). In the absence of South African guidelines for TC in irrigation water, the Canadian guideline of 1 000 cfu.100 mL^{-1} can be used as a comparison (Monaghan & Hutchison, 2010). The counts were higher than the Canadian guidelines during all of the sampling months, indicating an increased safety risk when irrigating fresh crops that are consumed raw (Monaghan & Hutchison, 2010). The *E. coli* counts ranged from 197 to 6 867 MPN.100 mL^{-1} (Fig. 9). The SAWQG and WHO *E. coli* limit of 1 000 organisms.100 mL^{-1} was only exceeded on 2 April and 28 May 2013 (WHO, 1989; DWAF, 1996b). The SAWQG and WHO guideline for *E. coli* of less than 1 000 organisms.100 mL^{-1} was met on all of the other sampling occasions (WHO, 1989; DWAF, 1996b). Although many of the river water samples exceeded the guidelines for irrigation of fresh produce, in many instances the levels were only slightly higher than the guidelines. This should be kept in mind, as water abstracted from rivers is usually not used directly for irrigation and often undergoes at least sand filtration before use. The overall microbial counts for ACC, TC and *E. coli* were a lot higher at the river (Sampling Point 1) during the UV treatment study (900 to 142 000 cfu.mL^{-1} for ACC, 2 723 to 241 960 MPN.100 mL^{-1} for TC and 195 to 6 867 MPN.100 mL^{-1} for *E. coli*) than in the baseline study (4 500 to 9 600 cfu.mL^{-1} for ACC, 3 468.5 to 61 600 MPN.100 mL^{-1} for TC and 110 to 2 098 MPN.100 mL^{-1} for *E. coli*). The baseline study was conducted from October 2012 to February 2013, the summer months in the Southern Hemisphere, while the UV treatment study was done from March 2013 to May 2013 (autumn and beginning of winter). According to Bruhn & Wolfson (2007) ultraviolet rays from the sun might kill bacteria on a warm and sunny day, leading to lower than expected counts. Heavy storms and rainfall have also been shown to contribute to higher microbial counts in rivers due to storm water overflows, pollution and runoff from pastures and wastewater treatment plant overflows (Kistemann *et al*., 2002; Hill *et al*., 2006).

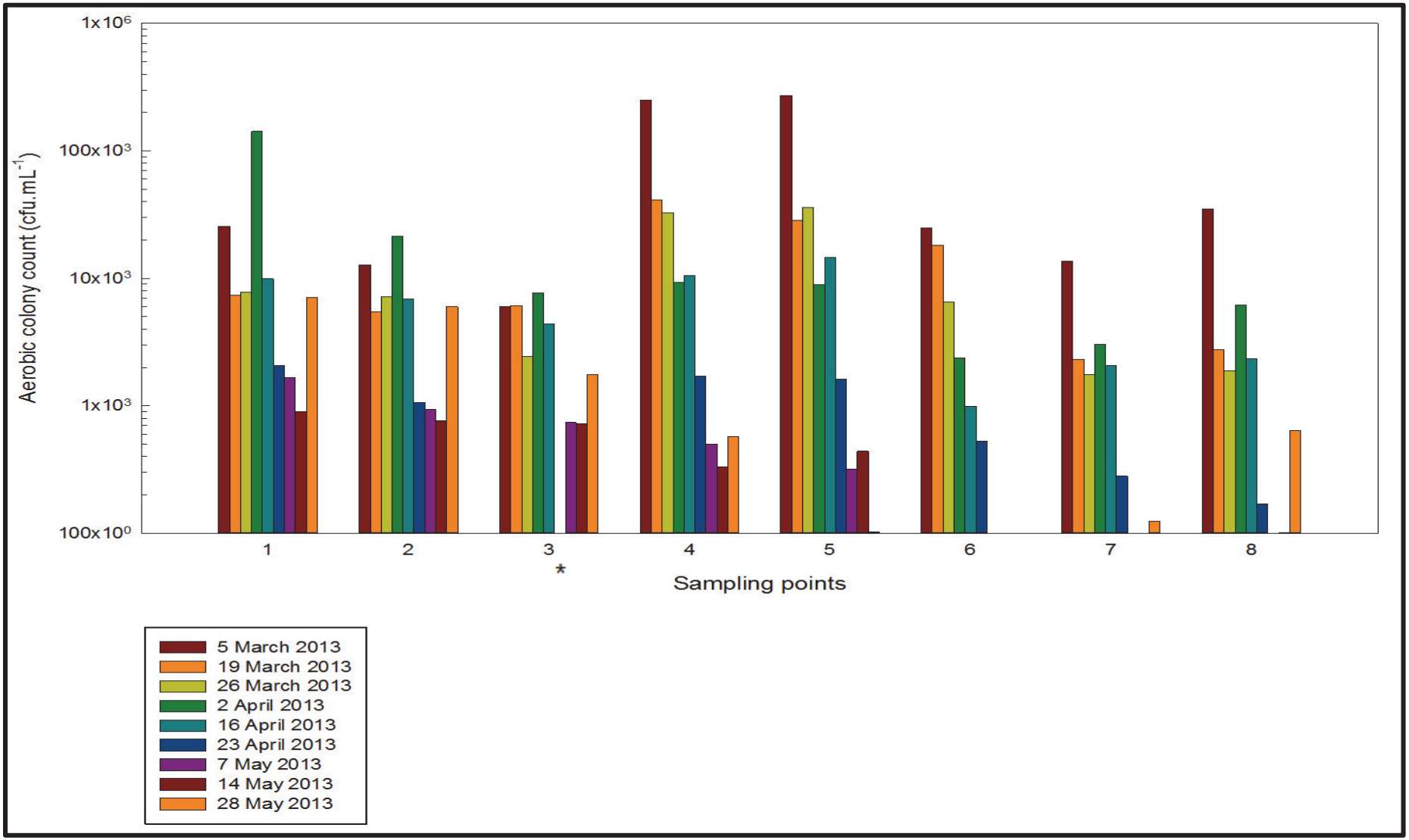


Figure A1.7 Aerobic colony counts at the eight different sampling points as obtained during the UV treatment study, from March to May 2013.

(*no counts reported at Sampling Point 3 on 23 April – sample bottle broke during transport)

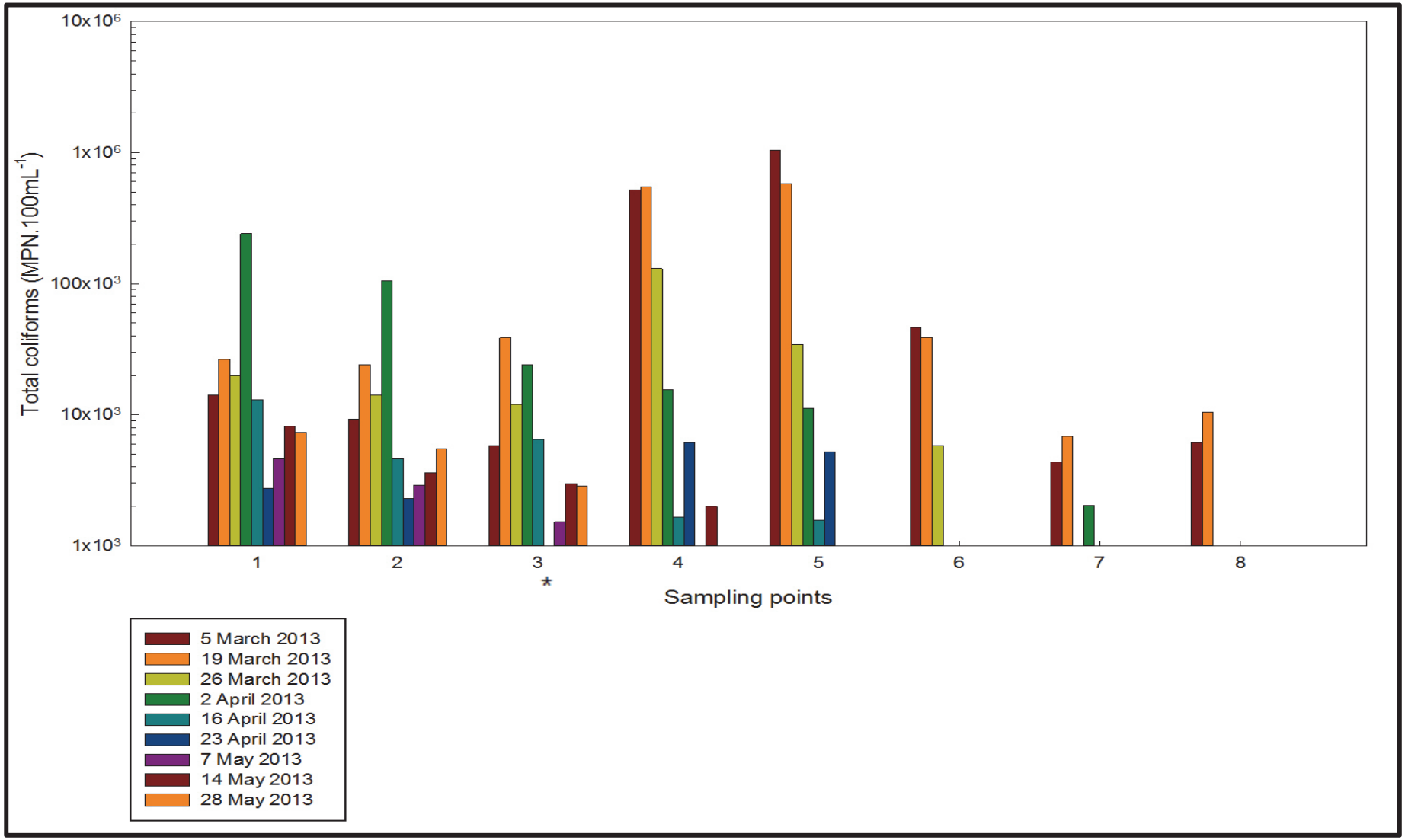


Figure A1.8 Total coliforms at the eight different sampling points as obtained during the UV treatment study from March to May 2013 (*no counts reported at Sampling Point 3 on 23 April – sample bottle broke during transport)

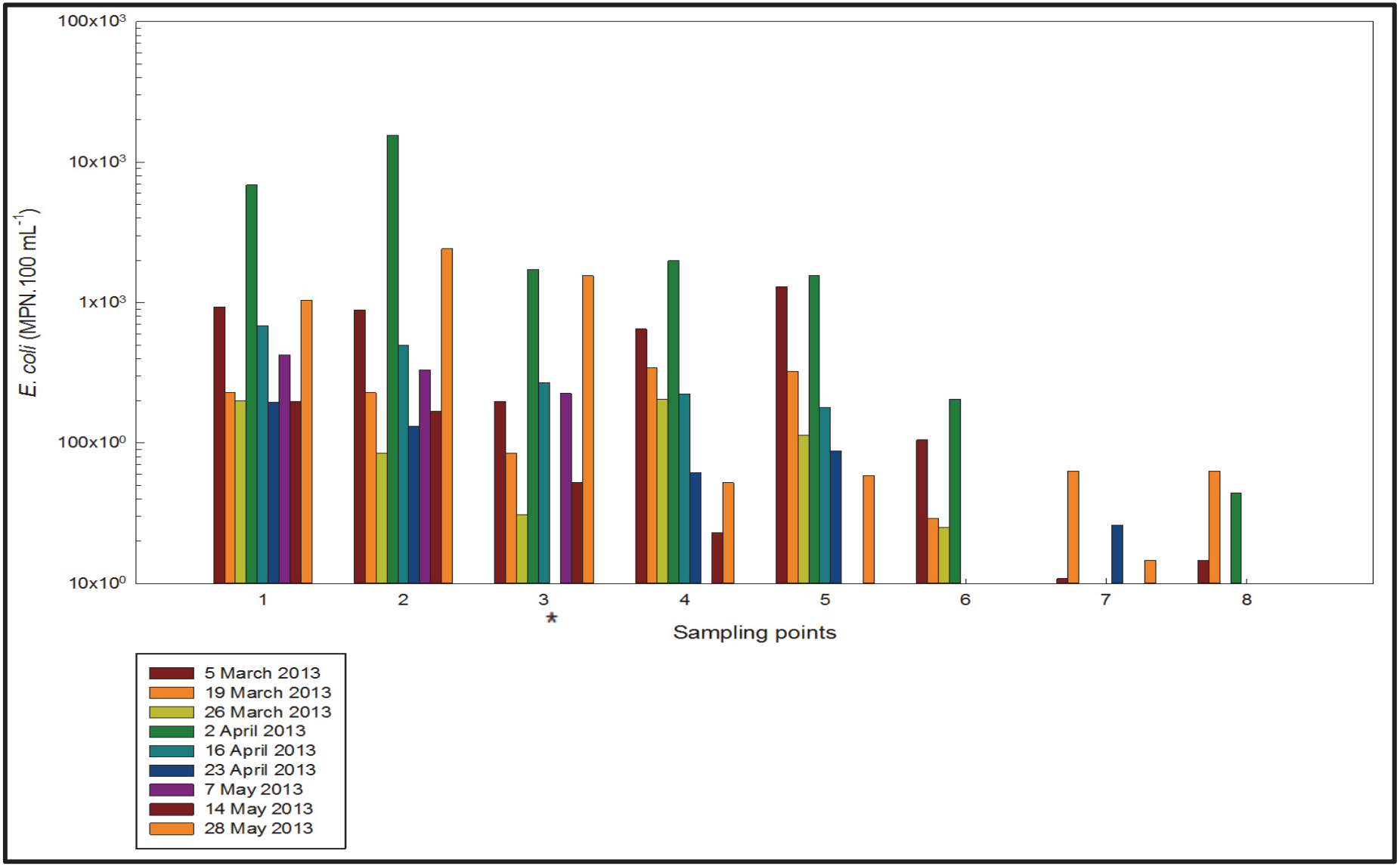


Figure A1.9 *Escherichia* coli at the eight different sampling points as obtained during the UV treatment study, from March to May 2013.(*no counts reported at Sampling Point 3 on 23 April – sample bottle broke during transport)

This might explain why counts are lower during the baseline study, which was conducted during the summer months (October 2012 to February 2013).

At Sampling Point 2 the ACC ranged between 760 and 21 300 cfu.mL^{-1} (Fig. A1.7). From Figure A1.7 it is clear that the ACC were very similar to those of the river water (Sampling Point 1) except on 2 April 2013 where ACC were a lot lower at Sampling Point 2 (21 300 cfu.mL^{-1}) than at Sampling Point 1 (142 000 cfu.mL^{-1}).

As mentioned in the baseline study, it was expected that the sand filters between the river and the inlet to the first holding dam would result in a slight reduction in the microbial load, but this was not the case (Hijnen *et al*., 2007). The TC varied between 2 282 and 104 620 MPN.100mL^{-1} (Fig. 8). Similar to the trend with ACC, the range for TC at Sampling Point 2 stayed more or less the same as at Sampling Point 1. The fact that the TC levels have not been reduced by the sand filtration means that the loads are still above the 1 000 cfu.100 mL^{-1} Canadian guideline, indicating an increased risk of using such water to irrigate crops (Monaghan & Hutchison, 2010). The *E. coli* counts ranged from 85 to 15 531 MPN.100 mL^{-1} (Fig. 9). The *E. coli* loads were very similar to those at the river (Sampling Point 1). The SAWQG and WHO guideline for irrigation water were only exceeded on 2 April and 28 May 2013 (increase from Sampling Point 1 to Sampling Point 2), thus the water could be regarded as safe for irrigation of fresh produce on the rest of the sampling occasions, in terms of the guidelines for *E. coli* (WHO, 1989; DWAF, 1996b).

The ACC at Sampling Point 3 varied between 720 and 7 400 cfu.mL^{-1} (Fig. A1.7). The ACC remained similar in loads from Sampling Point 2 to Sampling Point 3, with only a few visible decreases on 5 March, 2 April, 8 January 2013 and on 28 May 2013. The TC varied from 1 515 to 38 730 MPN.100mL^{-1} (Fig. A1.8). The TC levels at Sampling Point 3 were never below the 1 000 cfu.100 mL^{-1} guideline for safe irrigation water as set out in the Canadian guideline (Monaghan & Hutchison, 2010). The TC remained more or less constant from Sampling Point 2 to Sampling Point 3 on most of the sampling occasions (Fig. A1.8). The *E. coli* counts varied between 31 and 1 725 MPN.100 mL^{-1} (Fig. A1.9). The SAWQG and WHO guideline of 1 000 cfu.100 mL^{-1} was only exceeded on 2 April and 28 May 2013, thus at this stage of the irrigation cycle, the water could mostly be regarded as safe for irrigation of crops that are consumed raw in terms of *E. coli* (WHO, 1989; DWAF, 1996b). It can clearly be seen in Figure A1.9 that the *E. coli* loads generally decreased from Sampling Point 2 to Sampling Point 3.

The ACC varied between 330 and 251 000 cfu.mL^{-1} at Sampling Point 4 (Fig. A1.7). As part of the UV treatment study, no H_2O_2 was added to the water. It can clearly be seen in Figure A1.7 that ACC increased (0.5-1.5 logs increase) from Sampling Point 3 to Sampling Point 4 during March 2013. The ACC at Sampling Point 4 remained constant similar to that of Sampling Point 3 on 2 April and thereafter the loads decreased slightly from Sampling Point 3 to Sampling Point 4 (less than 1.0 log decrease). The TC counts ranged between 547.5 and 547 500 MPN.100 mL^{-1} (Fig. A1.8). The same trend was seen in the TC counts as with ACC, as it can clearly be seen in Figure A1.8 that TC loads increased from Sampling Point 3 to Sampling Point 4 during March 2013, but decreased thereafter from Sampling Point 3 to Sampling Point 4. The Canadian guidelines were only met on 7 and 28 May 2013, thus the water could be considered unsuitable for the irrigation of fresh crops which are consumed raw on the other sampling occasions (Monaghan & Hutchison, 2010). The rest of the time the Canadian guideline was exceeded after water passed through the sand filters, showing increases as high as 2.0 logs on certain sampling dates. *E. coli* counts varied from 31 to 1 725 MPN.100 mL^{-1} (Fig. A1.9). Unlike in the baseline study, in Figure A1.9 it can clearly be seen that the *E. coli* loads increased from Sampling Point 3 to Sampling Point 4 during March, remained relatively constant during April and decreased during May. The SAWQG and WHO guideline was met on all sampling occasions except on 2 April 2013, thus the water was presumed as safe for the irrigation of fresh produce in terms of *E. coli* counts (WHO, 1989; DWAF, 1996b). It was expected that microbial loads would be reduced between Sampling

Point 3 and Sampling Point 4, due to sand filtration that takes place (Hijnen *et al*., 2007). A possible explanation for the initial increases in ACC, TC and *E. coli* loads could be due to the sand filters which are used for extended periods without being re-sanded. As a result the filters might be clogged and not filtering properly. As a result of the increase in counts from Sampling Point 3 to Sampling Point 4 during March, the farmer decided to take action by adding chlorine to the sand filters (Sampling Point 4) three/four days prior to the sampling day 7 May 2013. Chlorine was added as a rapid solution to prevent any further increases in counts at Sampling Point 4. It was suspected that an extensive biofilm clogging had taken place and that bacteria were being "washed out" of the sand filter. After the chlorine was added, it was left in the sand filters for an undetermined time where after the entire system was flushed (Zettler, L. 2013, Owner, Limberlost Farms, Stellenbosch, South Africa, personal communication, 7 May 2013). This explains why the counts stopped increasing from Sampling Point 3 to Sampling Point 4 after 7 May 2013. Even though the addition of chlorine had a positive effect on the counts, chlorine could not indefinitely be added to the system since it is known to cause harmful by-products during water treatment (Tate & Arnold, 1990; Woo *et al*., 2002; Westerhoff, 2006; Momba *et al*., 2008). The week prior to sampling day 14 May 2013, the sand filters were resanded (Zettler, L. 2013, Owner, Limberlost Farms, Stellenbosch, South Africa, personal communication, 14 May 2013). After the addition of chlorine and resanding, no further increases in counts from Sampling Point 3 to Sampling Point 4 were observed.

In the UV treatment study, Sampling Point 5 was added to monitor the microbial levels in the water directly before the installed UV system. The UV dose reading ($mJ.cm^{-2}$) and % UVT was measured on each of the samplings days, since it may affect the efficacy of the UV apparatus in lowering the microbial counts present in the water (Werschkun *et al*., 2012). No H_2O_2 dosing was added during the UV treatment study so the effectiveness of the UV to destroy microbial growth could be monitored. The ACC ranged between 103 and 273 000 $cfu.mL^{-1}$ at Sampling Point 5 (before UV treatment) (Fig. A1.7). It can clearly be seen in Figure A1.7 that the ACC remained constant from Sampling Point 4 to Sampling Point 5 during all of the sampling occasions except on 28 May 2013, when the loads decreased. This was to be expected since no hurdle was in place between Sampling Point 4 and Sampling Point 5. The high counts in March which decreased thereafter can be explained by the chlorine addition and re-sanding as described above. The TC counts ranged from 12.1 to 1 046 200 $MPN.100\ mL^{-1}$ (Fig. A1.8). The same trend (high counts in March followed by decreases) was seen in the TC counts as with ACC. It can clearly be seen in Figure A1.8 that TC loads remained relatively constant from Sampling Point 4 to Sampling Point 5. This was to be expected since no hurdle was in place between Sampling Point 4 and Sampling Point 5. The *E. coli* counts varied between 1 and 1 533.1 $MPN.100\ mL^{-1}$ (Fig. A1.9). The *E. coli* loads remained more or less constant from Sampling Point 4 to Sampling Point 5 (Fig. A1.9). Once again this was to be expected since no hurdle was in place between Sampling Point 4 and Sampling Point 5.

After UV treatment at Sampling Point 6 the ACC varied from 44 to 25 000 $cfu.mL^{-1}$ (Fig. A1.7). It can clearly be seen in Figure 7 that ACC decreased from Sampling Point 5 to Sampling Point 6 (after passing through UV) during all of the sampling occasions. These reductions in ACC represented decreases ranging between 0.01 and 1.5 log-reductions. Even though the loads decreased after UV treatment, it was not always efficient in lowering the ACC to below SAWQG as set out for domestic use (DWAF, 1996a). The counts only fell within the SAWQG from 5 March to 16 April 2013. The TC ranged between 1 and 46 110 $MPN.100\ mL^{-1}$ (Fig. A1.8). It can clearly be seen in Figure A1.8 that TC loads decreased from Sampling Point 5 to Sampling Point 6 (after passing through UV) during all of the sampling occasions. These reductions in TC counts represented decreases ranging between 0.01 and 1.5 log-reductions. Even though the TC loads decreased after UV treatment, it was not always efficient in lowering the loads to below Canadian

guidelines (Monaghan & Hutchison, 2010). The Canadian guidelines were only exceeded during March 2013, with the remainder of the loads being below these guidelines of 1 000 cfu.100 mL^{-1} set for irrigation water (Monaghan & Hutchison, 2010). The *E. coli* counts varied between 1 and 206.4 MPN.100 mL^{-1} (Fig. A1.9). It can clearly be seen in Figure A1.9 that *E. coli* loads decreased from Sampling Point 5 to Sampling Point 6 (after passing through UV) during all of the sampling occasions. These reductions in *E. coli* counts represented decreases ranging between 0.5 and 1.5 log-reductions. The UV was successful in reducing all of the *E. coli* loads to below SAWQG, making it safe for the irrigation of fresh produce that are consumed raw (DWAF, 1996b). Microbial reduction throughout all of the sampling occasions was not constant (0.01 to 1.5 log-reductions). The efficiency of the UV treatment thus varies and this can possibly be ascribed to the fact that the initial counts in the water are extremely high (900 to 142 000 cfu.mL^{-1} for ACC, 2 723 to 241 960 MPN.100 mL^{-1} for TC 195 to 6 867 MPN.100 mL^{-1} for *E. coli* at Sampling Point 1), it might be due to biofilms or pieces of biofilm that are present in the water (from the sand filters or the walls of pipes) which are not completely destroyed, incorrect UV dosage or percentage variance in the waters' UVT (Werschkun *et al*., 2012). Log-reductions ranging from 0.77 to 1.80, 0.66 to 1.77 and 0.07 to 1.57 were achieved for ACC, TC and *E. coli*, respectively, during the UV treatment study. The average overall log-reduction achieved during the UV treatment study for ACC, TC and *E. coli* was 1.25, 1.16 and 0.90, respectively.

At Sampling Point 7 the ACC ranged from 70 to 13 700 cfu.mL^{-1} (Fig. A1.7). The ACC remained very similar from Sampling Point 6 to Sampling Point 7, with only occasional differences in counts (Fig. A1.7). On some of the sampling days, an increase in counts occurred, from Sampling Point 6 (after UV) to the pump house (Sampling Point 7) (2 April, 16 April and 28 May 2013). This could possibly be attributed to a contaminated piping system or the formation of a biofilm. The TC varied between 24.3 and 6 867 MPN.100 mL^{-1} (Fig. A1.8). A decrease in TC loads could clearly be seen in Figure A1.8 from Sampling Point 6 to Sampling Point 7. Even though an additional decrease in TC loads occurred, the Canadian guidelines (1 000 cfu.100 mL^{-1}) for the irrigation of fresh crops that are to be consumed raw were still exceeded on 5 and 19 March 2013 (Monaghan & Hutchison, 2010). *E. coli* counts ranged from 1 to 63 MPN.100 mL^{-1} (Fig. A1.9). A decrease in *E. coli* loads could be seen in Figure A1.9 from Sampling Point 6 to Sampling Point 7, except on 19 March, 23 April and on 28 May 2013. Although these increases occurred, they were relatively small (13.6 to 33.8 MPN.100 mL^{-1}) and did not result in any samples exceeding the SAWQG and WHO guideline of 1 000 organisms.100 mL^{-1} (WHO, 1989, DWAF, 1996b). These slight increases in *E. coli*, may, however, also be evidence of possible biofilms in the pipe system between Sampling Point 6 (after UV) and Sampling Point 7 (the pump house).

The point of irrigation, Sampling Point 8, was the most critical point in terms of meeting the guidelines for crops being irrigated that are to be consumed raw without any further hurdles implemented to reduce possible microbial loads. The ACC ranged between 44 and 35 000 cfu.mL^{-1} (Fig. A1.7). The ACC remained more or less constant from Sampling Point 7 to Sampling Point 8 (except on 5 March, 2 April and 28 May where slight increases occurred). Total coliforms counts varied from 16.8 to 10 462 MPN.100 mL^{-1} (Fig. A1.8) with only very slight increases on 5 March and 19 March. The counts were only above the Canadian guidelines of 1 000 cfu.100 mL^{-1} on 5 and 19 March 2013, making the water of the later sampling dates safe to use for the irrigation of fresh crops that are consumed raw (Monaghan & Hutchison, 2010). The *E. coli* counts varied from 1 to 63 MPN.100 mL^{-1} (Fig. A1.9) with slight increases only on 5 March and 2 April. The SAWQG and WHO guideline was met on all of the sampling dates, thus the water could be considered safe for irrigation of fresh crops in terms of *E. coli* counts (WHO, 1989; DWAF, 1996b). During some sampling weeks there was an increase in ACC, TC and *E. coli* loads in the water from the pump house (Sampling Point 7) to the point of irrigation (Sampling Point 8). This could possibly be attributed to a contaminated pipe system or the presence of a biofilm in the pipes

between these sampling points. As the irrigation system (from pump house to point of irrigation) experiences times of non-use, this could provide the opportunity for biofilm formation within the system. The efficiency of the sand filters and UV treatment were improved considerably after the re-sanding of the filters. This can be ascribed to improved filter performance and also an improvement in the UVT (from ca. 56 to 66) which would increase the UV efficiency slightly.

Conclusions and Recommendations

When taking all of the data collected during the baseline study and UV treatment study into consideration it is clear that the water extracted from the Eerste River is not suitable for the irrigation of fresh produce that are consumed raw, since it contains high microbial counts and it does not comply with any of the South African or Canadian guidelines used as set limits. The presence of bacteria of faecal origin such as *E. coli* is definitive evidence that the water is faecally contaminated, most probably due to sewage treatment plants that are not functioning properly. This is even more reason not to use the water for irrigational purposes without receiving any treatment.

Due to several sampling points being monitored throughout the irrigation system, it was possible to monitor the effect of different processes throughout the irrigation system. It is clear from the results that the first sand filtration step after abstraction from the river was not very effective in lowering the microbial load. This could possibly be due to extended usage period, without the filters being re-sanded. It was also seen that the counts generally did not change much through the holding dams, but ACC and TC increased while *E. coli* counts decreased through the sand filters preceding the H_2O_2 dosing. This could possibly be ascribed to biofilm build-up and clogging within the sand filters. The on-farm dosing of H_2O_2 was not very effective or consistent. Log-reductions between 0.0 and 1.5 and on one occasion 2.0 were seen between Sampling Point 4 and Sampling Point 5, but were mostly below 1.0 log-reduction. The average overall log-reduction achieved during the baseline study (H_2O_2 dosing) for ACC, TC and *E. coli* was 1.07, 1.13 and 0.65, respectively. It was also observed that a slight increase in counts occurred when the water was pumped (via the pump house) from the holding tank to the point of irrigation, indicating the possible existence of biofilms in the pipe system.

During the UV treatment study similar results were obtained as during the baseline study in that the initial sand filtration was ineffective in lowering microbial counts, little change was observed over the holding dams and microbial loads increased after the sand filtration subsequent to the holding dams. The increases in counts after the sand filter were rectified after the re-sanding thereof, highlighting the importance of this practice. The average overall log reduction achieved during the UV treatment study for ACC, TC and *E. coli* was 1.25, 1.16 and 0.90, respectively. The efficiency of the UV treatment thus varies and this could possibly be ascribed to biofilms or pieces of biofilm present in the water (from the sand filters or the walls of pipes) which are not completely destroyed, incorrect UV dosage or percentage variance in the waters' UVT (Werschkun *et al*., 2012). Another possibility is that the environmental strains of bacteria in this river have higher UV dose requirements than expected. It has previously been shown that several *E. coli* isolates from the Eerste River have increased antibiotic resistance, which may be an indication that they also differ in the resistance to UV (Huisamen, 2012).

The counts in the river water during the UV treatment study were mostly higher than during the baseline study. This could possibly be due to an increase in rainfall that occurred from March to May 2013. The baseline study was conducted from October 2012 to February 2013, the summer months in the Southern Hemisphere, while the UV treatment study was done March 2013 to May 2013 (autumn and beginning of winter). According to Bruhn & Wolfson (2007) ultraviolet rays from the sun might kill bacteria on a warm and sunny day, leading to lower than expected counts in the summer months. Heavy storms and rainfall have also been shown to contribute to

higher microbial counts in rivers due to storm water overflows, pollution and runoff from pastures and wastewater treatment plant overflows (Kistemann *et al*., 2002; Hill *et al*., 2006). This might explain why the counts were higher during the UV treatment study conducted during March to May 2013 (autumn and beginning of winter).

It is difficult to compare the efficacy of the two treatments since several external factors such as ambient temperature and rainfall might have played a role in the results according to literature even though it was not always reflected in the results. However, when all the results are taken into consideration it can be concluded that UV was the slightly more effective treatment method, since the average log-reductions achieved were slightly higher and would have been achieved without the addition of expensive chemical dosing and the risk of producing disinfectant by-products.

It would be highly recommended that monitoring of the irrigation system continue, to monitor the efficacy of the sand filters. This can be used as an indication of when re-sanding is required. Furthermore, it would be recommended that increased UV dosages be investigated and compared to dosages suggested in literature. It is also important to monitor the effect of biofilms in the irrigation pipe system on the counts at the point of irrigation, especially if the efficiency of the treatment system is enhanced. Otherwise the positive effect of the treatment system could be nullified if recontamination takes place in the subsequent pipe system.

A.2 EXPERIMENTAL PROCEDURES (LABORATORY-SCALE)

A.2.1 General materials and methods

Escherichia coli strain panel
All the *E. coli* strains used in the laboratory studies discussed in this report are presented in Table A2.1. The strain panel includes two American Type Culture Collection (ATCC) strains and four environmental *E. coli* strains. The environmental *E. coli* strains had been isolated from previous studies (Department of Food Science, Stellenbosch University) and stored in 40 % ($v.v^{-1}$) glycerol (Fluka Analytical, Germany) at -80 °C. Each culture was resuscitated in Nutrient Broth (NB) (Merck) (35°C for 24 h), after which single colonies were obtained by streaking on Eosin Methylene-Blue Lactose Sucrose Agar (L-EMB) (Oxoid, South Africa). An API 20E test (BioMèrieux, South Africa), Gram staining and catalase test was performed on each culture to confirm that the isolated strains were indeed *E. coli* strains.

Before each treatment an inoculum was prepared for each strain by diluting an overnight culture (incubated in NB at 35°C) to achieve a turbidity equal to 0.5 McFarland standard (BioMèrieux, South Africa). Diluents used in lab-scale studies were either Sterile Saline Solution (SSS) (0.85% $m.v^{-1}$ NaCl) or sterilised river water.

Table A2.1 Known characteristics of six *E. coli* strains used for laboratory-scale studies.

Strain	Source	AB resistance
ATCC 25922	Reference (ATCC)	None
ATCC 35218	Reference (ATCC)	AMP,C,STR
MJ58	Parsley	None
MJ56	Parsley	None
M53	Plankenburg river	T, TM, AMP, STR
F11.2	Plankenburg river	T

ATCC – American Type Culture Collection
AB – Antibiotic; T – Tetracycline; TM – Trimethoprim; Amp – Ampicillin; STR – Streptomycin

Microbiological analysis for lab-scale studies involving E. coli strains in saline
For all the studies where treatments were tested on pure *E. coli* strains, a dilution series was prepared both before (control), and after all specific treatments and time intervals. Enumeration of *E. coli* strains before and after specific treatments and time intervals were done on Violet Red Bile Agar (VRBA) (Biolab, South Africa). Plates were poured in duplicate, inverted and incubated at 35°C for 24h.

Treatment Solutions

A commercial form of PAA was used: Tsunami 100, composed of 31% acetic acid, 15% peroxyacetic acid and 11% hydrogen peroxide (Ecolab, South Africa). Sodium hypochlorite (NaOCl) representative of 15% ($m.v^{-1}$) available chlorine (Metsi Water Solutions, South Africa) were used for chlorine disinfection. Sterile sodium thiosulfate ($Na_2S_2O_3$, 1% ($m.v^{-1}$)) was used after each time interval according to the method of Mazzola *et al*., (2006) to quench the residual action of PAA and NaOCl to ensure that the exact contact times were attained.

All H_2O_2 stock solutions used in the laboratory-scale studies were prepared from 30% ($v.v^{-1}$) hydrogen peroxide (Merck, South Africa). A Spectroquant® Hydrogen Peroxide Cell Test (2.0-200 $mg.L^{-1}$) (Merck, South Africa) was used to confirm each of the respective concentrations. For PAA studies: "TEST strips" were used to indicate true concentrations of PAA solutions.

River water sampling

River water was sampled from the Plankenburg River (Stellenbosch) following standard sampling procedures (SANS 5667-6, 2006). Electrical conductivity was measured using a HI 8711 conductivity meter (Hanna Instruments, USA) adjusted to $mS.m^{-1}$. Water turbidity was measured using an Oreon AQ3010 turbidity meter (Thermo Scientific, USA) and expressed as NTU (nephelometric turbidity units). Water temperature and pH was measured using WTW pH meter (WTW, Germany). The ultraviolet transmission percentage (UVT%) of river water was measured by a Sense™ ultraviolet transmittance monitor (Berson, Germany).

Physio-chemical analyses of river water samples

Chemical oxygen demand (COD), alkalinity, total suspended solids (TSS) and volatile suspended solids (VSS) were determined as described for Pilot-scale studies. The results of these analyses were also compared to guidelines set for water intended to be used for fresh produce irrigation (Table 3.1).

Microbiological analyses of river water samples

Microbial analysis of river water samples were performed by using both standard plating methods as well as the standard membrane filtration (MF) method (USEPA, 2002). For the plating methods, serial dilutions were prepared in 9 mL SSS units. Serial dilutions were prepared in larger volumes (90 mL) of SSS for the MF analyses and 100 mL of diluted water samples were filtered through sterile cellulose nitrate membrane filters (0.45 µm) (Whatman, England) according to the U.S. Environmental Protection Agency (USEPA) method 1604 (USEPA, 2002).

The Aerobic colony count (ACC), Total coliform (TC) and *Escherichia coli* (EC) or Faecal coliform (FC) enumeration were done using standard plating methods (SANS 4832, 2007; SANS 4833, 2007). Aerobic colony counts were determined on plate count agar (PCA) (Biolab, South Africa) at 30°C for 48 h. Total coliforms (TC) were identified by counting purple-pink colonies on VRBA after a 24 h incubation period at 36°C. Faecal coliforms (FC) were identified by counting purple-pink colonies on VRBA after a 24 h incubation period at 44.5°C (Schraft & Watterworth, 2005). Where applicable, TC and *Escherichia coli* (EC) were enumerated on Chromocult® Coliform Agar Enhanced Selectivity (CES) (Merck, South Africa) at 35°C for 24 h. Blue colonies characteristic to EC were counted, while both blue and salmon to red were counted as TC.

River water flocculation

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

UV disinfection

All laboratory-scale studies that involved UV disinfection (as a single treatment or as part of a combination treatment) were conducted using a bench-scale collimator device (Berson, The Netherlands) (Fig. A2.1). The instrument utilised an Amalgam LP mercury vapour lamp (UV-Technik, Germany) with power output of 40 W and arc length of 25 cm. Light was predominantly emitted at 253.7 nm. UV light intensity at the sample surface was determined before each treatment using an ILT1400 radiometer (International Light Technologies, USA) coupled with a XRL140T254 detector (International Light Technologies, USA). Subsequently, the required time of exposure to deliver a desired UV dose was calculated according to the following equations (Morowitz, 1950; Hallmich & Gehr, 2010):

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

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

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

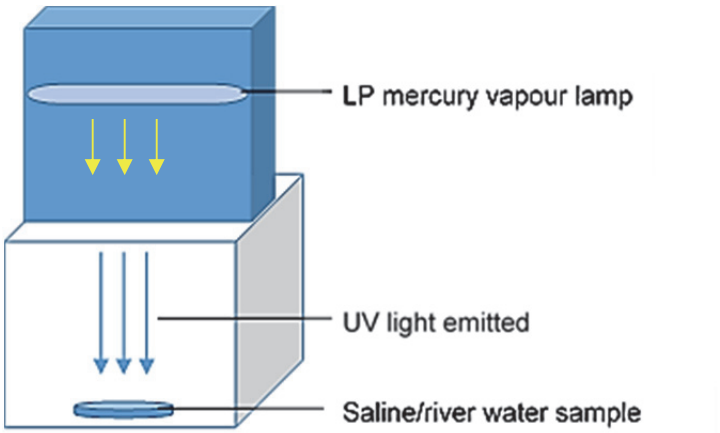


Figure A2.1 Schematic representation of the bench-top collimator beam device used for laboratory-scale UV and UV/H_2O_2 disinfection experiments.

Statistical analysis

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

A3. EFFICACY OF UV & UV/H_2O_2 TREATMENTS ON LABORATORY-SCALE DISINFECTION OF *E. COLI* STRAINS AND OF RIVER WATER

Research study design

To evaluate the potential of LP UV and UV/H_2O_2 combination treatments for the reduction of microbial loads in river water prior to irrigation, the effectiveness of several disinfection approaches was studied in a laboratory-scale experiment. A preliminary study in saline (utilising LP UV light at a single dose and H_2O_2 at a single concentration and contact time) was executed on two ATCC reference and four environmental *E. coli* strains (Table A2.1) to determine whether these respond differently in terms of log inactivation. Thereafter, a range of UV doses, as stand-alone treatment, and UV in combination with H_2O_2 at varying concentrations were tested against three of the *E. coli* strains. Furthermore, the influence of water quality on treatments showing potential for disinfection was investigated. Autoclaved river water was used to evaluate the influence of water quality, measured in terms of organic matter content (COD), ultraviolet transmission percentage (UVT%), turbidity, suspended solids content (TSS) and conductivity, on disinfection efficiency.

In a concluding study the effectiveness of UV irradiation for the disinfection of river water was assessed, taking into account the influence of changing water quality and a heterogenic microbial population. Prior to performing disinfection experiments on the river water, techniques for enumerating TC, FC and the ACC population were optimised. Where possible, results of the microbiological and water quality analyses were compared to guidelines for irrigation water quality set by DWAF (1996) (Table 3.1) and were used to interpret the effectiveness of the respective treatments.

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

Escherichia coli inoculums (0.5 McFarland standard equivalent) were prepared in sterile 0.85% SSS and aseptically transferred to a sterile petri dish before performing the respective treatments. In the case of UV irradiation as stand-alone treatment the petri dishes were immediately positioned in the centre of the sphere of UV light observed on the surface of the bottom section of the collimator device (Fig. A3.1). For the UV/H_2O_2 combination treatments, H_2O_2 was added to each petri dish and allowed to have contact with the inoculum before it was transferred to the collimator (Fig. A3.1). The contact time was initiated as soon as the H_2O_2 was mixed with the inoculum.

Six *E. coli* isolates (Table A.3.1), including two ATCC reference (ATCC 25922 and ATCC 35218) and four environmental strains (MJ58, MJ56, M53 and F11.2), were exposed to a lower-limit UV dose (4 mJ.cm^{-2}) to determine whether they respond differently to UV light irradiation. Additionally, the same strains (ATCC 25922, ATCC35218, MJ58, MJ56, M53 and F11.2) were subjected to a single concentration (20 mg.L^{-1}) of H_2O_2 for a predetermined contact period (10 min) before being irradiated at the same UV dose (4 mJ.cm^{-2}). One of the reference strains (ATCC 25922) and two of the environmental strains (F11.2 and MJ56) were further irradiated with incremented UV doses (8, 10 & 13 mJ.cm^{-2}) and were also exposed to both lower and higher H_2O_2 concentrations (2.5, 100 & 200 mg.L^{-1}) using the contact time (10 min) and UV dose (4 mJ.cm^{-2}) mentioned earlier (Table A3.1). Enumeration of the respective *E. coli* strains was performed both before and after the different treatments in order to determine the reduction achieved for specific UV doses and combination treatments. This was done by serially diluting the untreated and treated inoculums where after duplicate pour plates were prepared using VRBA (Fig. A3.1). Following incubation, the responses of the *E. coli* strains were expressed in terms of log inactivation. Experimental procedures were performed in triplicate.

Table A3.1 Ultraviolet and UV/H_2O_2 combination treatments executed on six *E. coli* strains in a laboratory-scale investigation

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

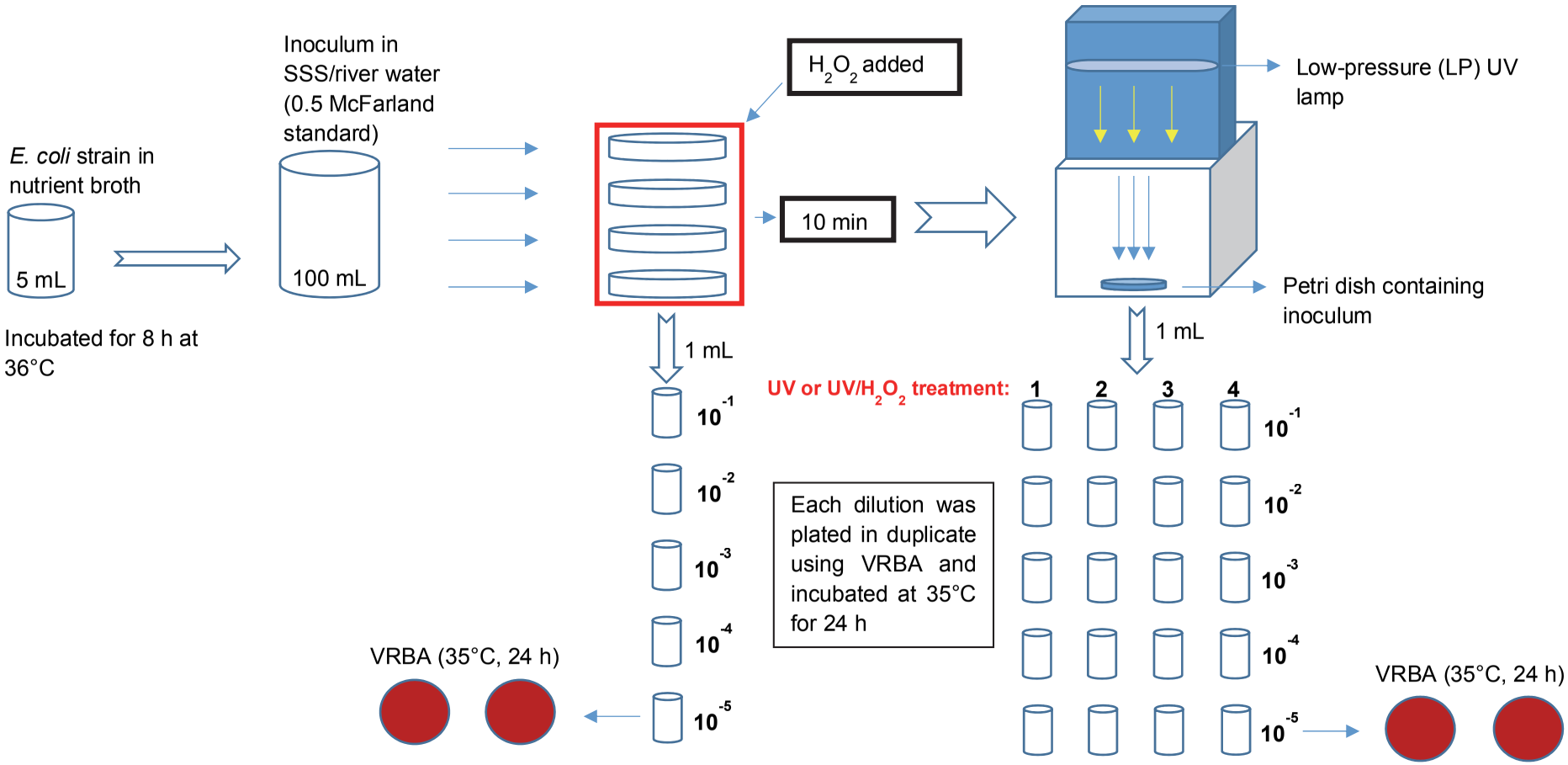


Figure A3.1 Experimental design used in Study A to determine the log inactivation achieved for each strain following the application of UV or UV/H_2O_2 disinfection treatments as summarised in Table 3.

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

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

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

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

Study D: UV disinfection of river water

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

The collimated-beam device was utilised to execute the treatments according to the procedure described in Study A. In this instance, larger volumes (depth 22 mm) were irradiated in sterilised 500 mL glass beakers (one for each dose) in order to mimic the conditions presented in a pilot-scale system in which the water depth around the UV lamp would be approximately 22 mm. The glass beaker was subsequently transferred to the lower section of the collimator device and irradiated with UV light at the respective UV doses (Fig. A3.1). Total and faecal coliforms and total aerobic bacteria (ACC) were enumerated before and after each UV treatment using the pour plate technique and media (VRBA and PCA) selected based on the findings of Study C (comparison of different techniques for enumerating the same microbial groups

Results

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

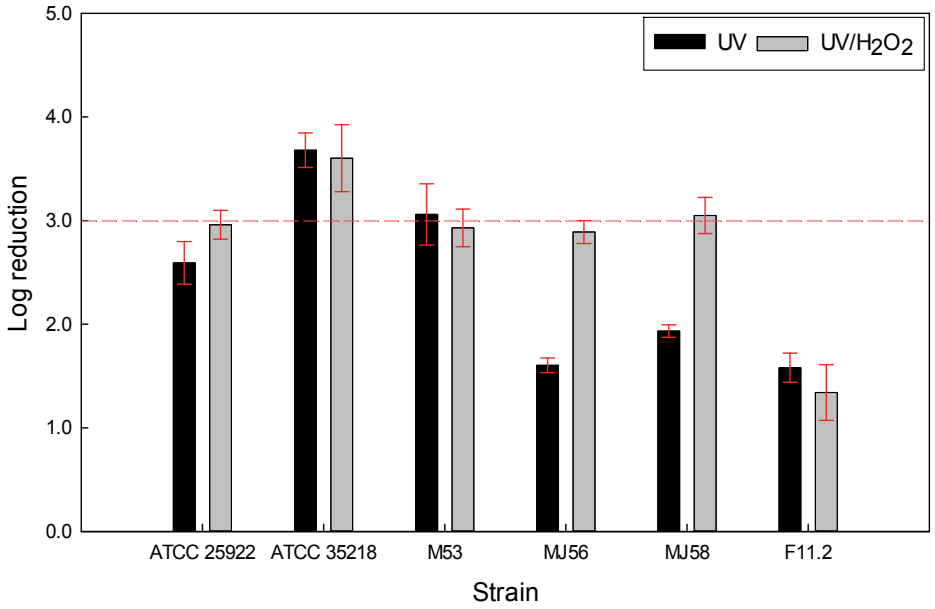


Figure A3.2 Disinfection efficiency of UV (4 mJ.cm^{-2}) and combined UV/H_2O_2 (4 mJ.cm^{-2}/20 mg.L^{-1}) treatments on six *E. coli* strains in SSS. Error bars were calculated based on standard deviation at a confidence interval of 0.95. * - No growth detected at lowest dilution (10^{-1}).

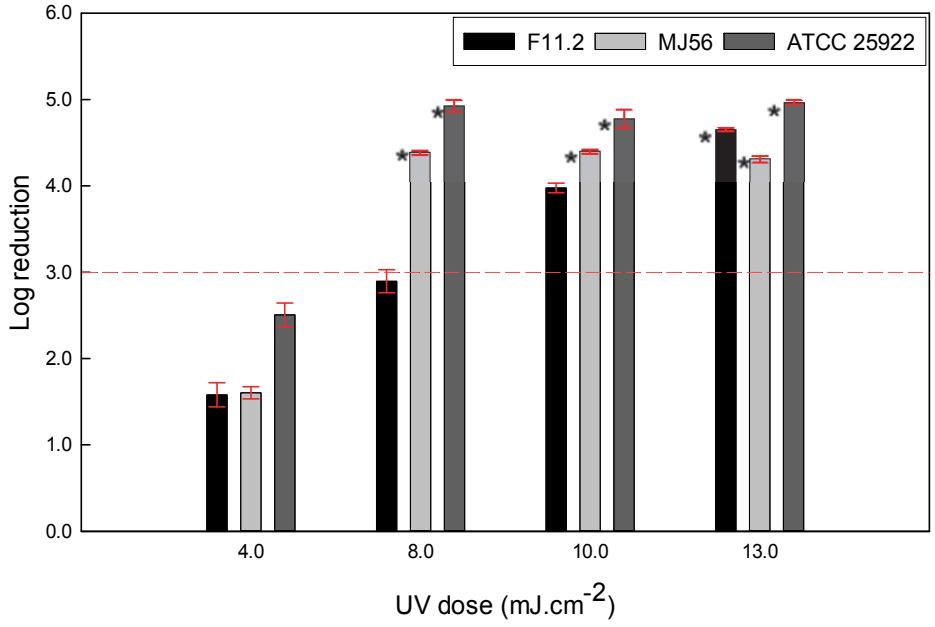


Figure A3.3 Effect of varying UV dose on the survival of three *E. coli* strains in SSS. Error bars were calculated based on standard deviation at a confidence interval of 0.95. * - No growth detected at lowest dilution (10^{-1}).

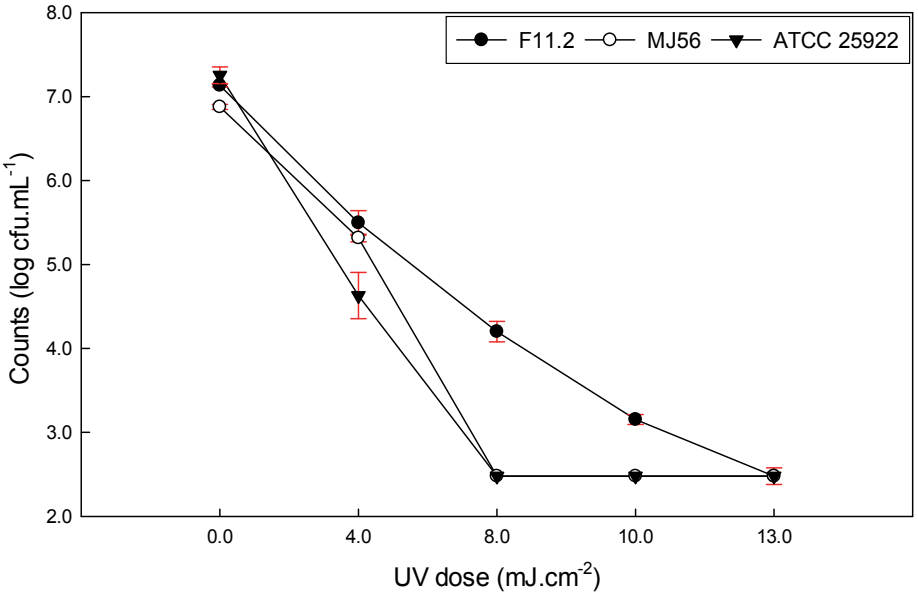


Figure A3.4 Effect of UV dose on the number of viable colonies of *E. coli* strains following treatment in SSS. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

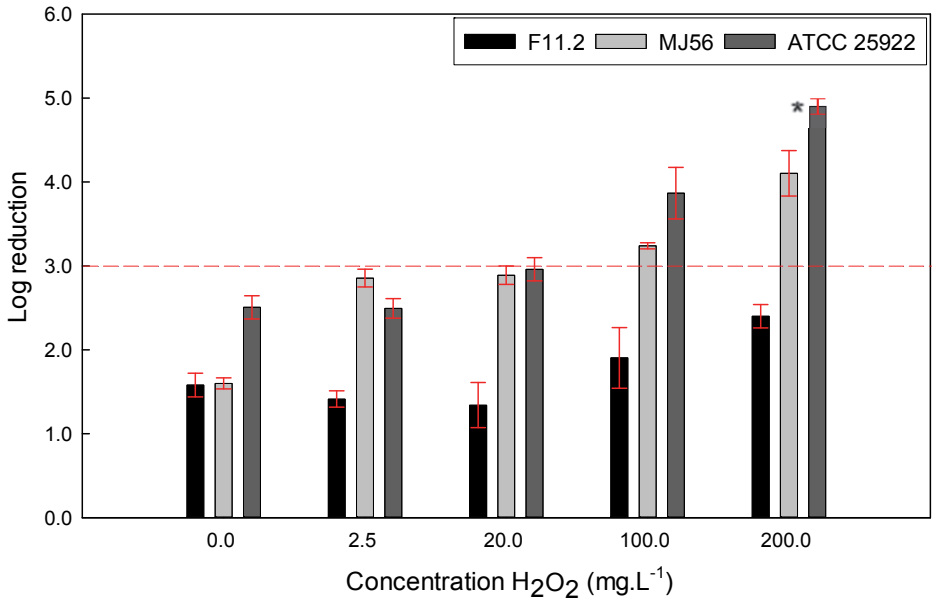


Figure A3.5 Effect of varying H_2O_2 concentrations used in combination with a UV dose of 4 mJ.cm^{-2} on the survival of three *E. coli* strains in SSS. Error bars were calculated based on standard deviation at a confidence interval of 0.95. * - No growth detected at lowest dilution (10^{-1}).

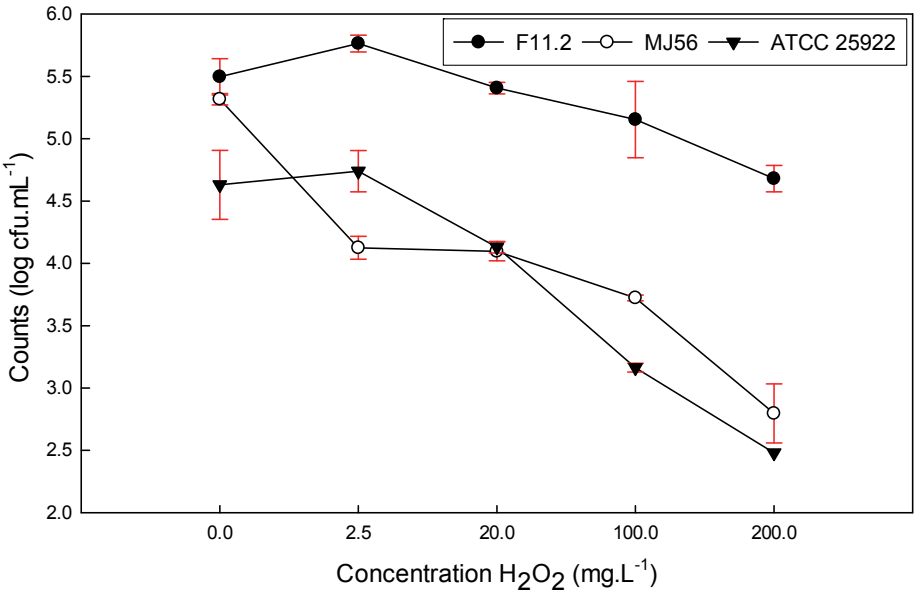


Figure A3.6 Effect of H_2O_2 concentration and a UV dose of 4 mJ.cm^{-2} on the number of viable colonies of *E. coli* strains following the application of UV/H_2O_2 combination treatments in SSS. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

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

Table A3.2 Physico-chemical properties of untreated and flocculated river water

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

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

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

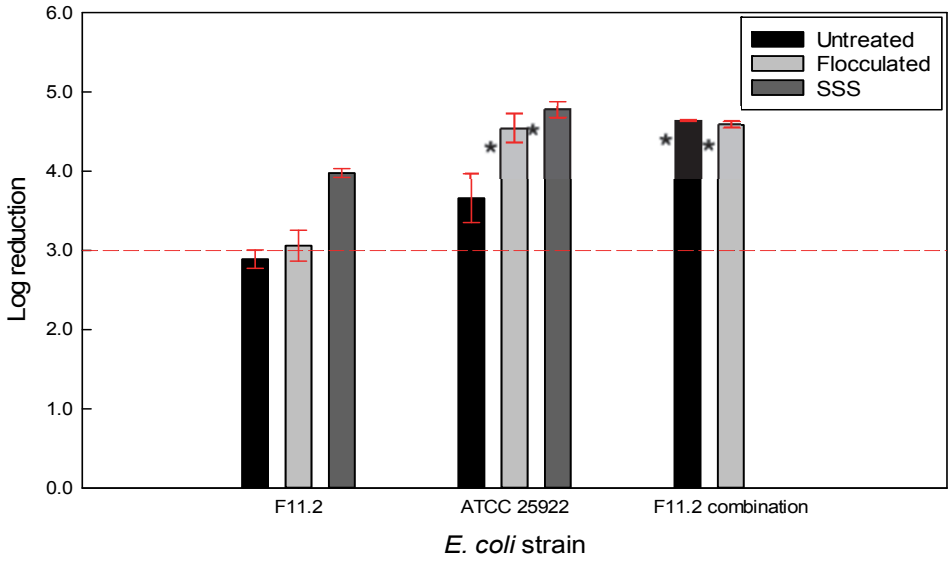


Figure A3.7 Log reduction of selected *E. coli* strains (F11.2 and ATCC 25922) exposed to a UV dose of 10 mJ.cm^{-2} in SSS and untreated and flocculated river water and a UV/H_2O_2 combination treatment (F11.2 combination) in river water (untreated and flocculated). Error bars were calculated based on standard deviation at a confidence interval of 0.95. * - No growth detected at lowest dilution (10^{-1}).

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

Study C: C ***tal aerobic microorgan***

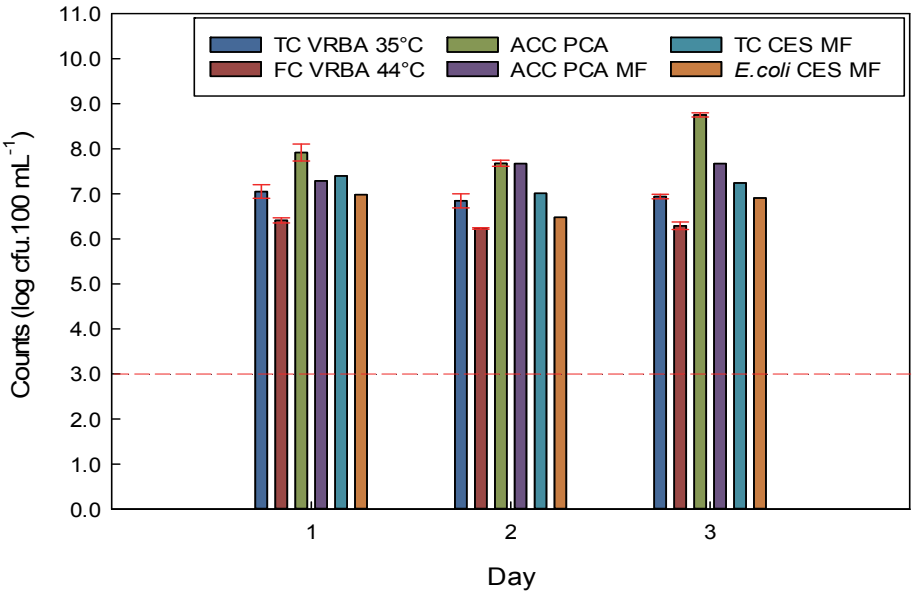


Figure A3.8 Microbial populations determined in river water on three sampling days using different methods of enumeration. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

TC – Total coliforms; FC – Faecal coliforms; ACC – Aerobic colony count.
MF – Membrane filtration.
VRBA – Violet Red Bile Agar; PCA – Plate Count Agar; CES - Chromocult® Coliform Agar Enhanced Selectivity.

Study D: UV disinfection of river water

River water analysis

Table A3.3 Physico-chemical and microbiological properties of river water used in trials 1-3

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

[a] Determined using VRBA (pour plate technique) and incubation temperature of 44°C
[b] Determined using VRBA (pour plate technique) and incubation temperature of 35°C
[c] Determined using PCA (pour plate technique) and incubation temperature of 30°C

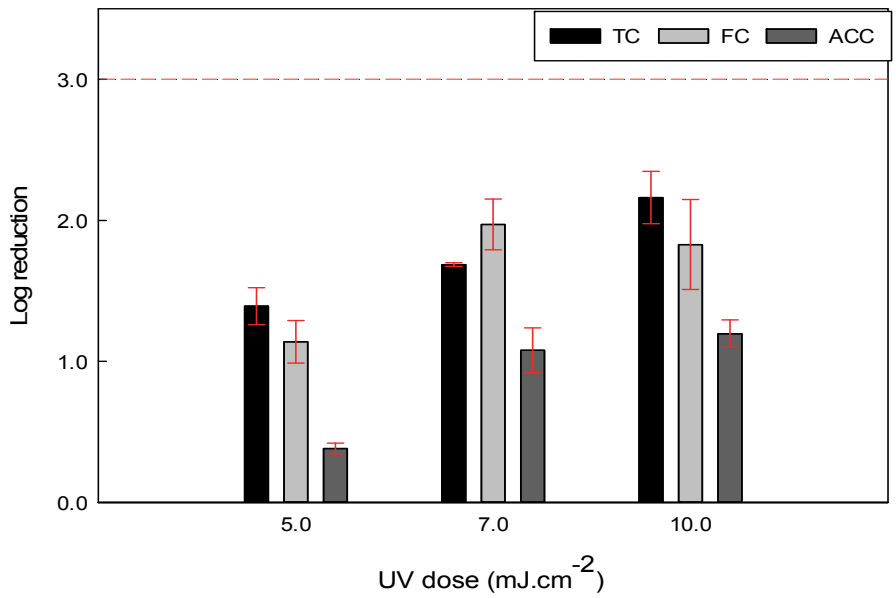


Figure A3.9 Microbial reductions achieved at three doses (5, 7 and 10 mJ.cm^{-2}) following laboratory-scale LP UV irradiation during experimental Trial 1. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

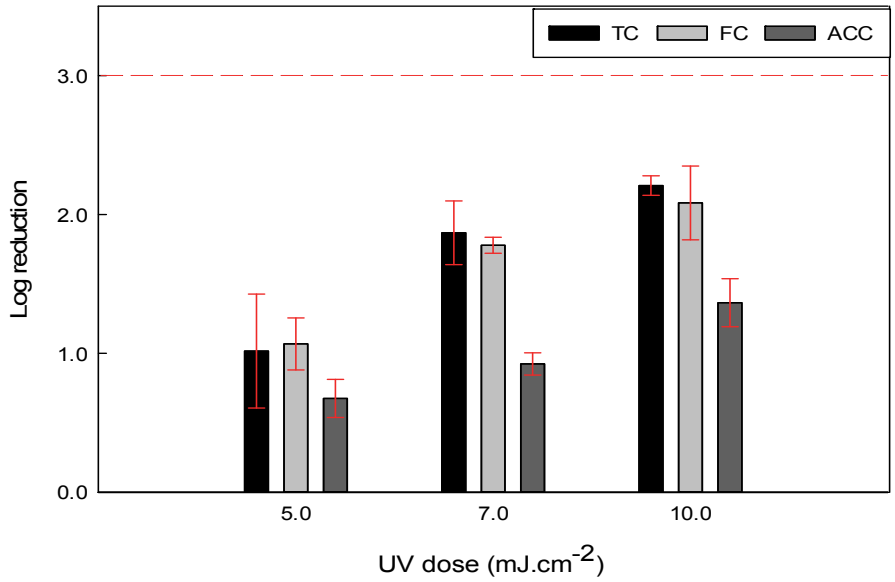


Figure A3.10 Microbial reductions achieved at three doses (5, 7 and 10 mJ.cm^{-2}) following laboratory-scale LP UV irradiation during experimental Trial 2. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

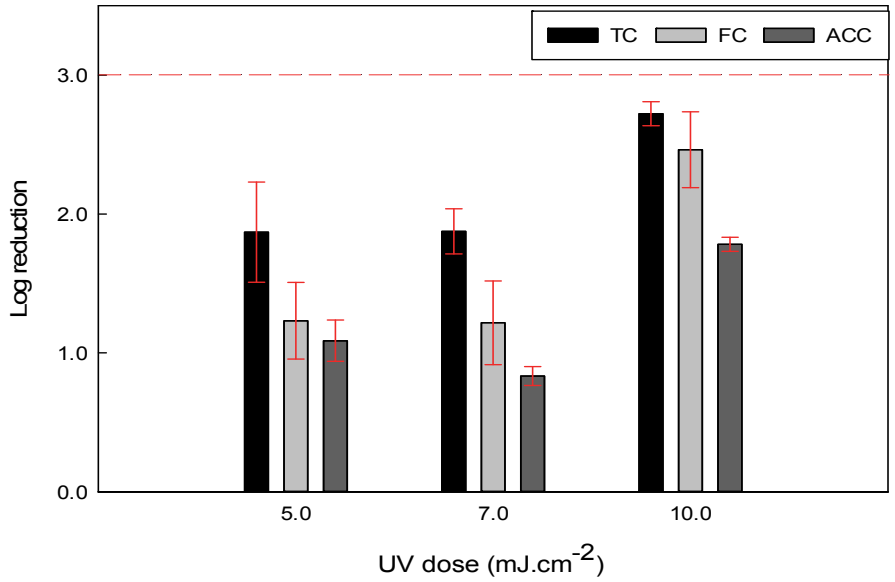


Figure A3.11 Microbial reductions achieved at three doses (5, 7 and 10 mJ.cm^{-2}) following laboratory-scale LP UV irradiation during experimental Trial 3. Error bars were calculated based on standard deviation at a confidence interval of 0.95.

Table A3.4 Log reductions achieved in river water following LP UV irradiation at a dose of 10 $mJ.cm^{-2}$ on three respective days

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

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

Table A3.5 Faecal coliform concentrations in river water following LP UV disinfection at three doses (5, 7 and 10 $mJ.cm^{-2}$) in a laboratory-scale study

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

Conclusions

In this study the disinfection potential of LP UV and UV/H_2O_2 treatments was investigated using six reference and environmental *E. coli* strains (Table A3.1). Initially, clear strain-to-strain variation in the resistance against both treatments (UV dose of 4 $mJ.cm^{-2}$ and UV/H_2O_2 combination of 4 $mJ.cm^{-2}$/20 $mg.L^{-1}$) was seen (Fig. A3.2). The general observation was that the reference strains were more sensitive. In each case, environmental isolate F11.2 was the most resistant and strain ATCC 35218 the most vulnerable. The use of popular reference strains might thus not be the most accurate method for the optimisation of disinfection treatments on laboratory-scale. In addition, the low doses utilised for both UV and the combination treatment were considered inadequate to generate a 3 log target reduction. In comparison to UV alone, the combination treatment showed potential for increased disinfection, yet the majority of strains reacted similarly towards the respective processes. Resistance mechanisms, including catalase activity and the use of efflux pumps, may allow *E. coli* strains to protect themselves against the combined effects of UV and H_2O_2.

In subsequent studies it was observed that increased UV doses and H_2O_2 concentrations resulted in overall greater reductions (Figures A3.3 & A3.4). Even for the most resistant strain a 3 log reduction was achieved at a UV dose of 10 $mJ.cm^{-2}$. Similarly, higher concentrations of H_2O_2 resulted in better disinfection but the effect was less profound. At a concentration of 200 $mg.L^{-1}$ (and UV dose of 4 $mJ.cm^{-2}$) the most resistant strain was reduced by 2.40 log units only (Figures A3.5 & A3.6). Variation in the responses of the different strains to incremented H_2O_2 concentrations was related to their catalase activity, although exceptions were observed (see results reported by Giddey *et al*. (2015) – Appendix A4.1.1). It became clear that the dynamics of

combined treatments are complex and therefore further research is required to optimise the conditions for AOPs.

In the following study, it was observed that the physico-chemical properties of both untreated and flocculated river water failed to meet the guidelines for water intended to be used for fresh produce irrigation (Table A3.2 & Table 3.1). Furthermore, significantly better reductions ($p<0.05$) were achieved in saline compared to sterile untreated river water for both test strains (ATCC 25922 and F11.2) (Fig A3.7). The impact of suspended and dissolved compounds on the availability of UV photons (and consequent disinfection) was thus clear. It was also apparent that the use of a polymeric coagulant at 7 $mg.L^{-1}$ resulted in a slightly increased water quality. The effect thereof on the delivered UV light, however, was only slight and could not result in greater reductions of a resistant environmental strain. When the same strain was exposed to a "shock" combination treatment, complete inactivation was achieved in both water samples (Fig A3.7). It is suggested that in the presence of high H_2O_2 concentrations the available UV light was influenced not as much by water quality, but rather it was absorbed by the chemical for the generation of reactive oxygen species.

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

Different methods for the enumeration of TC, FC and ACC were evaluated and compared in order to optimise methodologies for subsequent studies. For the respective microbial groups, the use of different media (VRBA, CES and PCA) and techniques (pour plates and membrane filtration) compared fairly well, irrespective of the absence of a standardised reference inoculum (Fig A3.8). Based on the results obtained, and those of similar studies reviewed in the literature, the pour plate technique was selected as suitable enumeration method.

The final study evaluated the potential of LP UV irradiation for the disinfection of three dissimilar water samples taken from the Plankenburg River (Figures A3.9; A3.10; A3.11). Firstly, it was observed (comparing Table A3.2 & Table 3.1) that the water did not comply with guidelines (DWAF, 1996). Faecal coliforms were detected at levels of 6.41, 6.23 and 6.29 log $cfu.100\ mL^{-1}$ in the three respective samples. Sources such as informal settlements and industrial operations probably contributed to the high levels of pollution observed in the river water. Following treatment, similar inactivation was generally observed for TC and FC while the heterotrophic bacteria population showed much greater resistance. Increased UV doses resulted in improved disinfection but the maximum (10 $mJ.cm^{-2}$) could not deliver a 3 log reduction of TC, FC or the total ACC population (Table A3.4). In addition, it was seen that a reduction exceeding 3 log would be required to produce water that could be safely used for the irrigation of fresh produce (Table A3.5). Considering the impact of water quality and microbiological aspects on UV disinfection it is recommended that doses > 10 $mJ.cm^{-2}$ be evaluated in future studies.

A4. LABORATORY-SCALE CHEMICAL DISINFECTION OF *E. COLI* STRAINS AND OF RIVER WATER

A4.1 Effect of H_2O_2 on environmental and reference *E. coli* strains and river water

A4.1.1 Effect of H_2O_2 on eleven environmental and reference *E. coli* strains

The results obtained as part of this investigation was published in the following article:
Title: Impact of hydrogen peroxide treatment on environmental *Escherichia coli* strains
Authors: Giddey, K.F., Kidd, M., Britz, T.J., Sigge, G.O. and Lamprecht, C.
Journal: Journal of Applied and Environmental Microbiology Vol.3, No.2 (2015): 49-57
It's available free of charge at http://pubs.sciepub.com/3/2/4 DOI:10.12691/jaem-3-2-4

A4.1.2 Effect of H_2O_2 on river water

Research study design

This study was performed on river water sampled from the Plankenburg River. Three concentrations (250, 300 and 350 $mg.L^{-1}$) of H_2O_2 and five time intervals (0 (initial counts), 30, 60, 90 & 120 min) were tested in order to determine the effect that H_2O_2 had on the ACC (aerobic colony counts), total coliforms and *E. coli* counts in the river water. River water was sampled and ACC, total coliforms and *E. coli* counts were analysed before and after the addition of H_2O_2 in order to determine the log reduction achieved after specific time intervals.

Results

Table A4.1.1 Physico-chemical data for the two river water samples taken from Plankenburg river

Sample	pH	Temperature (°C)	Alkalinity ($mg.L^{-1}$)	Electrical conductivity ($mS.m^{-1}$)	COD ($mg.L^{-1}$)
Sample 1	6.95	15.1	150	0.46	5.4
Sample 2	6.71	14	100	0.47	9

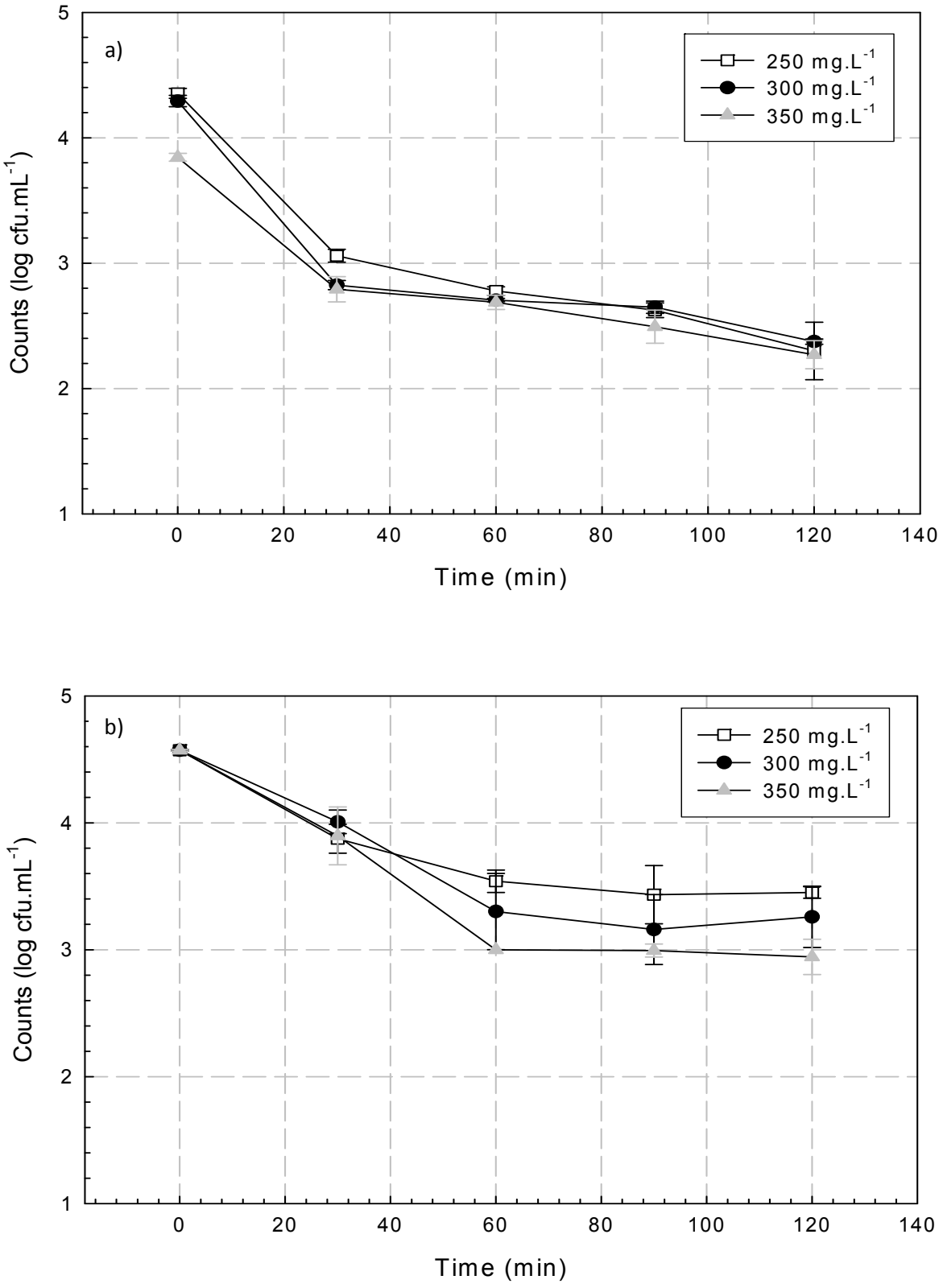
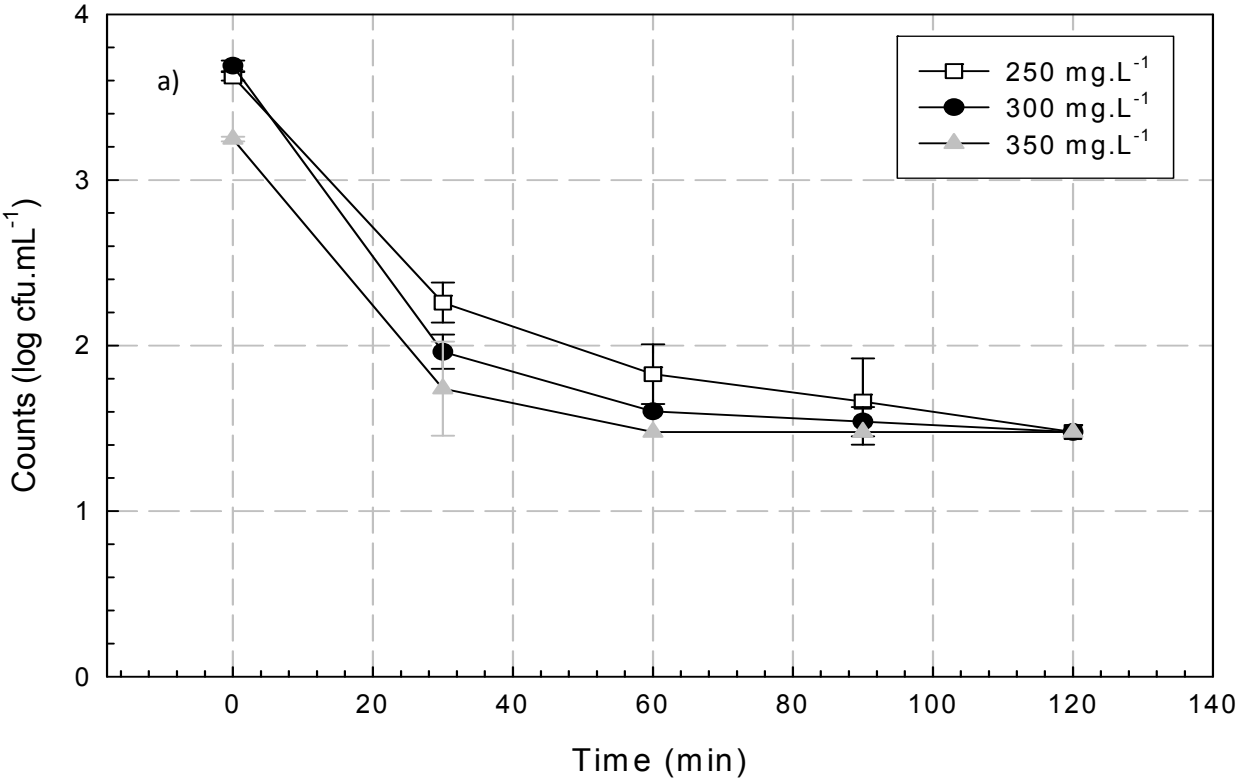


Figure A4.1.1 Effect of H_2O_2 on ACC levels in sampled river water at specific time intervals (a) Sample 1; (b) Sample 2. Error bars represent error at a 95% confidence interval.



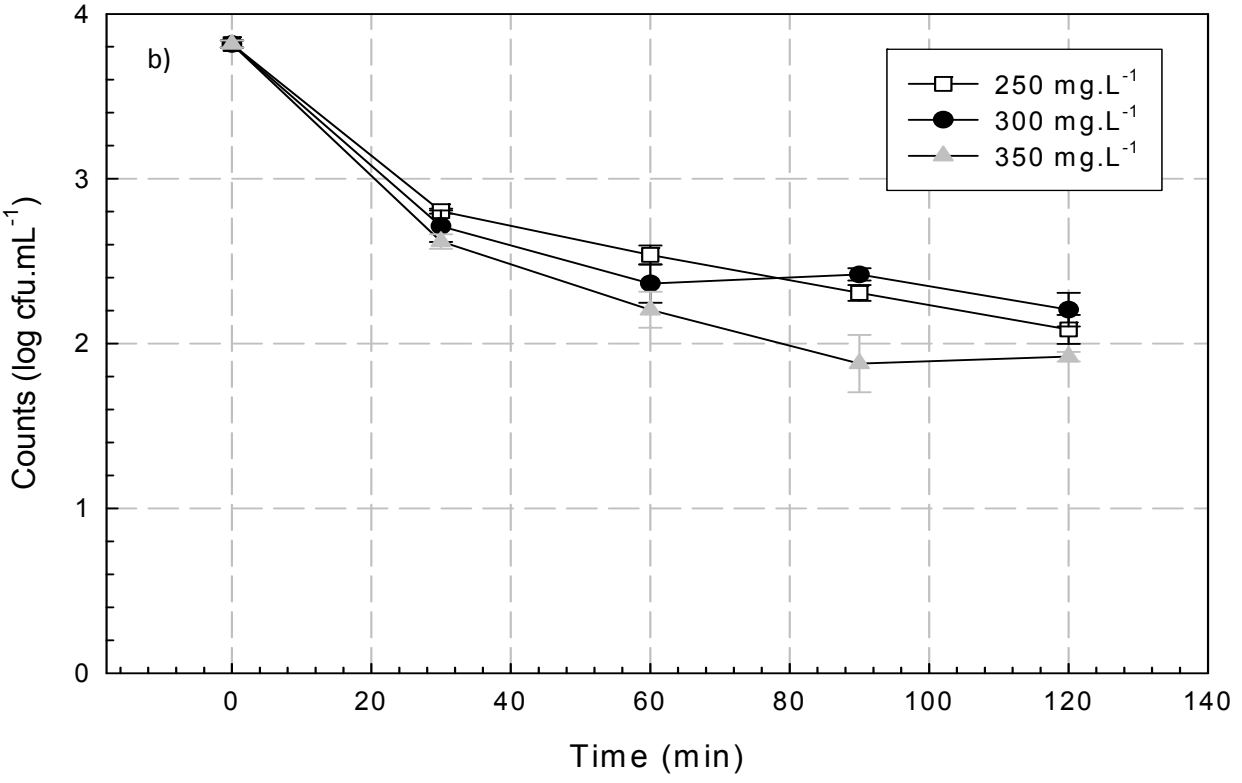


Figure A4.1.2 Effect of H_2O_2 on total coliforms in sampled river water at specific time intervals (a) Sample 1; (b) Sample 2. Error bars represent error at a 95% confidence interval.

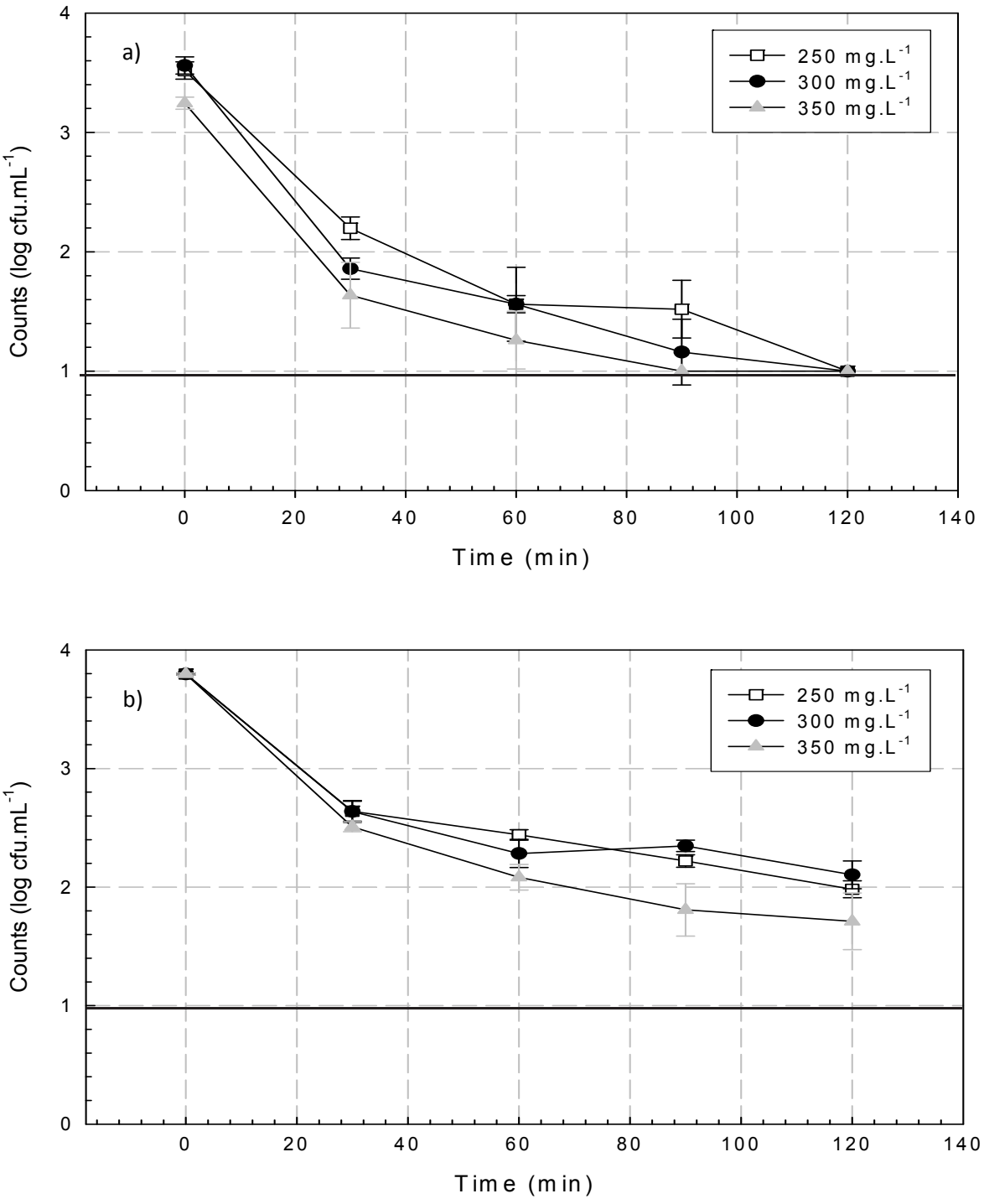


Figure 4.1.3 Effect of H_2O_2 on *E. coli* in sampled river water at specific time intervals (a) Sample 1; (b) Sample 2. The solid line represents the DWA guidelines of less than 1000 faecal coliforms per 100 mL. The method only required a 1 mL sample, therefore, if more than 1 log was present in 1 mL the estimated value would be above the limit of 1 000 faecal coliforms in 100 mL (DWAF, 1996). Error bars represent error at a 95% confidence interval.

Conclusions

In the river water disinfection trials (Section A4.1.2) a trend was seen which indicated that the higher H_2O_2 concentrations resulted in a higher log reduction (Figures A4.1.1 to A4.1.3), which was expected. It was observed that a 120 min contact time would be needed to achieve better log reductions, since the counts were still decreasing (slowly) in the last 30 min for most of the treatments. When comparing the overall microbial inactivation from river sample 1 and 2, it was observed that the same log reductions were not achieved in the different samples. There are three reasons that could explain why this was observed. Firstly, the initial microbial counts differed for sample 1 and 2 and this could influence the log reductions achieved, where a higher log reduction was achieved in the sample with the lower initial counts. Secondly, the two river samples probably contained different bacterial strains and species, which could have reacted differently to the H_2O_2 treatment. The samples were not sampled on the same day and therefore the time lapse may have influenced the microbial levels present in the sample. Thirdly, other unknown substances may be present in the water (from runoff) that may interact or interfere with the H_2O_2 treatment. None of the treatments resulted in water of acceptable quality for fresh produce irrigation (Fig. 4.1.3)

A4.2 Effect of Peracetic acid on environmental and reference *E. coli* strains

Research study design

In this research study the resistance of six *E. coli* strains to various PAA concentrations and contact times was investigated. The investigation of PAA as a possible alternative for Chlorine was based on preliminary studies done as part of this project that indicated that PAA (used at concentrations applied in fresh produce industry) are very effective against an *E. coli* strain in saline (N. Blom BTech project title: To determine the efficacy of commercial sanitising agents on various *E. coli* strains, Cape Peninsula University of Technology, 2014). Note that all disinfection trials were conducted in triplicate and 'no growth' was recorded as 30 colony forming units per millilitre (cfu.mL^{-1}) at the lowest dilution investigated to facilitate statistical analysis.

In Study A, the sensitivity of two environmental *E. coli* strains to PAA concentrations, lower than those recommended for commercial application, was investigated. The recommended PAA concentration for commercial applications is up to 50 mg.L^{-1} for 5 min for fresh produce, where PAA is to serve as a sanitising agent for example in washing water. Environmental *E. coli* strains M53 and F11.2 were selected to test against the commercially recommended PAA concentration of

48 mg.L^{-1} and also against lower doses of 6, 12 and 24 mg.L^{-1}. Strains were enumerated after 5 min of exposure to PAA in 0.85% (m.v^{-1}) sterile saline solution (SSS). The selection of the lower concentrations of PAA investigated in this study was based on previous studies on wastewater disinfection (Freese *et al*., 2003; Veschetti *et al*., 2003; Dell'Erba *et al*., 2004; Koivunen & Heinonen-Tanski, 2005a; Luukkonen *et al*., 2014).

In Study B, strain-to-strain variation between environmental and reference *E. coli* strains (Table A2.1) was evaluated in SSS. All *E. coli* isolates were exposed to 6 mg.L^{-1} PAA and enumerated at time intervals of 5, 15 and 25 min.

In Study C, the optimisation of PAA treatments in terms of treatment concentrations and contact times was studied in saline solution. The effect of longer contact times and lower PAA concentrations was investigated. The most resistant strain from Study B was selected for this trial and was tested against 0.5, 1.5, 3.0, 4.5 and 6.0 mg.L^{-1} PAA. *G*rowth was evaluated after 5, 15 and 25 min.

Study D focussed on the influence of river water quality on PAA efficiency during two separate trials (Trial 1 & Trial 2). This was done by using both sterile river water as well as flocculated river water. The river water samples (untreated and flocculated river water) were inoculated with environmental *E. coli* F11.2 and treated with a PAA dose of 4.5 mg.L^{-1} (Trial 1) for a contact time of 25 min, as well as a PAA concentration of 6 mg.L^{-1} (Trial 2) for an exposure time of 25 min.

Results

Study A: Efficacy of commercial and lower PAA concentrations on E. coli inactivation

Manufacturers recommend high concentrations of up to 50 mg.L^{-1} PAA for short contact times to sanitise fresh produce items prior to consumption. Figure A4.2.1 shows the log reductions of two environmental *E. coli* strains (M53 and F11.2) after treatment with four different PAA concentrations for 5 min. The reductions obtained at 6 mg.L^{-1} for M53 and F11.2 were 1.95 log and 1.59 log, respectively (Fig. A4.2.1).

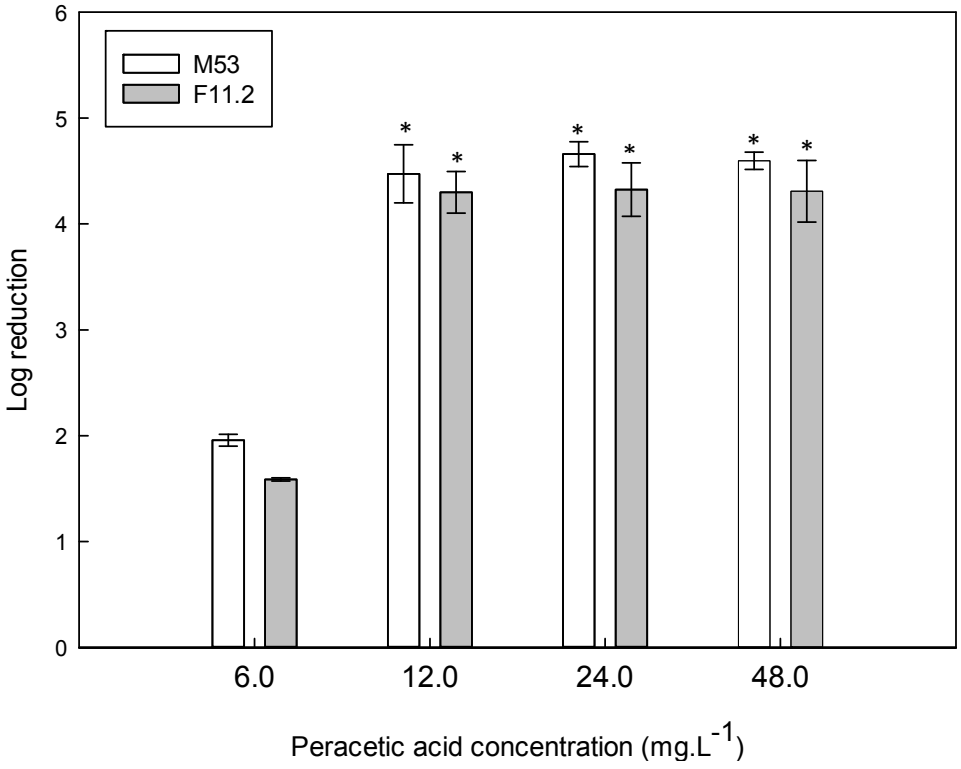


Figure A4.2.1 Log reductions observed after 5 min at different PAA concentrations for environmental *E. coli* strains M53 and F11.2 in saline solution. Error bars are calculated from standard deviation at a 95% confidence level. * - No growth detected at lowest dilution (10^{-1}).

Study B: Strain-to-strain variation between environmental and reference E. coli strains

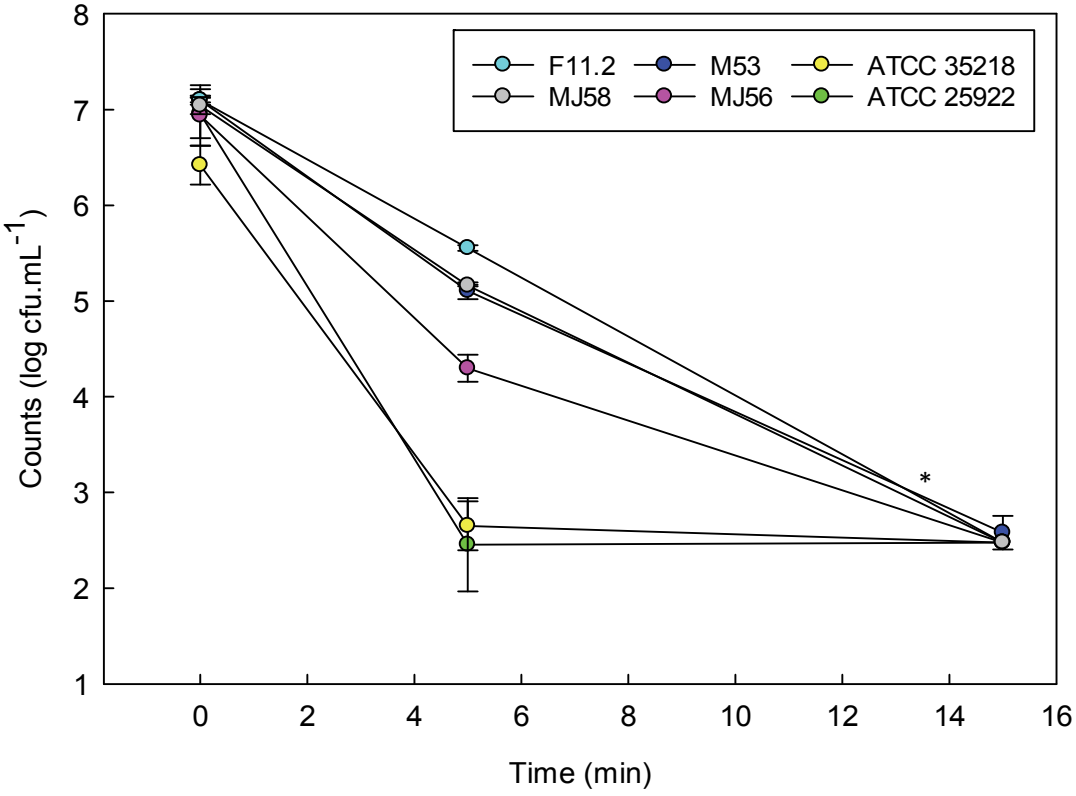


Figure A4.2.2 Inactivation curve for six *E. coli* strains against 6 mg.L^{-1} PAA at 5 and 15 min in saline. Error bars are calculated from standard deviation at a 95% confidence level. * - No growth detected at lowest dilution (10^0).

Study C: Optimisation of PAA treatments in terms of concentration and contact time

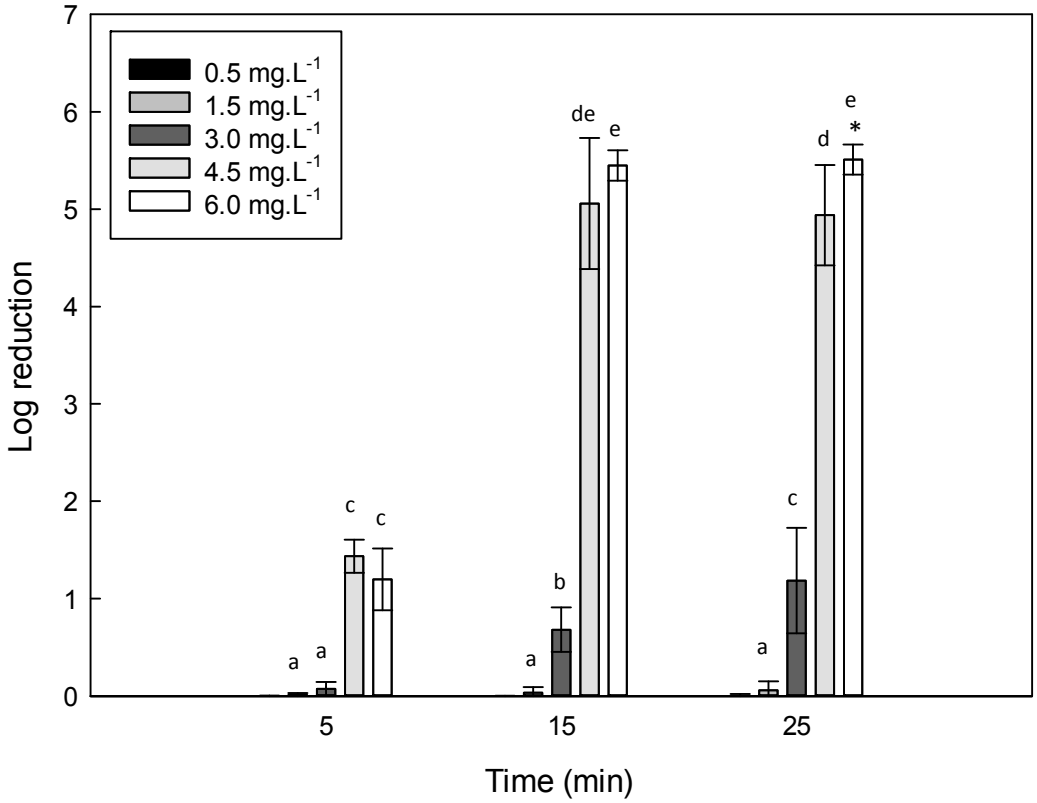


Figure A4.2.3 Log reductions observed for *E. coli* F11.2 at five PAA concentrations after 5, 15 and 25 min in saline. Error bars are calculated from standard deviation at a 95% confidence level. Statistical analysis was done using the mixed model repeated measures ANOVA and the Fisher LSD post hoc test. * - No growth detected at lowest dilution (10^0).

Study D: Influence of the water quality on PAA treatment efficiency in river water

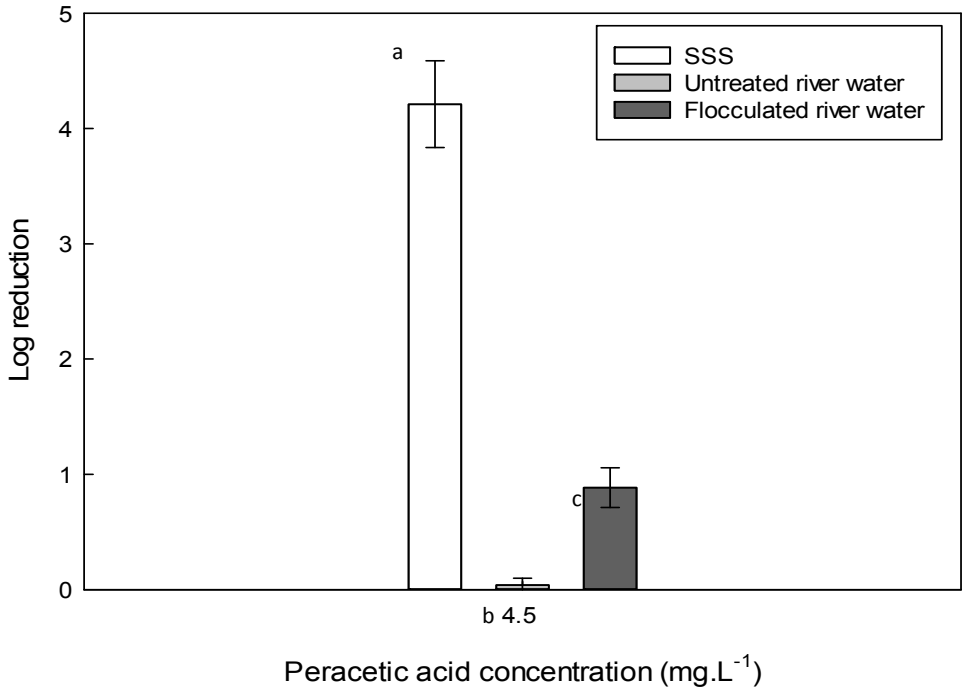


Figure A4.2.4 Log reductions observed for *E. coli* F11.2 in SSS compared to untreated and flocculated river water during disinfection Trial 1. The strain was exposed to 4.5 $mg.L^{-1}$ peracetic acid for a contact time of 25 min. Error bars are calculated from standard deviation at a 95% confidence level. Statistical analysis was done using the one way ANOVA and the Fisher LSD post hoc test.
SSS – sterile saline solution;

Table A4.2.1 Water quality parameters of river water before and after flocculation during peracetic acid disinfection Trial 1 and Trial 2

	Trial 1		**Trial 2**	
	Untreated	**Flocculated**	**Untreated**	**Flocculated**
pH	7.22	ND	7.02	ND
COD ($mg.L^{-1}$)	79	74	18	18
TSS ($mg.L^{-1}$)	8.75	0.73	7.30	0.50
Turbidity (NTU)	16.82	15.24	6.73	1.00
Alkalinity ($mg.L^{-1}$ $CaCO_3$)	100.0	137.5	37.5	25
Conductivity ($mS.m^{-1}$)	57	60	88	89
UVT%	35.2	36.5	76	89

ND – Not determined

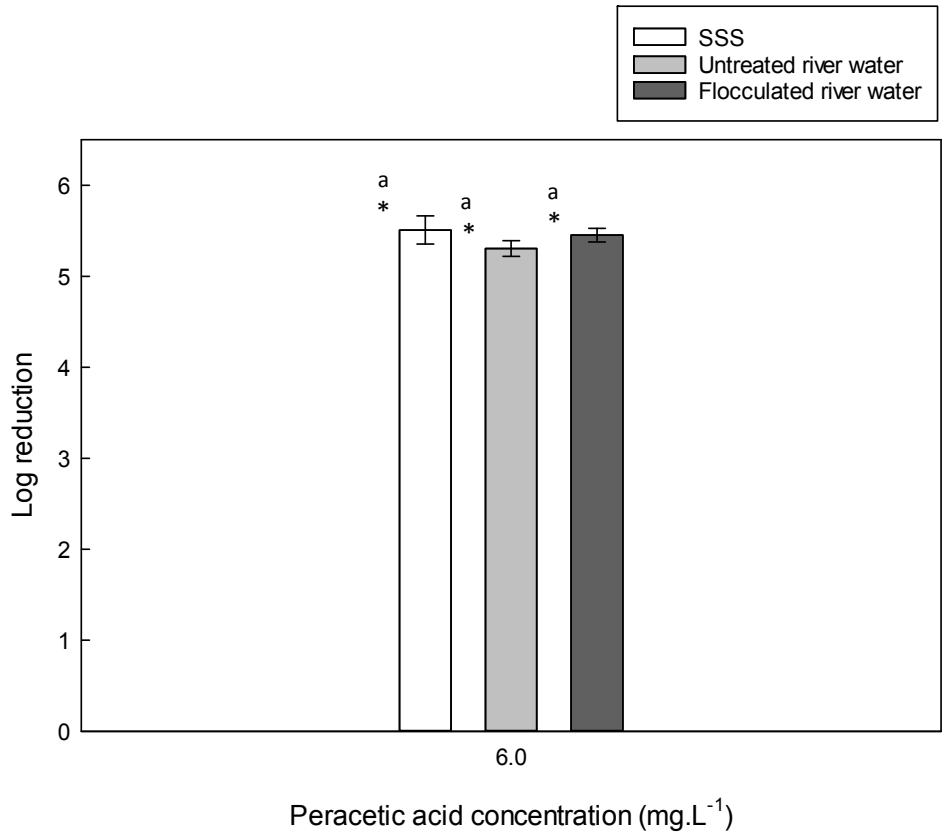


Figure A4.2.5 Log reductions observed for *E. coli* F11.2 in SSS compared to untreated and flocculated river water during disinfection Trial 2. The strain was exposed to 6.0 $mg.L^{-1}$ peracetic acid for a contact time of 25 min. Error bars are calculated from standard deviation at a 95% confidence level. Statistical analysis was done using the one way ANOVA and the Fisher LSD post hoc test. * - No growth detected at lowest dilution (10^0).

Conclusions

Results indicated that the *E. coli* strains investigated responded differently to PAA disinfection and the variability of strains within the same species was, thus, clearly evident. *Escherichia coli* isolates that served as reference strains were in all cases more sensitive to PAA disinfection than environmental *E. coli* strains (Fig. A4.2.2). Bacteria are generally equipped with various defence mechanisms against chemical oxidative stresses. The development of these defence strategies is an adaptive response as a result of continual stresses posed by the environment and the resistance of environmental strains can be generated by multiple mechanisms. Thus, it is important to use environmental *E. coli* strains during disinfection optimisation rather than reference *E. coli* strains, as the latter may be very sensitive to biocides and not a good representation of the actual river water population.

A wide range of PAA dosages and contact times were effective against *E. coli* removal (Figures A4.2.1 & A4.2.3). The *E. coli* strains tested were inactivated at commercial concentrations and very short contact times (Fig. A4.2.1). Therefore, the effect of lower PAA doses and longer contact times was investigated in an attempt to decrease the cost of dosing (Fig. A4.2.3). Having said this, longer contact times may delay the production rate (limit the capacity of the plant) of treated water at commercial scale by increasing the size of the tanks or storage dams required for the longer contact times. At lower PAA doses and longer contact times, it was evident

that the degree of *E. coli* disinfection was concurrently dependent on both the PAA concentration and contact time. Low PAA doses ranging from 0.5-3.0 $mg.L^{-1}$ were ineffective (< 1.18 log reduction) over a 25 min contact period. At these concentrations, a tolerance limit was exhibited by *E. coli*. This is explained by the fact that a slight increase in the concentration to 4.5 and 6.0 $mg.L^{-1}$, resulted in significant disinfection after 15 and 25 min (> 4 log). The disinfection efficiencies at a contact time of 5 min were much lower than reductions obtained after 15 and 25 min showing that a longer contact time can contribute to increased disinfection.

Variation in water quality was evident on different sampling days (Table A4.2.1). The possible neutralisation of PAA possibly occurred in water with an alkalinity of 100-137.5 $mg.L^{-1}$ $CaCO_3$ (Fig. A4.2.4). If so, the germicidal capability of PAA was significantly decreased. *Escherichia coli* inactivation was, however, not limited in water representing alkalinity levels between 25.0-37.5 $mg.L^{-1}$ $CaCO_3$ (Fig. A4.2.5).

Overall, PAA can be suggested as an efficient and cost effective disinfection method for contaminated river water due to its high efficacy at low concentrations and contact times. The optimum PAA dosage and contact time suggested as a treatment option for contaminated river water would be 4.5-6.0 $mg.L^{-1}$ for a contact period of 25 min. This recommendation is subject to change as water quality plays a major role in the disinfection efficiency of PAA and also, the water quality varies over time. Therefore, water quality of any specific source will need to be considered individually before a treatment regime is implemented. The concentration of PAA can be increased easily since there are no concerns regarding environmental impacts. This may, however, increase costs. The microbial population in river water may react differently to PAA disinfection than the *E. coli* strains tested in this study. Therefore, further investigation into how effective PAA is against an unknown mixed microbial population in river water, taking into account varying water quality properties may give an indication of concentrations and contact times needed for irrigation water disinfection.

The effect of another well-known chemical disinfectant, chlorine, should be investigated in also considering its potential for river water disinfection. The effectiveness of this chemical disinfectant towards a wide range of microorganisms has been reported numerously.

A4.3 Effect of chlorine on environmental and reference *E. coli* strains

Research study design

The efficacy of chlorine, using both HTH calcium hypochlorite ($Ca(OCl)_2$ and sodium hypochlorite (NaOCl), on the survival of various *E. coli* strains was investigated. This was done by means of four independent studies..

During Study A, the effect of $Ca(OCl)_2$ on three *E. coli* strains (ATCC 25922, M53, MJ58) was evaluated. Of the three strains, one served as a reference strain (ATCC 25922) and the other strains were environmental isolates (M53 and MJ58) that had previously been isolated from different sources (Table A2.1). A stock solution, representing 1 000 $mg.L^{-1}$ ($Ca(OCl)_2$) available chlorine, was prepared from granular HTH $Ca(OCl)_2$ prior to disinfection. Thereafter, each strain was dosed separately with chlorine concentrations of 6, 9 and 12 $mg.L^{-1}$ and the effect thereof was evaluated after contact periods of 30, 60, 90 and 120 min.

During Study B, the effect of NaOCl on six *E. coli* strains (Table A2.1) was studied. Two of these strains (ATCC 25922 and ATCC 35218) served as reference strains and were compared to four environmental strains (M53, MJ56, F11.2 and MJ58). Each strain was treated individually with 6, 9 and 12 $mg.L^{-1}$ chlorine and *E. coli* growth was determined after four time intervals (30, 60, 90 and 120 min). Based on these results, the most resistant strain was selected to evaluate its performance against increased chlorine concentrations (14 and 24 $mg.L^{-1}$ for 30 min) in SSS.

In Study C, the two chlorine sources, NaOCl and $Ca(OCl)_2$, were compared. The efficacy of these two disinfectants was compared using the most resistant strain from Study B. The *E. coli* suspension was dosed with 12 $mg.L^{-1}$ chlorine (derived from both NaOCl and $Ca(OCl)_2$, respectively) and a contact time of 120 min was allowed. The residual chlorine concentration remaining after 120 min disinfection was measured using a cell test kit (Merck, Germany). The United States Environmental Protection Agency (2004) recommends a limit of ≤ 1 $mg.L^{-1}$ residual chlorine for reclaimed water intended for land irrigation. This was the residual limit chosen for this study as there are no residual levels specified for the irrigation of fresh produce. Also, to analyse the possible effect of pH, the pH was recorded after chlorine disinfection.

During Study D, the effect of river water quality on chlorine disinfection was investigated. Sterile saline solution as well as sterilised (untreated and flocculated) river water were inoculated with *E. coli* MJ58 prior to chlorine disinfection. Each sample was exposed to 12 $mg.L^{-1}$ chlorine for 120 min. The chlorine residual was measured shortly after disinfection using a cell test kit (Merck, Germany). Physico-chemical analyses were performed on untreated and flocculated river water samples (pH, COD, TSS, electrical conductivity, alkalinity, turbidity and UVT%).

Results

Study A. The effect of $Ca(OCl)_2$ on E. coli inactivation

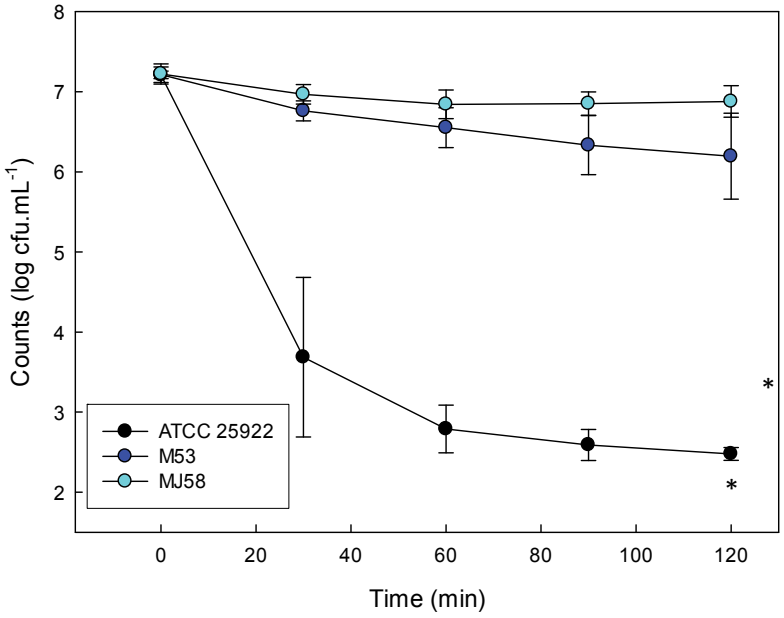


Figure A4.3.1 Inactivation curve for three *E. coli* isolates (ATCC 25922, M53 and MJ58) against 6 $mg.L^{-1}$ chlorine ($Ca(OCl)_2$) over 30, 60, 90 and 120 min contact period in saline. Error bars were calculated from the standard deviation at a 95% confidence level.

* - No growth detected at the lowest dilution (10^{-1}).

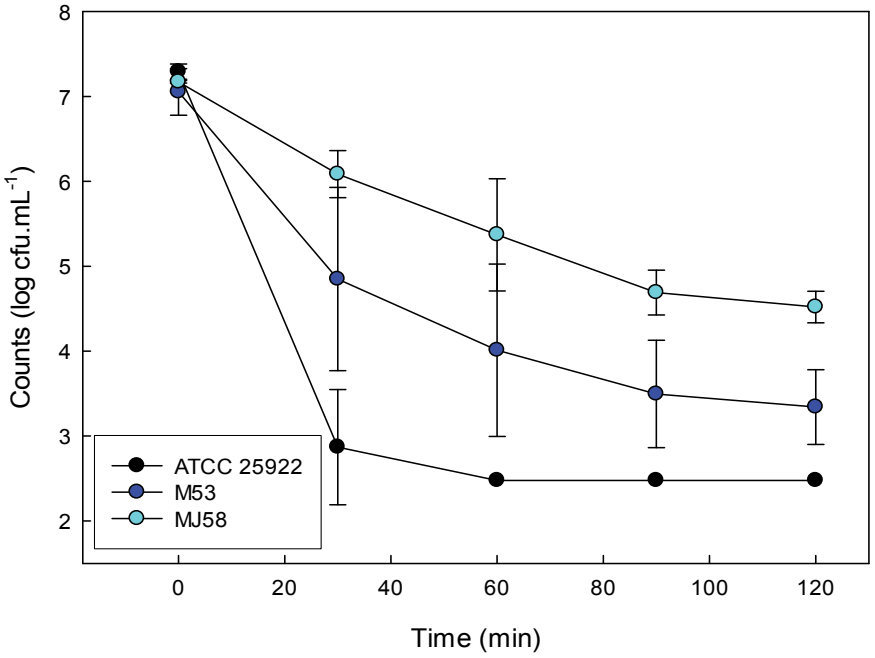


Figure A4.3.2 Inactivation curve for three *E. coli* isolates (ATCC 25922, M53 and MJ58) against 9 $mg.L^{-1}$ chlorine ($Ca(OCl)_2$) over 30, 60, 90 and 120 min contact period in saline. Error bars were calculated from the standard deviation at a 95% confidence level.

* - No growth detected at the lowest dilution (10^{-1}).

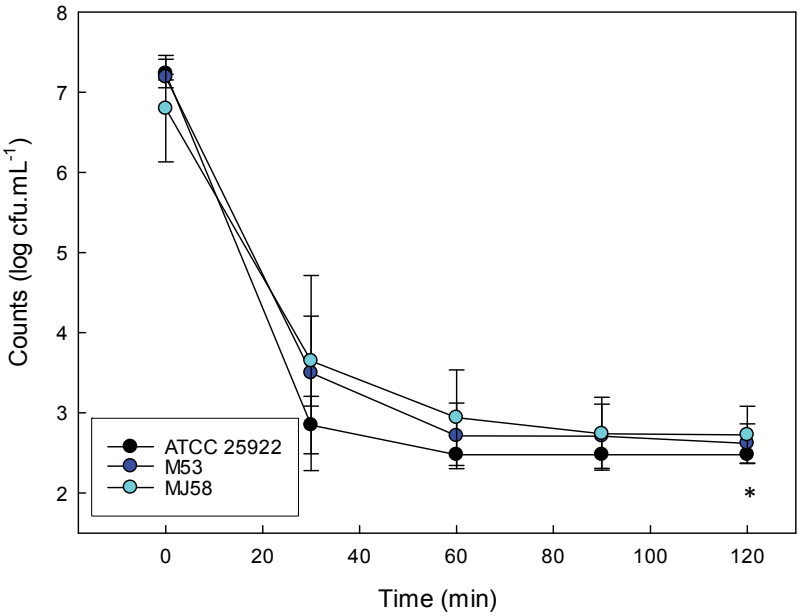


Figure A4.3.3 Inactivation curve for three *E. coli* isolates (ATCC 25922, M53 and MJ58) against 12 mg.L^{-1} chlorine ($Ca(OCl)_2$) over 30, 60, 90 and 120 min contact period in saline. Error bars were calculated from the standard deviation at a 95% confidence level.

* - No growth detected at the lowest dilution for ATCC 25922 (10^{-1}).

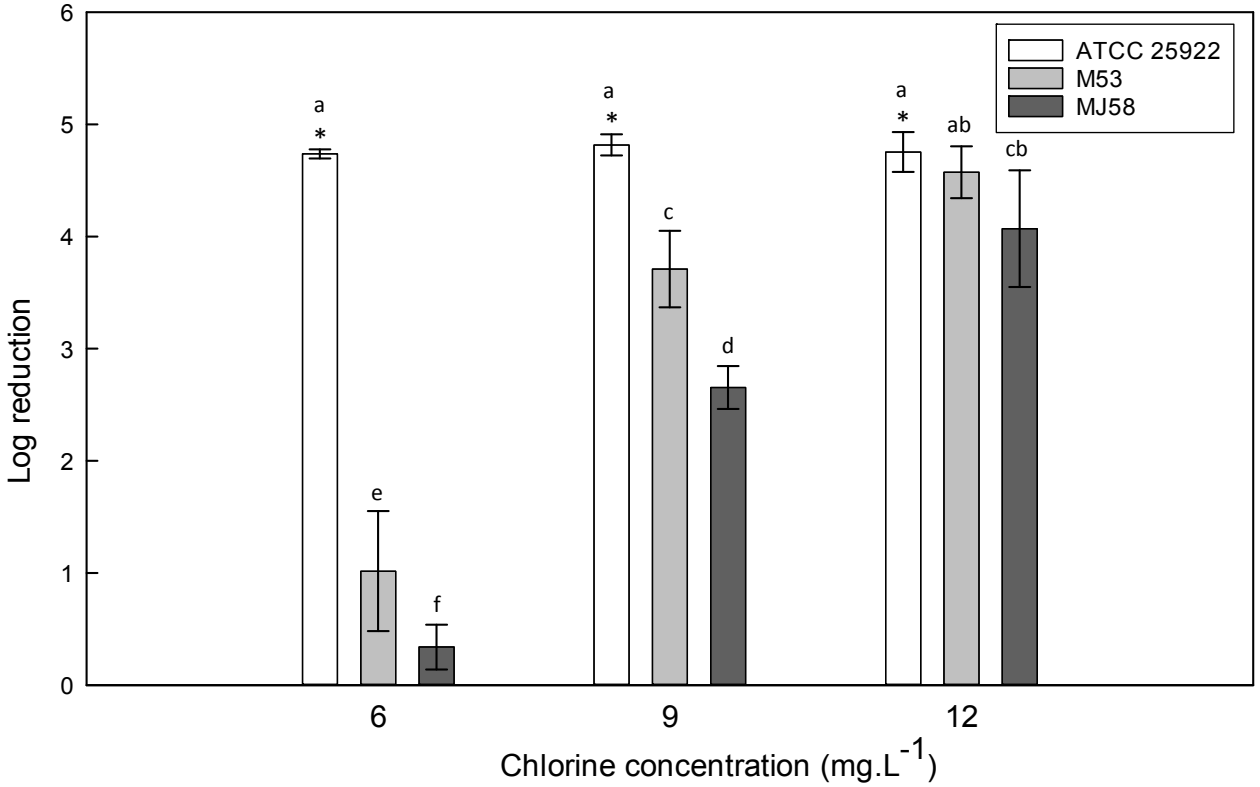


Figure A4.3.4 Log reductions achieved for ATCC 25922, M53 and MJ58 at different chlorine concentrations ($Ca(OCl)_2$) of 6, 9 and 12 mg.L^{-1} over a contact period of 120 min in saline. Error bars were calculated from the standard deviation at a 95% confidence level. Statistical analysis was done using the two way ANOVA and the Fisher LSD post hoc test.

* - No growth detected at lowest dilution investigated (10^{-1}).

Study B. The effect of NaOCl on E. coli inactivation

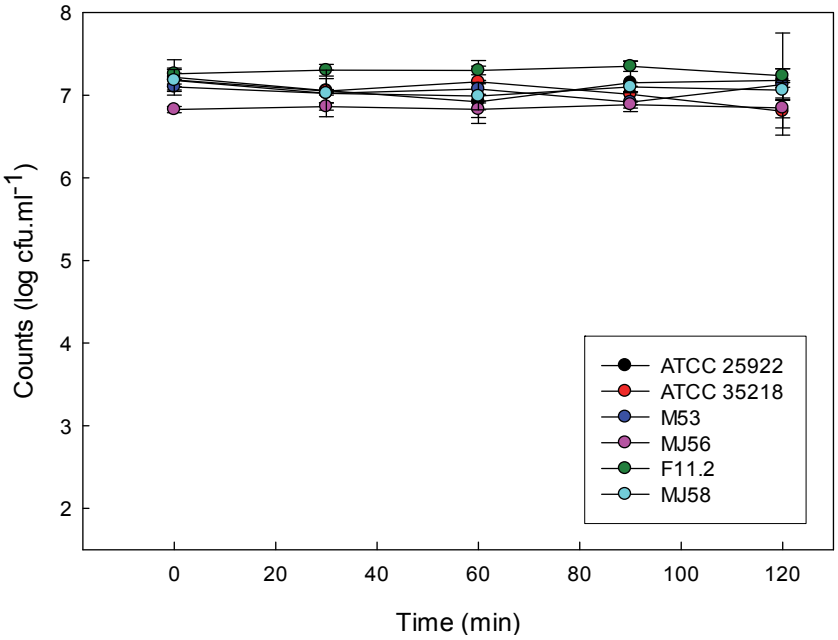


Figure A4.3.5 Inactivation curves of six *E. coli* strains (ATCC 25922, ATCC 35218, M53, MJ56, F11.2 and MJ58) against 6 mg.L^{-1} chlorine (NaOCl) at different time intervals (30, 60, 90 and 120 min) in saline. Error bars are calculated from standard deviation at a 95% confidence level.

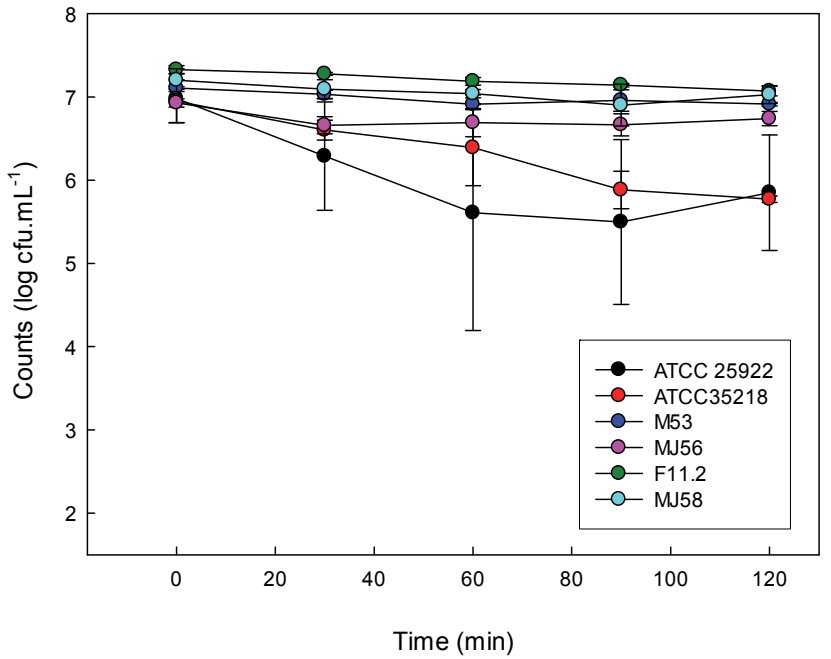


Figure A4.3.6 Inactivation curves of six *E. coli* strains (ATCC 25922, ATCC 35218, M53, MJ56, F11.2 and MJ58) against 9 mg.L^{-1} chlorine (NaOCl) at different time intervals (30, 60, 90 and 120 min) in saline. Error bars are calculated from standard deviation at a 95% confidence level.

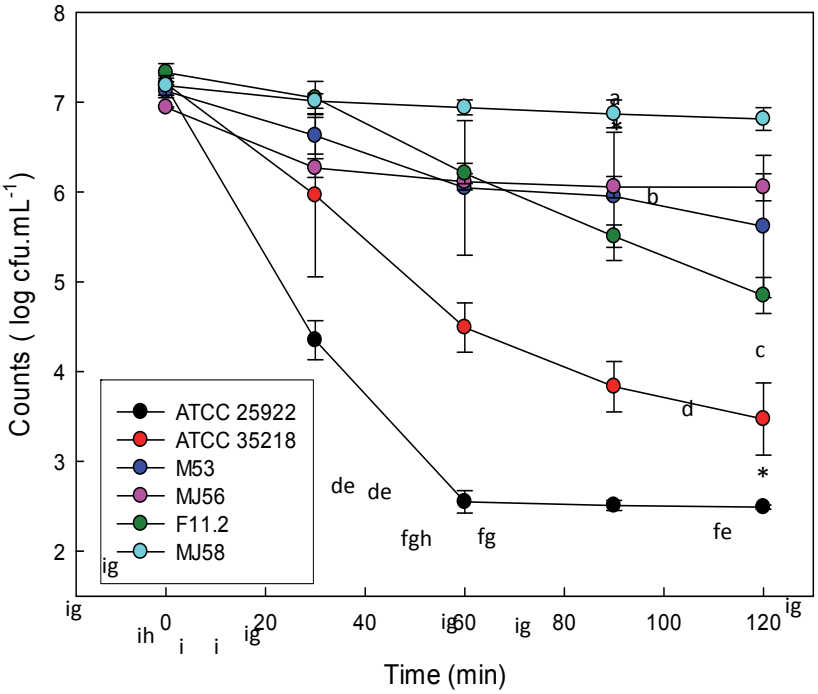


Figure A4.3.7 Inactivation curves of six *E. coli* strains (ATCC 25922, ATCC 35218, M53, MJ56, F11.2 and MJ58) against 12 mg.L^{-1} chlorine at different time intervals (30, 60, 90 and 120 min). Error bars are calculated from standard deviation at a 95% confidence level. * - No growth detected at lowest dilution (10^{-1})

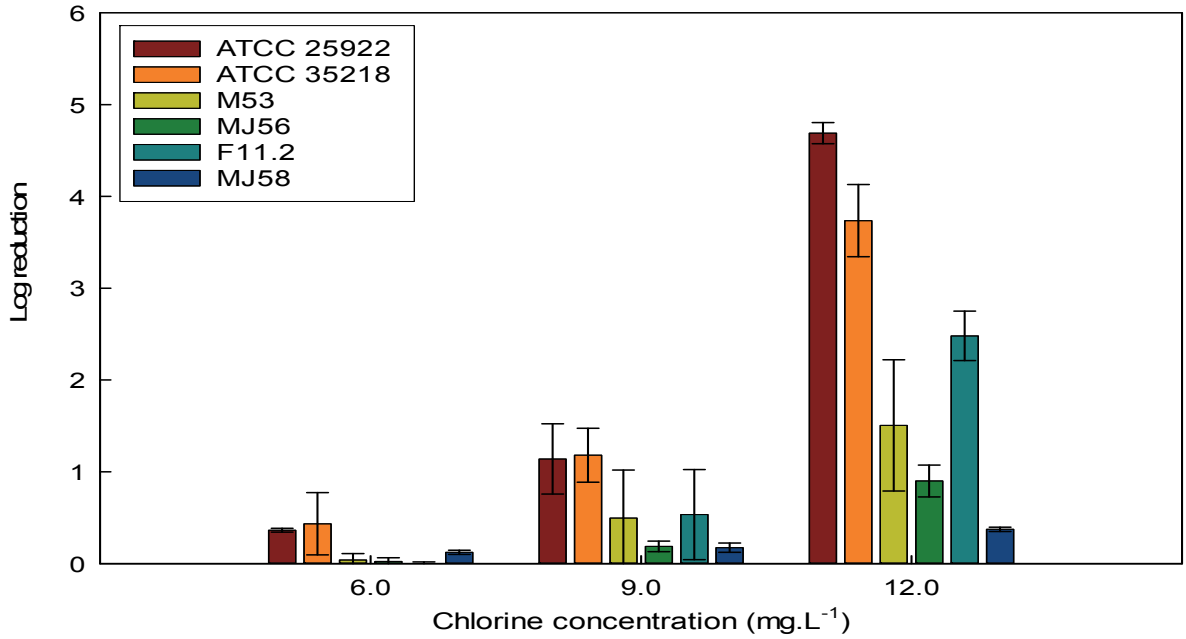


Figure A4.3.8 Log reductions obtained for six *E. coli* strains (ATCC 25922, ATCC 35218, M53, MJ56, F11.2 and MJ58) at different chlorine concentrations (NaOCl) over a contact period of 120 min in saline. Error bars are calculated from standard deviation at a 95% confidence level. Statistical analysis was done using the two way ANOVA and the Fisher LSD post hoc test. * - No growth detected at lowest dilution investigated (10^{-1}).

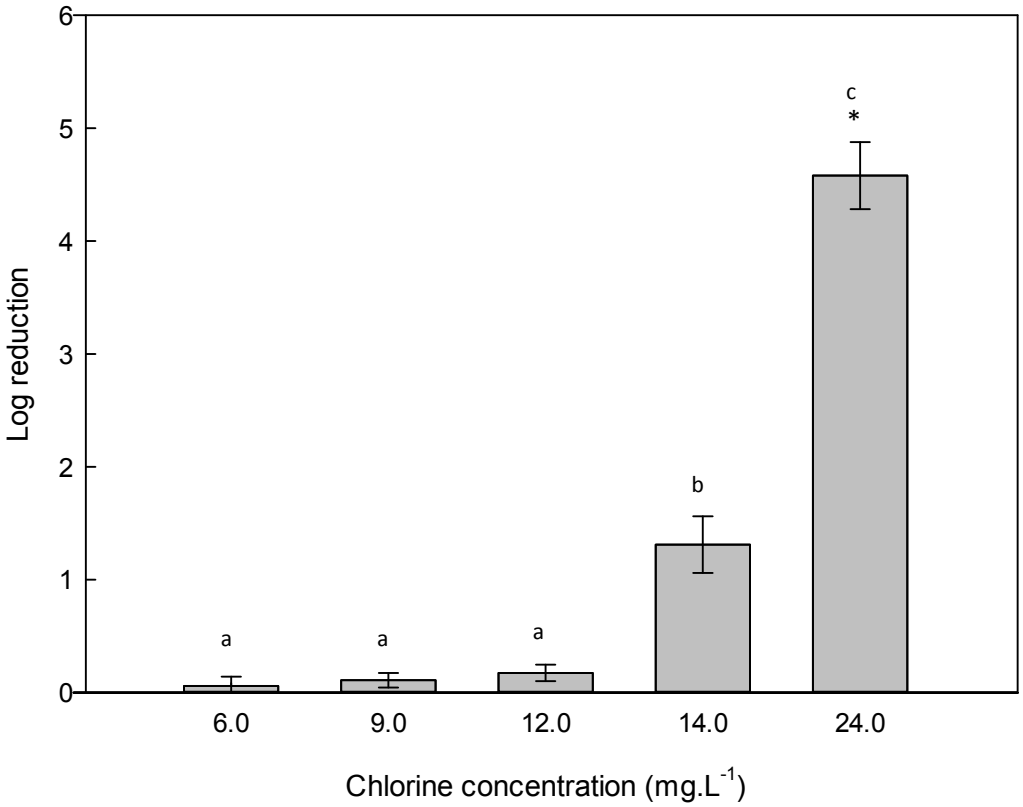


Figure A4.3.9 Log reductions after 30 min for *E. coli* MJ58 against the following chlorine concentrations (NaOCl) in saline: 6, 9, 12, 14 and 24 $mg.L^{-1}$. Error bars were calculated from the standard deviation at a confidence level of 95%. Statistical analysis was done using the one way ANOVA and the Fisher LSD post hoc test. * - No growth detected at lowest dilution investigated (10^0).

Study C. Comparing disinfection capabilities of NaOCl and $Ca(OCl)_2$ on E. coli survival

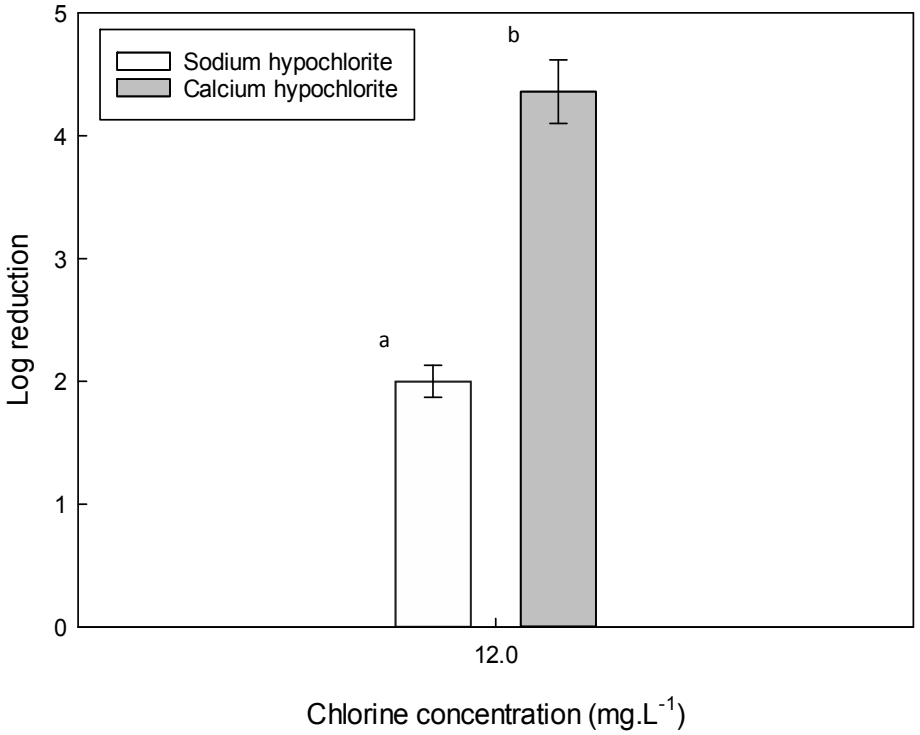


Figure A4.3.10 Log reductions obtained against 12 $mg.L^{-1}$ for 120 min on *E. coli* MJ58 for sodium and calcium hypochlorite in SSS. Error bars are calculated form standard deviation at a 95% confidence level. Statistical analysis was done using the one way ANOVA and the Fisher LSD post hoc test.

Table A4.3.1 Residual chlorine and pH values after disinfection

	Residual chlorine ($mg.L^{-1}$)	
Time (min)	**NaOCl**	**$Ca(OCl)_2$**
30	0.86	0.82
60	0.86	0.84
90	0.60	0.62
120	0.66	0.57
pH		
After disinfection	7.12	7.12

Study D. The effect of water quality on chlorine disinfection

Table A4.3.2 River water properties and chlorine residual before and after flocculation

		Untreated	**Flocculated**
pH		7.22	7.30
COD ($mg.L^{-1}$)		79	74
TSS ($mg.L^{-1}$)		8.75	0.73
Turbidity (NTU)		16.8	15.2
Alkalinity ($mg.L^{-1}$ $CaCO_3$)		100.0	137.5
Conductivity ($mS.m^{-1}$)		57	60
UVT%		35.2	36.5
	SSS	**Untreated**	**Flocculated**
Residual chlorine ($mg.L^{-1}$)	0.63	2.34	2.11

SSS –Sterile saline solution

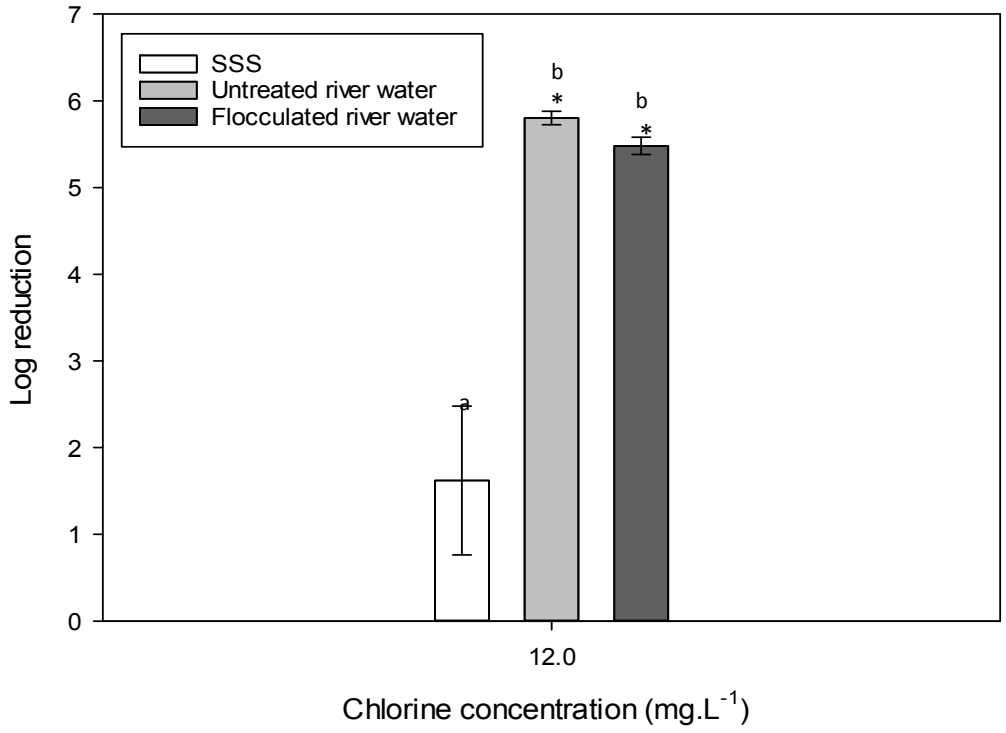


Figure A4.3.11 Comparison between chlorine disinfection in SSS, untreated and flocculated river water inoculated with *E. coli* MJ58 after chlorine exposure (12 $mg.L^{-1}$) for 120 min. Error bars were calculated from the standard deviation at a confidence level of 95%. Statistical analysis was done using the one way ANOVA and the Fisher LSD post hoc test.

SSS – Sterile saline solution; * - No growth detected at lowest dilution investigated (10^0).

Conclusions

Strain variation was prominent during chlorine studies (Figures A4.3.1-A4.3.8). Reference strains were always more sensitive than environmental strains as reference strains showed higher log reductions (Figures A4.3.4 & A4.3.8). The reference strain ATCC 25922 was the most sensitive strain and environmental *E. coli* MJ58 showed the lowest reduction trend throughout chlorine disinfection in saline. Greater resistance by environmental strains indicates the variability in susceptibility towards chlorine. From this study it was evident that strains from the same species may differ in their response to chlorine and implies the development of various resistance mechanisms to withstand oxidative stress. The most resistant strain (MJ58) was completely inactivated in saline at a chlorine dosage of 24 $mg.L^{-1}$ (NaOCl) and 30 min (Fig. A4.3.9). Chlorine doses that ranged from 6-12 $mg.L^{-1}$ (NaOCl) were inadequate to effectively reduce *E. coli* strains in saline (Figures A4.3.5-A4.3.7).

Of the two chlorine sources investigated during saline studies, $Ca(OCl)_2$ was much more effective on *E. coli* than NaOCl (Fig. A4.3.10). Generally, the NaOCl solutions can degrade over time. Therefore, it is very important to ensure the use fresh NaOCl solutions and confirm the actual free chlorine concentration prior to the application towards contaminated river water.

Chlorine disinfection is pH-dependent. Although the pH after disinfection was the same (Table A4.3.2) in this study, the different chlorine sources had slightly different initial pH levels, directly after the addition NaOCl and $Ca(OCl)_2$ to saline (Bester, 2015). The exceptionally low alkalinity (buffer capacity) of SSS was probably responsible for this phenomenon. More alkaline initial pH levels (> pH 8) in saline solutions containing NaOCl resulted in significant differences in *E. coli* disinfection between NaOCl and $Ca(OCl)_2$. The addition of chlorine to well-buffered systems is of utmost importance since the optimum pH range for chlorine disinfection range between 7.2-7.4. Fortunately, the pH of river water used as part of study D (pH 7.22 – 7.30) falls within the range for optimal chlorine functioning during chlorine disinfection. Of the two chlorine sources evaluated, NaOCl is preferred for commercial-scale applications. Calcium hypochlorite requires additional installations to filter the insoluble material before disinfection. Therefore, it is suggested that further investigation into chlorine disinfection on river water is based on the use of NaOCl as a chlorine source.

In study D the influence of water quality on chlorine disinfection was investigated. River water displaying a COD load between 74 and 79 $mg.L^{-1}$ did not influence chlorine disinfection (12 $mg.L^{-1}$ for 120 min) and no *E. coli* growth was detected (> 5 log reduction) (Table A4.3.2; Fig. A4.3.11). The chlorine concentration of 12 $mg.L^{-1}$ (NaOCl) met the demand posed by organic particles as well as microorganisms in river water. Note that at lower chlorine dosages, the effect of COD would have been more prominent. Adding to this, river water was well-buffered and contributed to effective *E. coli* disinfection compared to ineffective *E. coli* removal in saline solution (low buffer capacity). The residual chlorine levels were > 2 $mg.L^{-1}$ in river water samples treated with 12 $mg.L^{-1}$ chlorine. Maintaining a low residual concentration is important. The application of lower dosages will probably result in lower residual levels consequently limiting the risk posed to the environment (by-product formation) and ultimately fresh produce items.

The resistance of the heterogenic population in river water to chlorine may differ from the isolates investigated in this study and therefore may vary in their reactions to the chlorine doses evaluated in this study. Therefore, further research investigating the efficacy of chlorine on river water disinfection is necessary. Effective *E. coli* inactivation was achieved by chlorine at the water quality reported in this study. Therefore, chlorine can be considered a potential disinfectant for contaminated river water. The residual chlorine concentration is a limiting factor when choosing an optimum chlorine concentration for river water disinfection. A predetermined chlorine

concentration could not be suggested for river water disinfection as the selected chlorine dosage should rather be based on river water quality and subsequent chlorine demand.

A4.4 Disinfection efficacy of peracetic acid and chlorine treatments of river water at laboratory-scale

Research study design

In this study, river water was disinfected with PAA and chlorine on five separate days. Liquid sodium hypochlorite (NaOCl) served as the chlorine source during this study. Table A4.4.1 displays the dosages, contact times and microorganisms investigated during each trial. After chlorine disinfection (120 min), the residual concentration ($mg.L^{-1}$) was measured using a cell test kit (Merck, Germany). Note that water quality analysis was performed on river water prior to disinfection. This included the evaluation of temperature, pH, COD, alkalinity, TSS, electrical conductivity, turbidity and UVT%.

Table A4.4.1 Information regarding concentration, contact time and microorganisms investigated during five disinfection trials testing chlorine and PAA efficacy

	Concentration ($mg.L^{-1}$)	Contact time (min)	Microorganisms studied
Trial 1-3	PAA – 4.5	PAA – 15, 25	Heterotrophic microorganisms Total coliforms *Escherichia coli*
	Chlorine – 6.0	Chlorine – 30, 60, 90, 120	
Trial 4-5	PAA – 3.0	PAA – 15, 25	
	Chlorine – 3.0	Chlorine – 30, 60, 90, 120	

PAA – Peracetic acid

Figure A4.4.1 displays the general procedure followed during chlorine and PAA disinfection of river water samples. Eighty millilitres of river water was used for treatment studies at laboratory-scale. Firstly, control plates were prepared (10^{-1}-10^{-4}) to determine the initial microbial load present in river water (Fig.A4.4.1). Thereafter, the river water was dosed with the disinfectant (chlorine or PAA) at the particular concentration investigated (Fig. A4.4.1). A dilution series (10^{-1}-10^{-4}) was prepared after disinfection in SSS. Each dilution (as well as an undiluted sample) was transferred in duplicate to petri dishes after which the appropriate agar was added (Fig. A4.4.1). This was followed by duplicate plating and the bactericidal effect on microorganisms was determined at different time intervals for each disinfectant evaluated. Contact times of 15 and 25 min were used during PAA disinfection compared to the four time intervals evaluated during chlorine disinfection (30, 60, 90 and 120 min) (Fig. A4.4.1). Note that only total coliforms were studied at different time intervals. The levels of *E. coli* and heterotrophic microorganisms were only determined before (control) and after a total time of 120 min (chlorine) and 25 min (PAA). For each water sample, disinfectant trials were conducted in triplicate.

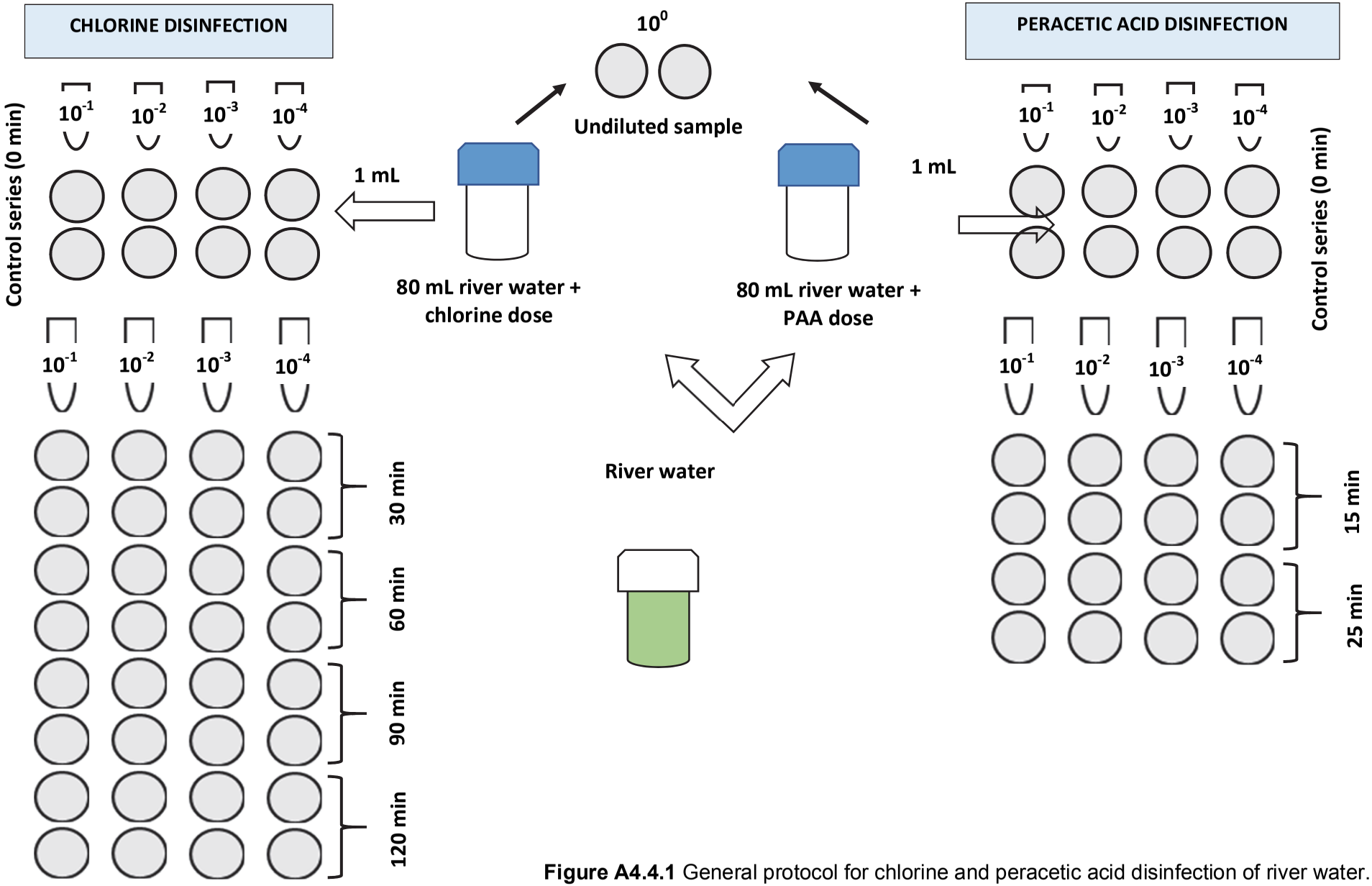


Figure A4.4.1 General protocol for chlorine and peracetic acid disinfection of river water.

Results

Table A4.4.2 Physico-chemical and microbiological parameters of river water before disinfection trials 1-5.

Parameters	**Trial 1**	**Trail 2**	**Trail 3**	**Trial 4**	**Trial 5**
Temperature (°C)	18	18.5	16	15	17
pH	7.32	6.73	7.43	7.12	6.90
COD ($mg.L^{-1}$)	30	1 094	21	14	108
Alkalinity ($g.L^{-1}$ $CaCO_3$)	119	95	88	75	87.5
TSS ($mg.L^{-1}$)	8.0	8.0	8.0	3.3	4.5
ECO ($mS.m^{-1}$)	52.9	64.6	35.7	41.5	49.6
Turbidity (NTU)	12.05	13.34	12.51	5.76	4.11
UVT (%)	49.9	15.1	62.3	36.9	53.3
ACC (cfu.100 mL^{-1})	18.32×10^6	18.58×10^6	13.47×10^6	7.60×10^5	9.63×10^5
TC (cfu.100 mL^{-1})	1.51×10^6	1.69×10^6	1.66×10^5	1.61×10^4	1.66×10^4
EC (cfu.100 mL^{-1})	1.97×10^5	5.40×10^5	6.38×10^4	2.85×10^3	3.52×10^3

ACC – Aerobic colony count; ECO – Electrical conductivity; TC – Total coliforms; EC – *Escherichia coli*

Effect of chlorine and PAA on microbial inactivation in river water (Trials 1-3)

Table A4.4.3 Log values (cfu.mL^{-1}) before and after chlorine and PAA disinfection Trial 1

	ACC	**TC**	**EC**
Chlorine (6.0 mg.L^{-1})			
Before treatment	5.26±0.04	4.00±0.05	3.30±0.0046
After treatment (120 min)	3.24 ±0.095	None	None
Peracetic acid (4.5 mg.L^{-1})			
Before treatment	5.26±0.04	4.28±0.21	3.30±0.0046
After treatment (25 min)	2.85±0.022	1.65±0.40	None

Table A4.4.4 Log values (cfu.mL^{-1}) before and after chlorine and PAA disinfection Trial 2

	ACC	**TC**	**EC**
Chlorine (6.0 mg.L^{-1})			
Before treatment	5.19±0.13	4.19±0.045	3.74±0.0061
After treatment (120 min)	3.13±0.043	None	None
Peracetic acid (4.5 mg.L^{-1})			
Before treatment	5.19±0.13	4.22±0.17	3.74±0.0061
After treatment (25 min)	5.25±0.14	4.61±0.33	4.06±0.13

Table A4.4.5 Log values (cfu.mL^{-1}) before and after chlorine and PAA disinfection Trial 3

	ACC	**TC**	**EC**
Chlorine (6.0 mg.L^{-1})			
Before treatment	5.12±0.090	3.00±0.16	2.80±0.071
After treatment (120 min)	3.72±0.023	None	None
Peracetic acid (4.5 mg.L^{-1})			
Before treatment	5.12±0.090	3.34±0.14	2.80±0.071
After treatment (25 min)	3.73±0.11	None	None

Table A4.4.6 Residual chlorine levels after chlorine disinfection trials 1-3. Chlorine residuals were recorded after a contact period of 120 min.

	Trial 1	**Trail 2**	**Trail 3**
Residual chlorine ($mg.L^{-1}$)	2.30	0.30	2.10

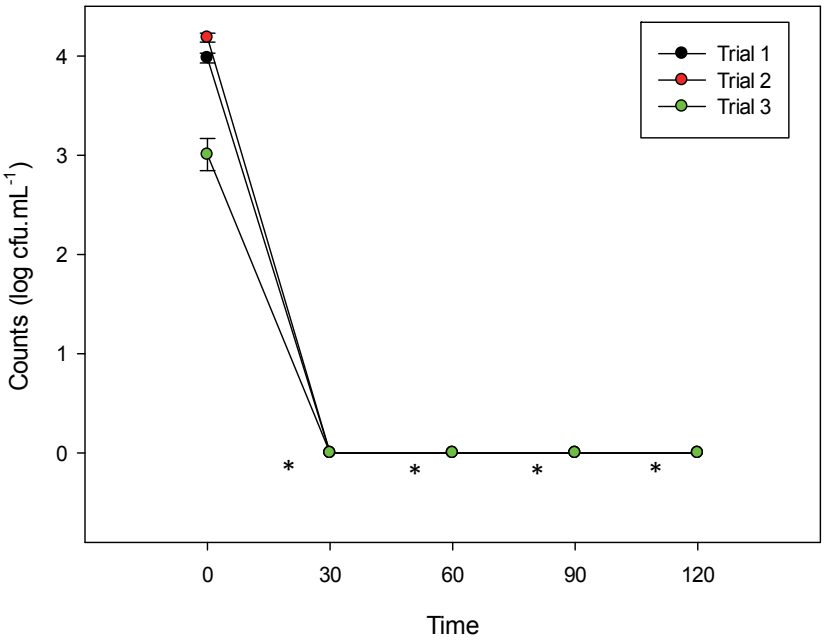


Figure A4.4.2 Inactivation curves for total coliform organisms determined at different time intervals (30, 60, 90 & 120 min) during chlorine (6.0 $mg.L^{-1}$) disinfection Trials 1, 2 and 3. Error bars are calculated from standard deviation at a 95% confidence level. * - No growth detected at lowest dilution (10^0)

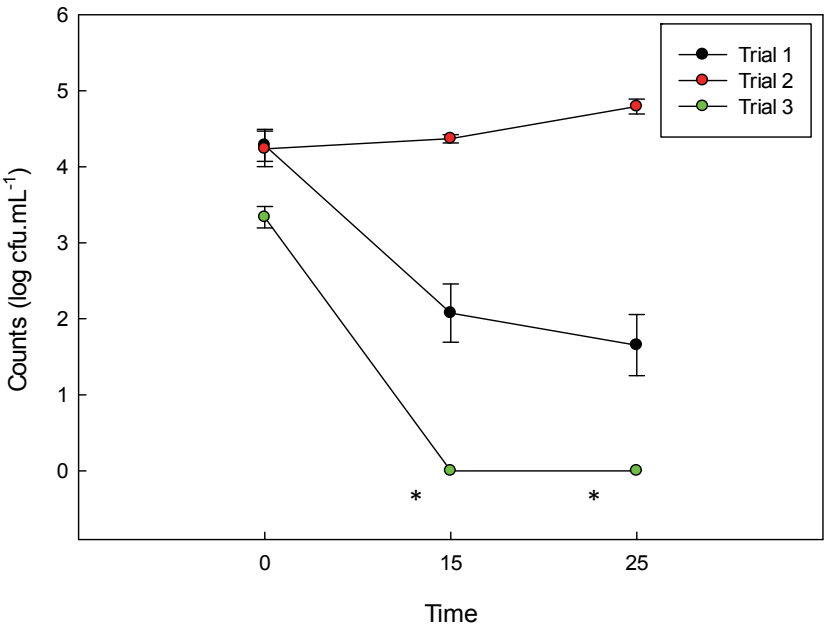


Figure A4.4.3 Inactivation curves for total coliform organisms at 15 and 25 min PAA (4.5 mg.L^{-1}) disinfection for Trial 1, 2 and 3. Error bars are calculated from standard deviation at a 95% confidence level. * - No growth detected at lowest dilution (10^0)

Effect of chlorine and PAA on microbial inactivation in river water (Trial 4 & 5)

Table A4.4.7 Log values (cfu.mL^{-1}) before and after chlorine and PAA disinfection Trial 4

	ACC	**TC**	**EC**
Chlorine (3.0 mg.L^{-1})			
Before treatment	3.88±0.066	2.12±0.11	1.45±0.059
After treatment (120 min)	0.73±0.30	None	None
Peracetic acid (3.0 mg.L^{-1})			
Before treatment	3.88±0.066	2.27±0.052	1.47±0.067
After treatment (25 min)	2.55±0.068	0.40±0.63	None

Table A4.4.8 Residual chlorine concentrations detected after 120 min chlorine disinfection for Trial 4 and 5

	Trial 4	**Trial 5**
Residual chlorine (mg.L^{-1})	1.47	0.79

Table A4.4.9 Log values (cfu.mL^{-1}) before and after chlorine and PAA disinfection Trial 5

	ACC	TC	EC
Chlorine (3.0 mg.L^{-1})			
Before treatment	3.96±0.16	2.17±0.037	1.53±0.12
After treatment (120 min)	2.71±0.034	None	None
Peracetic acid (3.0 mg.L^{-1})			
Before treatment	3.96±0.16	2.26±0.11	1.53±0.12
After treatment (25 min)	3.34±0.038	1.26±0.31	None

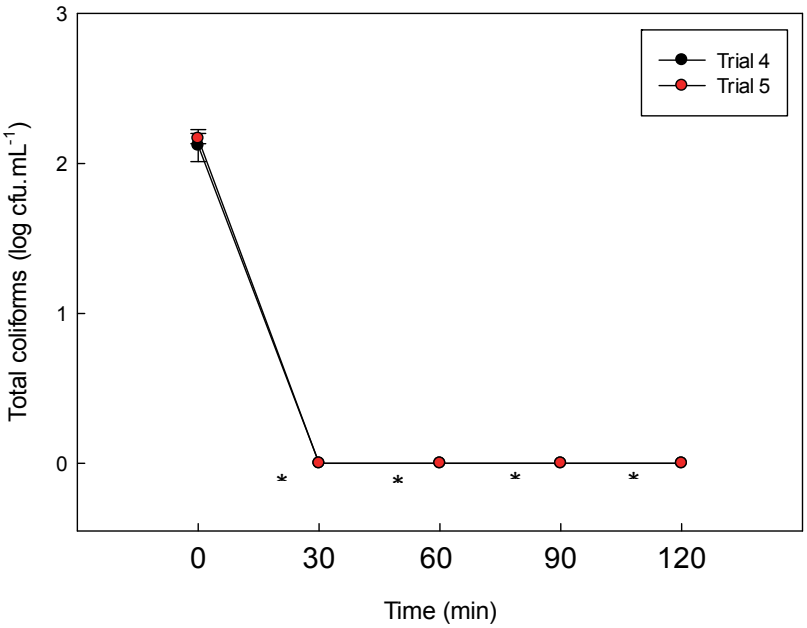


Figure A4.4.4 Inactivation curves for total coliform organisms determined at different time intervals (30, 60, 90 & 120 min) during chlorine (3.0 mg.L^{-1}) disinfection Trials 4 and 5. Error bars are calculated from standard deviation at a 95% confidence level. * - No growth detected at lowest dilution (10^0).

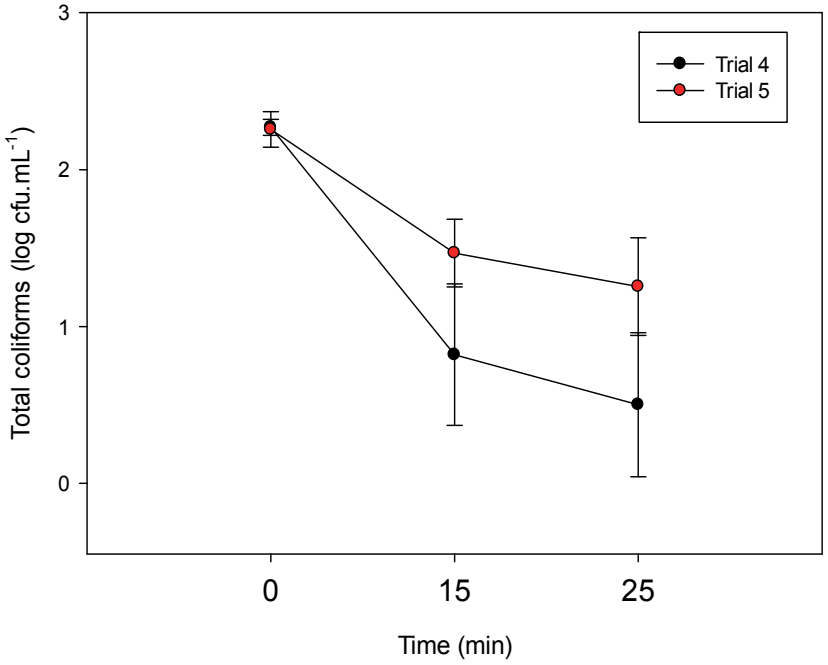


Figure A4.4.5 Inactivation curves for total coliform organisms at 15 and 25 min PAA (3.0 mg.L^{-1}) treatment for Trial 4 and 5. Error bars are calculated from standard deviation at a 95% confidence level. * - No growth detected at lowest dilution (10^0).

Conclusions

In this study it was seen that water quality played an essential role during chemical disinfection of river water. Results showed that the Plankenburg River is extensively polluted and displayed EC levels up to 1 690 000 cfu.100 mL^{-1} (Table A4.4.2). This river is regarded unsuitable for irrigation as results exceeded the faecal coliform guideline (Table 1) for irrigation water (WHO, 1989; DWAF, 1996). This raises the concern of disease outbreaks as the Plankenburg River is frequently used for irrigation by farmers.

The river water analyses indicated that the microbiological quality of the water varied vastly between different days (Table A4.4.2). During disinfection the EC population in river water was eliminated for both Chlorine and PAA in all trials except trial 2 (Tables A4.4.3-A4.4.5; A4.47 & A4.49) and conformed to the faecal coliform guideline for irrigation water in South Africa (DWAF, 1996) and also the target reduction target of 3-4 logs. The heterotrophic microorganisms were more resistant to chemical disinfection (Tables A4.4.3-A4.4.5; A4.4.7 & A4.4.9). Note that this group is a heterogeneous population that involves various strains and species that may show different levels of resistance to chlorine and PAA. It is suggested that the effect of chemical disinfection on other pathogens, should also be investigated to ensure the safety of water prior to crop irrigation.

Fluctuation in the physio-chemical parameters also occurred (Table A4.4.2). Of all the physio-chemical characteristics evaluated, the COD had the greatest influence on chemical disinfection. The COD load of the Plankenburg River ranged between 14-1 094 mg.L^{-1}. The disinfection efficiency of PAA was greatly influenced at high COD levels (1 094 mg.L^{-1}) in Trial 2, to such an extent that an increase in microbial growth occurred during the treatment period (Figure

A4.4.3). This was not the case with chlorine and this chemical was effective over the range of COD levels recorded in this study (Figures A4.4.2 & A4.4.4).

With regards to chlorine disinfection, the residual limit chosen for this study (≤ 1 $mg.L^{-1}$) was only met twice in this study (Tables A4.4.6 & A4.4.8). This result was related to high COD levels detected on these days (Table A4.4.2). Residual levels that exceeded this limit can pose a risk if discarded into the environment due to the risk of possible by-product formation.

Chlorine and PAA results were comparable in some cases (EC levels after disinfection), although chlorine was the better disinfectant in this study (Figures A4.4.2-A4.4.5). A chlorine concentration of ≤ 3.0 $mg.L^{-1}$ for a contact time of at least 30 min is suggested for river water disinfection, depending on the water quality on the particular day. Together with its low cost and high availability, chlorine will be a feasible option for irrigation water disinfection at commercial-scale (in terms of microbiological quality), although the environmental effects, formation of DBP's and increasing chlorine resistance of micro-organisms are possible concerns. On the other hand, PAA doses exceeding 4.5 $mg.L^{-1}$ are recommended for river water disinfection. The efficiency of this chemical is influenced by high COD levels in water. Increased PAA dosages negate these inferences during microbial disinfection. Higher PAA dosages imply higher costs, however, its use poses a lower risk to the environment than chlorine. In conclusion, water quality is ever changing, therefore required chemical dosages would also be subject to change. River water resources displaying a different character composition than the Plankenburg River could also react differently to chemicals and this should be considered in future studies.

A5. EFFECT OF COMBINATION TREATMENTS ON RIVER WATER (LABORATORY SCALE)

Research study design

This study investigated the efficacy that PAA (Peracetic acid), Cl (NaOCl) and H_2O_2 in combination with UV had in reducing the microbial load of river water from the Plankenburg river, without any sand filtration prior to treatment. On each of three separate days (study 1-3), three combination trials were conducted and compared to a UV treatment. For each of the three trails conducted on a day, one of three chemicals was added to a river water sample and then after 25 min intervals, exposed to LP UV irradiation at a dose of 13 $mJ.cm^{-2}$. The microbial load was enumerated before and after UV treatment.

Peracetic acid (PAA) was applied at a dose of 4 $mg.L^{-2}$, Cl at 6 $mg.L^{-1}$ and H_2O_2 at a dose of 2.5 $mg.L^{-1}$. After each treatment the effect on the total ACC population (ACC), total coliforms (TC) and faecal coliforms (FC) were evaluated following standard plate count procedures (Appendix A2). The physico-chemical properties of the river water samples were determined using standard methods (Appendix A2).

Results

Table A5.1. Physicochemical and microbiological properties of river water measured before each of the combination treatment studies (no. 1-3). (Treatments that the water were subjected to in each trail is summarised in the Table subscript.)

Parameter	Study 1 (Trails 1-3)	Study 2 (Trails 4-6)	Study 3 (Trails 7-9)
Physico-Chemical:			
UVT %	68	63.3	11.3
Turbidity	6.7	4.3	151
Conductivity	42	33	52
pH	6.6	6.7	6.4
Alkalinity	75	50	40
COD	23	20	56
TSS	7	7	21
VSS	3	5	16
Microbial (cfu.100mL^{-1}):			
ACC	8.7×10^5	7.8×10^5	2.0×10^6
TC	1.0×10^5	7.7×10^4	3.2×10^5
FC	4.7×10^4	2.8×10^4	1.5×10^5

Note: Trials 1,2 &3 ; trials 4, 5 &6 and trials 7, 8 &9 were conducted on the same days
Trial no: 1, 4 and 7 – PAA (4ppm)/UV 13$mJ.cm^{-2}$;
Trial no: 2, 5 and 8 – Cl (6ppm)/UV 13$mJ.cm^{-2}$
Trial no: 3, 6 and 9 – H_2O_2 (2.5ppm)/UV 13$mJ.cm^{-2}$

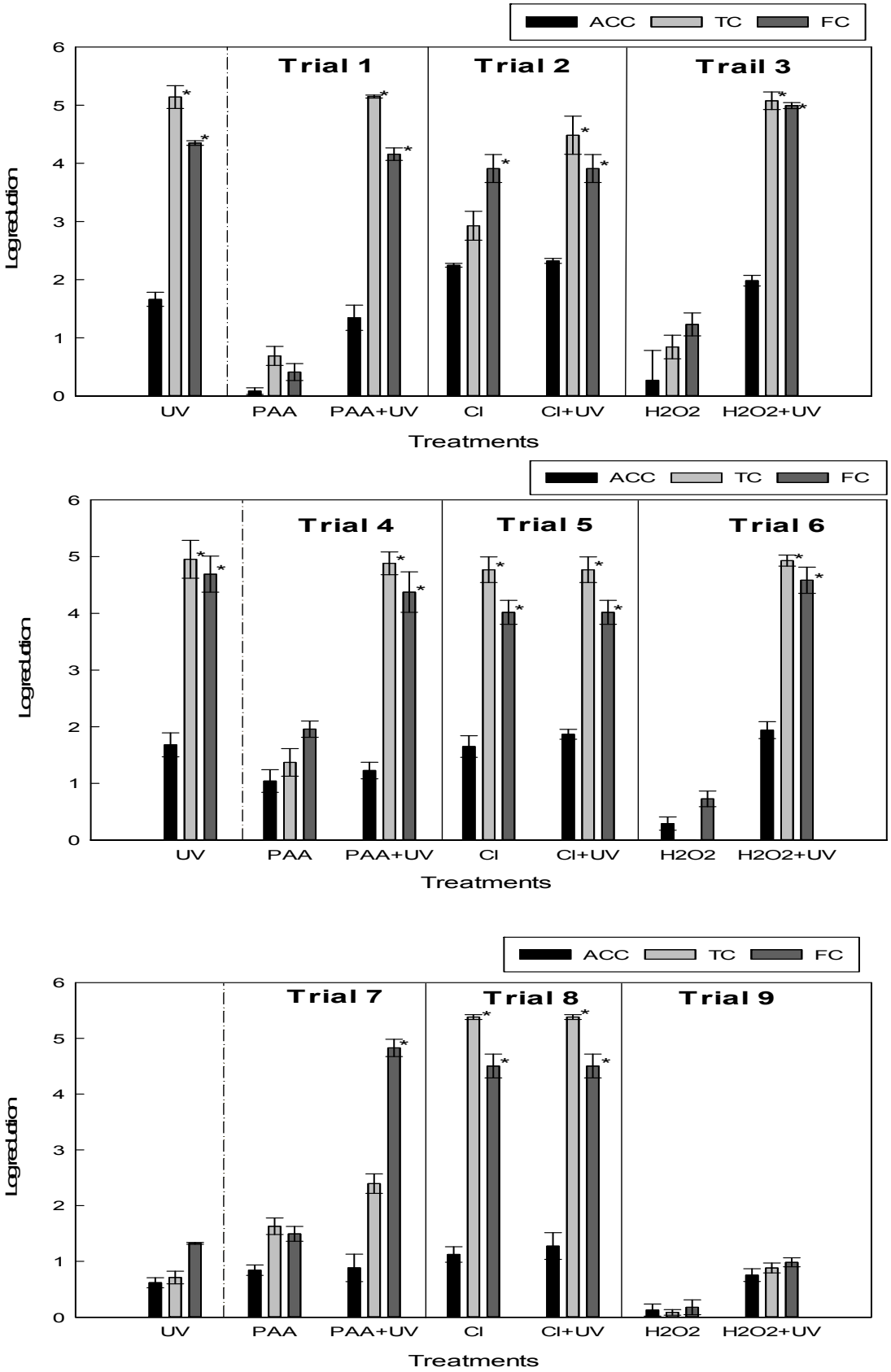


Figure A5.1 Log reductions obtained with combination treatments tested during Study 1 (Trials 1-3); Study 2 (Trials 4-6); and Study 3 (Trials 7-9). Error bars are calculated from standard deviation at a 95% confidence level. * - No growth detected at lowest dilution (10^0).

Conclusions

Microbial analysis of the river water (Table A5.1) indicated that the water used in all three the studies did not meet the FC guideline of 1000 CFU.100mL^{-1} (DWAF 1996) for fresh produce irrigation water before treatment. The log reductions obtained for each trial (Fig. A5.1) also indicated clearly that variation in the physico-chemical properties of river water can have a significant influence on treatment efficiency. The water used in Studies 1 and 2 (Trials 1-6) were, in terms of physico-chemical characteristics, of fairly good quality, with high UVT% and low COD, turbidity and TSS values (Table A5.1). These properties all contributed to the UV treatment (both single and in combination with PAA, Cl and H_2O_2) proving to be very effective in inactivating the FC and (in most cases) TC at the fairly low dose of 13 mJ.cm^{-2}. In Study 3 it was clear that factors such as the low UVT% and extremely high turbidity influenced the efficacy of the UV treatment. Less than a two log reduction was obtained for the FC content which would still result in water above the suggested limit. Under these conditions, however, FC and TC reductions during the chlorine treatments (single and combination) (Trial 8) were not negatively influenced and still resulted in total inactivation (as observed for Trials 2 & 5). The combination treatment involving PAA also successfully inactivated FC in all trials), although not as effective as a single treatment (Trials 1,4,7). H_2O_2 treatment proved to be very ineffective, with no significant differences observed between the combination treatment (H_2O_2/UV) and UV alone in all three studies. The potential effect that PAA can have, under poor water quality conditions, on improving UV irradiation (probably as a result of an advanced oxidation process), is evident in Trial 7. The effect of all these combination treatments, do, however, need to be tested at pilot-scale with MP UV irradiation to determine their larger-scale applicability.

A6. CAPACITY BUILDING

Students in training as part of project K5/2174:

Organisation	Student	Gender	Race	Degree
Cape Peninsula University of Technology	Nettania Blom	F	W	B Tech Food Technology
Stellenbosch University	Madelize Kotze	F	W	MSc Food Sc
Stellenbosch University	Kirsty Giddey	F	W	MSc Food Sc
Cape Peninsula University of Technology	Leonie Bredenhann	F	W	B Tech Food Technology
Stellenbosch University	Francois Olivier	M	W	MSc Food Sc
Stellenbosch University	Carmen Bester	F	W	MSc Food Sc
Stellenbosch University	Brandon van Rooyen	M	W	MSc Food Sc

SUMMARY OF STUDENTS RESEARCH

Name: **Nettania Blom**
Degree: **B Tech Food Technology (CPUT)**
Graduated: **November 2014**
Thesis title: **To determine the efficacy of commercial sanitising agents on various *E. coli* strains**

Disinfection of fresh produce is routinely carried out to prevent the spread of human pathogens. To this aim, the relative disinfection efficiency of chlorine (CL1 & CL2), peracetic acid (PA1 & PA2) and citric acid (CA) against three *Escherichia coli* strains (M53, ATCC 25922 and MJ58) were studied in laboratory-scale experiments.

For each strain an inoculum equal to 0.5 MacFarland standard was prepared. All treatments were done in triplicate. Serial dilutions before and after treatment was pour-plated with VRBA in duplicate and incubated for 24 hours at 35°C. All the data was analysed using Analysis of Variance (ANOVA).

A peracetic acid (PA1) dosage of 0.03% with a contact time of 5 minutes, peracetic acid (PA2) dosage of 0.03% for 2 minutes and citric acid (CA1) dosage of 0.39% for 5 minutes resulted each in an average 7 log reduction. Chlorine & dosage at 0.03% (CL1) and 0.06% (CL2) with a contact time of 5 minutes achieved an average 1.5-2 log reduction.

The results indicated that PA1, PA2 and CA1 are more effective sanitising agents than chlorine against *E. coli*. It was found that CL1 and CL2 are less effective against environmental strains. Peracetic acid is therefore recommended the best treatment option to inactivate the two environmental and one reference strains. In addition, a key advantage of PA compared to chlorine is the lack of harmful disinfection by-products. However, attention must be paid to the cost of PA.

Name: **Madelize Kotze**
Degree: **MSc Food Science (SU)**
Graduated: **March 2015**
Thesis title: **Investigating the efficacy of medium pressure UV and hydrogen peroxide as on-farm treatment methods to reduce the microbial load of irrigation water**

Many South African farmers are forced to use water from nearby rivers for crop irrigation, since it is the most affordable and sometimes only source of water available to them. During this research project, a baseline study was performed on a farm irrigating fresh produce with water obtained from the Eerste River. The baseline study was done over a five month period, at six preselected sampling points, to determine the microbial and physico-chemical parameters of the water so a baseline could be established to compare the results to when the ultraviolet (UV) apparatus was installed (February 2013). Aerobic colony count (ACC), total coliforms (TC) and *Escherichia coli* (*E. coli*) were tested for during the microbiological study, while the physico-chemical analysis comprised of temperature, pH, conductivity, chemical oxygen demand (COD), alkalinity and total soluble solids (TSS). The UV treatment study was also performed over a five month timeline, at eight different sampling points (original six sampling points, with additional sampling points before and after UV). The same microbiological tests were performed during the UV treatment study, but turbidity and percentage ultraviolet transmittance (% UVT) were performed additionally during physico-chemical analysis.

During the baseline study ACC, TC and *E. coli* counts as high as 9 600 cfu.mL^{-1}, 13 799 MPN.100 mL^{-1} and 2 098 MPN.100 mL^{-1} were isolated at the river (Sampling Point 1), respectively. While performing the UV treatment study ACC, TC and *E. coli* counts as high as 142 000 cfu.mL^{-1}, 241 960 MPN.100 mL^{-1} and 6 867 MPN.100 mL^{-1} were isolated at the river, respectively. As a result it was concluded that the Eerste River was mostly unsuitable for irrigation of fresh produce that are consumed raw. The higher counts in the river, during the UV treatment study might be attributed to the increase in rainfall that occurred in the sampling months (March to July 2013).

The counts as measured at the point of irrigation are considered of greater importance, since the counts present in the river might still decrease to below the guideline levels after passing through sand filters and the addition of hydrogen peroxide (current mode of treatment) or after passing through the UV in the UV treatment study. The ACC, TC and *E. coli* counts during the baseline study were as high as 8 800 cfu.mL^{-1}, 24 196 MPN.100 mL^{-1} and 85 MPN.100 mL^{-1} at the point of irrigation (Sampling Point 6), respectively. After hydrogen peroxide addition average log-reductions ranging between 0.65 and 1.13 were seen, but reduction was never constant. The counts at the point of irrigation remained more or less constant as at the river due to contamination that occurred at the sand filters, making the water unsuitable for irrigation of fresh produce in terms of ACC and TC counts. In the UV treatment study ACC, TC and *E. coli* counts were as high as 35 000 cfu.mL^{-1}, 10 462 MPN.100 mL^{-1} and 63 MPN.100 mL^{-1} at the point of irrigation (Sampling Point 8), respectively. Average log-reductions in the range of 0.90 to 1.25 were achieved, but it was inconsistent. After treatment with chlorine and re-sanding of the sand filters, no further contamination occurred and counts decreased to below guideline limits', making the water safe for irrigational use in terms of all of the microbiological parameters. Not only is UV treatment more effective in reducing microbiological counts than H_2O_2, it is also relatively less expensive in the long term. Hydrogen peroxide treatment of water amounts to a very high capital expense every month, whereas UV may seem expensive when starting up, but the monthly operating cost thereafter is marginally less than for H_2O_2.

It is of great importance to farmers to find a treatment that would bring the counts in the river water too below the guideline limits required for safe irrigation since pathogens can be carried over from

water onto fresh produce, resulting in an increase in produce-associated foodborne outbreaks and loss of consumer trust.

Name: **Kirsty Giddey**
Degree: **MSc Food Science (SU)**
Graduated: **March 2015 (*cum laude*)**
Thesis title: **Investigating the efficacy of hydrogen peroxide against isolated environmental *E. coli* strains**

Surface water used for irrigation is often highly contaminated on a microbial level. Using contaminated surface water for the irrigation of fresh produce can lead to foodborne disease outbreaks and *Escherichia coli* has been a major cause of foodborne outbreaks associated with fresh produce over the past few years. There are many possible on-farm treatment options available to decrease the high microbial loads present in surface water, one of these is H_2O_2 and various factors can influence its use. The aim of this study was to determine the efficacy of H_2O_2 on different *E. coli* strains.

Water from the Plankenburg River was sampled and treated with (250, 300 and 350 $mg.L^{-1}$) H_2O_2 and the impact at 0, 30, 60, 90 and 120 min was then evaluated. It was found that the log reductions differed between samples. Log reductions ranged between 1.60-2.63 for Aerobic colony counts (ACC), total coliforms and *Escherichia coli.* The water was not considered safe for irrigation use although it had been treated with H_2O_2.

Reference (ATCC) and environmental *E. coli* strains were individually treated with H_2O_2 (250, 300 and 350 $mg.L^{-1}$) at 0, 30, 60, 90 and 120 min. Log reductions for the ATCC strains ranged between 2.13-5.48. This indicated strain-strain variation between the different reference strains tested. Log reductions for the environmental *E. coli* strains ranged from 2.17-3.93. *Escherichia coli* M53 and MJ56 were the most resistant and most sensitive environmental strains to the H_2O_2 treatment, respectively. Once again it was observed that strain-strain variations existed between the log reductions achieved. Overall, it was observed that the ATCC *E. coli* strains were more sensitive to the H_2O_2 treatments when compared the environmental strains. This indicates that ATCC strains should not be used for H_2O_2 treatment optimisation.

Certain factors can influence the efficacy of H_2O_2 such as concentration and organic matter (chemical oxygen demand) present in the water. Different H_2O_2 concentrations were evaluated (50, 350, 700 and 1 000 $mg.L^{-1}$) on two *E. coli* strains (M53 and W1371). Results indicated that 50 $mg.L^{-1}$ was not effective as less than 1 log reduction was achieved after 120 min. When 350 and 700 $mg.L^{-1}$ were used similar log reductions were achieved (1.78-2.27), which was not expected. Using 1 000 $mg.L^{-1}$ was considered an effective concentration that resulted in no growth present after 120 min. *Escherichia coli* strain W1371 carried EPEC virulence factors (potential pathogen). This was included in the study in order to determine how a strain carrying virulence factors would react to H_2O_2. *Escherichia coli* W1371 was considered resistant to the H_2O_2 treatment and log reductions were similar to that achieved for M53.

The catalase activity of the *E. coli* strains was studied to determine if a link existed between catalase activity and H_2O_2 resistance. Although a trend was observed between heat-stable catalase activity and H_2O_2 resistance, there were exceptions. It was concluded that high catalase activity does not always coincide with H_2O_2 resistance and that other mechanisms might also contribute to *E. coli* survival.

Overall, it was observed that there are certain factors that influence the efficacy of H_2O_2 as a treatment option. It can be concluded that environmental *E. coli* strains are generally more

resistant to the H_2O_2 treatment compared to ATCC *E. coli* strains, this needs to be considered when using H_2O_2 or other chemical disinfectants as a treatment option.

Name: **Leonie Bredenhann**
Degree: **B Tech Food Technology (CPUT)**
Graduated: **December 2015**
Thesis title: **To determine the efficacy of chlorine and UV-C treatment on various *E. coli* strains in wash water used in the fruit and vegetable industry (Graduated December 2015)**

Water used in the Western Cape is contaminated with various *Escherichia coli* strains and as a result, fresh fruits and vegetables are contaminated. The aim of this study was to determine the log_{10} reduction of various *E. coli* strains (M53 & ATCC25922) in response to chlorine and UV-C treatment, to identify the optimum method or combination of methods. The effect of photo-reactivation was evaluated, as well as the effect of pH on the efficacy of chlorine.

The inoculum was prepared by inoculating one pure colony into 5 mL NB and incubated for 24 hours at 35°C. Thereafter 4 mL was transferred into 220 mL SS and compared to 0.5 McFarland standard. The untreated SS was used to serve as the control. A 50 mL sample was subjected to only chlorine (25 ppm for 5 minutes), a combination of chlorine (25 ppm for 4 minutes) and UV-C (4mJ.cm^2 for 1 minute), UV-C (4mJ.cm^2 for 1 minute) and chlorine (25 ppm for 5 minutes) and UV-C (4mJ.cm^2 for 1 minute) treatment alone.

The combination of chlorine and UV-C, UV-C and chlorine treatment and UV-C treatment alone were subjected to photo-reactivation of 3 hours at the light intensity of 3.5 kLux, with and without the addition of sodium thiosulfate to quench the chlorine reaction.

Chlorine treatment was subjected to pH of 6.18 (pH 5.80 before autoclaving), 6.61 (pH 6.50 before autoclaving) and 6.98 (pH 7.20 before autoclaving).

After the various treatments, serial dilutions were prepared and pour plated with VRB agar and incubated inverted for 24 hours at 35°C. All data was analysed using ANOVA.

Even though chlorine is one of the most used disinfectant in the food industry, the results indicate that chlorine treatment is the least effective for the disinfection of water. It only resulted in a 1.29 log reduction. The most effective method is the combination of UV-C and chlorine treatment, resulting in a 2.98 log reduction. UV-C treatment alone resulted in a similar log reduction (2.81), but taking the ability of microorganisms to repair over time and thus the log increase of 1.77 after photo-reactivation time of 3 hours into account, it is advised to not use UV-C treatment on its own for the disinfection of water. For optimum chlorine treatment it is advised to ensure the pH of the solution is ideal. The pH of 6.61 resulted in a 2.57 log reduction, whereas the pH of 6.18 only obtained a 1.55 log reduction.

Name: **Carmen Bester**
Degree: **MSc Food Science (SU)**
Graduated: **March 2016 (*cum laude*)**
Thesis title: **Investigating the efficacy of chlorine against isolated environmental *E. coli* strains**

Growing concerns continue as fruits and vegetable contamination by disease-causing bacteria (*Escherichia coli* (*E. coli)*), viruses and protozoa lead to increasing disease outbreaks around the world. Groundwater and more popularly, surface water, are common sources of irrigation of fresh produce. Irrigation with poor water quality can transfer human- and plant pathogens to the surface

of fresh produce items simultaneously introducing the risk of food-borne diseases to humans. Surface water sources such as rivers are of variable water quality and selected South African rivers have shown to be unsuitable for irrigation as these are highly contaminated with faecal coliforms. *Escherichia coli* is a well-known water quality indicator and its presence is a sign of faecal contamination in surface waters. Various water disinfection strategies have been applied to eliminate or inactivate the presence of *E. coli* in water systems. Chemical disinfection by means of two chlorine sources (sodium hypochlorite (NaOCL) and calcium hypochlorite ($Ca(OCl)_2$) on selected *E. coli* strains was investigated at laboratory scale: a) The efficacy of NaOCl on six *E. coli* strains and b) the effect of $Ca(OCl)_2$ on three *E. coli* strains were studied. Differences were seen between NaOCl and $Ca(OCl)_2$ disinfection. During the NaOCl trails, the main disinfection period occurred within the first 60 min of disinfection and $Ca(OCl)_2$ disinfection showed highest reductions within the first 30 min of disinfection. American Type Culture Collection (ATCC) strains 25922 and 35218 were the most sensitive to chlorine disinfection and MJ58 (environmental strain) showed highest resistance to both NaOCl and $Ca(OCl)_2$ disinfection. Calcium hypochlorite had a much higher disinfection capability than NaOCl. This was noticed when a log reduction of 0.17 was obtained for MJ58 after NaOCl disinfection compared to a 2.65 log reduction when $Ca(OCl)_2$ was applied at similar concentration (9 $mg.L^{-1}$ available chlorine). However, for ATCC 25922, small differences were observed between NaOCl and $Ca(OCl)_2$ disinfection when 12 $mg.L^{-1}$ available chlorine was used. Reference strains were more sensitive to chlorine disinfection than environmental strains. Altogether, better disinfection performance was observed when $Ca(OCl)_2$ was used in comparison to NaOCl results. Flocculation of river water and the influence it has on the physio-chemical- and microbiological properties of river water was investigated: a) raw river water, b) flocculated river water and c) filtered river water. Flocculation did not cause significant changes in the physio-chemical- and microbiological characteristics of river water samples, however, filtration after flocculation had a better, but still a small effect on river water samples. The only noticeable difference on physio-chemical properties of water was observed for turbidity that has decreased with 5.42 NTU after filtration. The microbiological properties of river water was only slightly influenced by flocculation following filtration (0.25 log reduction for total coliforms and 0.40 log reduction for aerobic colonies).

Name: **Francois Olivier**
Degree: **MSc Food Science (SU)**
Graduated: **March 2016 (*cum laude*)**
Thesis title: **Investigating the efficacy of UV and UV/H_2O_2 against isolated environmental *E. coli* strains**

The disinfection efficacy of UV irradiation and combined UV/H_2O_2 AOPs was tested against six *E. coli* strains. Two reference strains (ATCC 25922 and ATCC 35218), two strains isolated from parsley and two strains isolated from river water were exposed to an UV dose of 4 $mJ.cm^{-2}$ as stand-alone treatment in PSS. Also, the *E. coli* strains were subjected to a H_2O_2 concentration of 20 $mg.L^{-1}$ for a period of 10 min prior to exposure to a UV dose of 4 $mJ.cm^{-2}$ as AOP. Three strains were then selected and exposed to UV doses of 8, 10 and 13 $mJ.cm^{-2}$ and H_2O_2 concentrations of 2.5, 100 and 200 $mg.L^{-1}$ under the same conditions. Initial experiments indicate that the reference strains were particularly sensitive to both UV and UV/H_2O_2 treatments with reductions of 3-log being easily achieved. Environmentally adapted *E. coli* was found to be more resistant with the strain isolated from the Plankenburg River representing reductions of 1.58- and 1.34-log following the initial UV and UV/H_2O_2 treatments, respectively. It was also observed that AOPs may present increased disinfection efficacy in comparison to UV irradiation alone.

Furthermore, Increased UV doses resulted in improved disinfection with a 3-log target reduction being achieved at a dose of 10 $mJ.cm^{-2}$ for the most resistant strain. A similar trend was observed for the UV/H_2O_2 treatment, however, a maximum reduction of 2.40-log was achieved for the most resistant strain at a H_2O_2 concentration of 200 $mg.L^{-1}$. Adding to this, the effect of coagulation, settling and filtration on the microbiological, physical and chemical qualities of river water was investigated to determine its influence on the efficiency of the suggested treatments. Prior to filtration, the addition of coagulant at a concentration of 7 $mg.L^{-1}$ had no substantial influence on any of the mentioned parameters. Following filtration however, the turbidity of coagulated water decreased with 5.42 units to 7.22 NTU while an increase in UVT% was observed. The COD of the sample decreased with 15.30 $mg.L^{-1}$. Results further show that coagulation and subsequent filtration had no significant effect on the levels of total coliforms and aerobic colonies in river water. Thus, while the effect of coagulation on the microbial parameters of river water may be insignificant it is worthwhile to investigate the effect thereof on the physicochemical properties of water as parameters such as UVT%, turbidity and COD are strongly related to the disinfection potential of the treatments.

Name: **Brandon van Rooyen**
Degree: **MSc Food Science (SU)**
Graduated: **handing in in December 2016**
Thesis title: **Investigating the efficacy of chemical based disinfectants and UV against environmental *E. coli* strains in river water at pilot-scale**

In this study the efficacy of combined treatments (Chemical/UV) were investigated first at laboratory level on river water as well as on resistant *E. coli* strains (in saline). (The resistant *E. coli* strains were chosen based on the results reported as part of the other sections of this WRC project.) Optimised treatments were then applied at a pilot-scale installation to determine their efficacy in the reduction of the microbial load (ACC) as well as total and faecal coliforms) in larger volumes of river water (up to 2500 L). Log reductions obtained with combination treatments were compared to log reductions obtained with UV treatment alone and chemical treatment alone.

A7. KNOWLEDGE DISSEMINATION

Summary of all scientific outputs related to Water Research Commission project K5/2174 – Scoping study on different on-farm treatment options to reduce the high microbial contaminant loads of irrigation water to reduce the related food safety risk:

Outputs to date:

International conferences **(poster)**

1) GIDDEY, K., LAMPRECHT, C., BRITZ, T.J. & SIGGE, G.O. 2014. Investigating the efficiency of hydrogen peroxide against isolated environmental *E. coli* strains. 17th IUFoST World Congress of Food Science and Technology, Montreal, Canada, 17-21 August 2014.
2) LAMPRECHT, C., OLIVIER, F., BESTER, C. & SIGGE, G.O. 2016. Variation in *Escherichia coli* strain resistance against UV irradiation and chemical biocides. FoodMicro 2016: 25th International ICFMH Conference, Dublin, Ireland, 19-22 July, 2016.

Local conferences **(oral presentation)**

1) GIDDEY, K. 2014. Investigating the efficiency of hydrogen peroxide against isolated environmental *E. coli* strains. Stellenbosch University Water Institute & WISA Water Reuse Division One-day Symposium and SUWI Water Forum, Spier Conference Centre, Stellenbosch, 15 October 2014.
2) BESTER, C., LAMPRECHT, C. & SIGGE, G.O. 2015. Comparison of the efficacy of three chemical disinfectants against isolated environmental and reference *E. coli* strains. 21st SAAFoST Biennial International Congress and Exhibition, Durban, 6-9 September 2015.
3) OLIVIER, F, LAMPRECHT, C. & SIGGE, G.O. 2015. Optimisation of ultraviolet (UV) light irradiation as disinfection technology for the treatment of river water prior to irrigation. 21st SAAFoST Biennial International Congress and Exhibition, Durban, 6-9 September 2015.
4) OLIVIER, F, LAMPRECHT, C. & SIGGE, G.O. 2015. Disinfection of microbiologically polluted river water using medium-pressure ultraviolet irradiation. Stellenbosch University Water Institute & WISA Water Reuse Division One-day Symposium and SUWI Water Forum, Spier Conference Centre, Stellenbosch, 8 October 2015.
5) BESTER, C., LAMPRECHT, C. & SIGGE, G.O. 2015. Comparing the disinfection efficacy of chlorine and peracetic acid against reference and environmental *E. coli* strains at laboratory-scale. Stellenbosch University Water Institute & WISA Water Reuse Division One-day Symposium and SUWI Water Forum, Spier Conference Centre, Stellenbosch, 8 October 2015.

Popular articles

1) SIGGE, G.O., LAMPRECHT, C. & OLIVIER, F. 2015. The potential of UV for the treatment of polluted irrigation water. South African Food Science and Technology (FST), accepted for publication in Volume 4, Number 2 (May 2015 issue).

Scientific articles

1) GIDDEY, K.F., KIDD, M., BRITZ, T.J., SIGGE, G.O. & LAMPRECHT, C. 2015. Impact of hydrogen peroxide treatment on environmental *Escherichia coli* strains. *Journal of Applied & Environmental Microbiology*, **3**(2), 49-57.
2) BESTER, C, LAMPRECHT, C. & SIGGE, G.O. 2015/2016. The comparison between chlorine and peracetic acid disinfection against environmental *Escherichia coli* strains at laboratory-scale. Submitted to Journal of Applied and Environmental Microbiology – pending review.
3) OLIVIER, F., LAMPRECHT, C. & SIGGE, G.O. 2015/2016. Efficiency of Medium-pressure (MP) Ultraviolet (UV) irradiation for the Disinfection of River Water Considering the Influence of Water Quality and Damage-repair Potential. Submission to Journal of Applied and Environmental Microbiology – pending review

MSc degrees awarded:

1) KOTZE, M. 2015. Investigating the efficacy of medium pressure UV and Hydrogen Peroxide as on-farm treatment methods to reduce the microbial load of irrigation water. MSc Food Science, Stellenbosch University, March 2015 Graduation.
2) GIDDEY, K. 2015. Investigating the efficacy of hydrogen peroxide against isolated environmental *E. coli* strains. MSc Food Science, Stellenbosch University, March 2015 Graduation.
3) BESTER, C. Investigating the efficacy of sodium hypochlorite, calcium hypochlorite and peracetic acid on environmental *Escherichia coli* strains. MSc in Food Science, Stellenbosch University, March 2016 Graduation.
4) OLIVIER, F. Evaluating the potential of ultraviolet (UV) irradiation for the disinfection of microbiologically polluted irrigation water. MSc in Food Science, Stellenbosch University, March 2016 Graduation.

BTech degrees awarded:

1) BLOM, N. 2014. To determine the efficacy of commercial sanitising agents on various *E. coli* strains. BTech Food Technology, Cape Peninsula University of Technology, December 2014 Graduation.
2) BREDENHANN, L. 2015. To determine the efficacy of chlorine and UV-C treatment on various *E. coli* strains in wash water used in the fruit and vegetable industry. BTech Food Technology, Cape Peninsula University of Technology, December 2015 Graduation.