Hort Innovation

Final Report

Improving safety of vegetable produce through on-farm sanitation, using Electrolysed Oxidising (EO) water

Project leader:

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Delivery partner:

University of South Australia

Project code:

VG15068

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Improving safety of vegetable produce through on-farm sanitation, using Electrolysed Oxidising (EO) water
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Content

Summary

The overall objective of this project was to test whether Electrolysed Oxidising (EO) water could be used to increase the quality of vegetable irrigation water and facilitate safe use of microbiologically impaired waters.

Outbreaks caused by fresh produce contaminated by water and soil-borne pathogens are a serious human health issue and a threat to the Australian vegetable industry. There are numerous possible causes of microbial contamination, including the use of contaminated irrigation water. Various disinfection methods are available to treat irrigation water, including the use of EO technology. A previous Hort Innovation project identified EO water as a very effective technology to treat fresh produce post-harvest but detailed information about its effectiveness to improve irrigation water quality and limit pre-harvest contamination is currently not available.

The main achievements of this project were:

- Comparison of the efficacy of EO water with that of other chlorine-based disinfection options for treating irrigation water contaminated by relevant water-borne pathogens. EO water was as effective as sodium hypochlorite and more effective than chlorine dioxide under comparable conditions.

- Identification of the boundary water conditions within which EO water treatment is effective. EO water treatment was effective within the normal range of irrigation water pH. The presence of organic matter (such as manure) reduced the efficacy of EO water treatment but increasing the concentrations of EO water was effective in overcoming the reduced efficacy associated with organic matter contamination.

- Pre-harvest EO water treatment of irrigation water to reduce microbial contamination was tested on lettuce and spinach leaves under greenhouse and field conditions. Single applications of high concentration EO water or long-term applications of lower concentration EO water were not detrimental to lettuce or spinach plants and resulted in substantially reduced microbial load on treated plant leaves.

- The effects of EO water irrigation on soil properties and soil microorganisms were assessed over time with vegetable growing soils from across Australia. Long-term application of EO water resulted in no or minimal change in soil properties (pH, electrical conductivity) for all soils tested; in comparison, irrigation with sodium hypochlorite had significant effects on pH for the majority of soils tested.

- Preliminary characterization of an electrochemical irrigation water treatment (Booster reactor treatment) and subsequent greenhouse and field trials showed substantial reduction in microbial load of water and lettuce leaf samples; however, limited reductions were observed for spinach leaves grown under glasshouse conditions.

This project has allowed us to identify conditions under which EO technology can significantly reduce microbial contamination of irrigation water and has demonstrated the potential for EO water or Booster-treated irrigation water to enhance the safety of fresh produce by reducing the microbial load.

Keywords

Electrolysed Oxidising water; lettuce; spinach; free available chlorine; foodborne pathogens.

Introduction

Contamination of fresh vegetables by human pathogens constitutes a serious threat to human health and to the sustainability of the Australian vegetable industry. Treatment of irrigation water with Electrolysed Oxidising (EO) water has the potential to reduce product contamination in the field (pre-harvest) and during post-harvest treatment, and may bring additional benefits such as broadening the options for irrigation source waters and reducing plant pathogen impacts.

There is mounting evidence that water-borne pathogens represent an important pathway of contamination during both pre- and post-harvest vegetable production. Cause-effect relationships between irrigation water and food-borne outbreaks have generally been poorly documented due to the time lag and multiple steps between production and outbreaks but this is beginning to change as new technologies facilitate more robust tracking. An increasing number of specific cases linked to contaminated irrigation water have been reported, including a *Salmonella* outbreak from tomatoes in the USA and *E. coli* outbreaks from lettuce in Sweden. The microbiological quality of irrigation water is thus a major concern for the vegetable industry, especially for produce that is consumed fresh and uncooked. This is a key concern for vegetable growers in Australia, where available water supplies vary widely in quality and large-scale recalls of fresh produce have occurred in recent years (e.g. www.foodstandards.gov.au/industry/foodrecalls/recalls/Pages/Pre-packaged-salad-leaves.aspx).

The risks associated with the use of microbiologically contaminated irrigation water are related to:

i) The persistence of pathogenic organisms on crops for several weeks under field conditions;

ii) The possibility that pathogens can enter the plant tissues and therefore be protected from post-harvest surface sanitisation of the vegetables;

iii) The fact that irrigated soil and biofilms in water distribution pipes may act as a reservoir for opportunistic human pathogenic bacteria.

The microbiological characteristics of irrigation water are regulated for fresh and recycled water in Australia. Although a long list of pathogens of interest is listed in the water quality guidelines, trigger values are only reported for thermo-tolerant coliforms (<10 colony forming units (cfu)/100 mL for raw human food crops). These limits are more stringent for recycled water (<1 cfu/100 mL for *E. coli* and *Clostridium perfringens*) and can restrict the water sources considered acceptable for irrigation. Stringent limit can help mitigate risks, but source water quality varies temporally and more needs to be done to provide safe and reliable water supplies for high-risk horticultural crops. The cost of intensive monitoring is also an issue. Recycled water quality is routinely tested at the point of discharge by the operators of wastewater treatment facilities but is rarely tested again at the point of use, and other sources of water are generally not tested on a regular basis.

To overcome these issues, sanitisation of irrigation water using disinfectants provides an attractive solution to expand the choice of source waters that can safely be used for vegetable production. EO water offers an alternative to traditional chemical treatment methods, with several potential benefits including the presence, in addition to chlorine, of reactive oxygen species that are toxic to microorganisms, the possibility of treating phytopathogens in the field and the possibility for additional use of EO water for post-harvest treatment (Hort Innovation report VG09086). However, the effectiveness of EO water as an irrigation water treatment has not yet been fully evaluated and its impacts on soil microorganisms are also yet to be discerned. This project was designed specifically to fill this knowledge gap.

Methodology

The project was divided into five activities to achieve the project objectives.

Activity 1: Literature review

A literature review was conducted to document the efficacy of pre-harvest water treatment using available sanitisers to reduce pathogen levels on high-risk vegetables. This review covered the range of traditional and emerging available technologies for water disinfection (see Appendix 1: VG15068 Milestone report 102 appendices), whilst noting that there is limited published information on irrigation water disinfection. A revised version of this literature review was published in Critical Reviews in Environmental Science and Technology in 2019 (see Appendix 2).

Activity 2: Establishment of boundary conditions for the efficient use of EO water

EO water produced using potassium chloride (KCl) or sodium chloride (NaCl) was tested to confirm comparable efficacy. Two other disinfection materials (sodium hypochlorite and chlorine dioxide) were also tested. Water samples were prepared with a range of pH, organic matter content, and pathogen loads. Three organisms, representative of important pathogenic bacteria, were inoculated into the test water: *E. coli* (ATCC 25922), *Listeria innocua* 6a (ATCC33090), and *Salmonella enterica* serovar Enteritidis 11RX. The kill rates (assessed using standard plating methods for viable microorganisms and molecular methods to detect viable but not culturable microorganisms) for different EO treatment concentrations were determined across the range of water conditions established. Residual total and free chlorine were also measured. These results were used to define the range of water conditions within which EO water can effectively improve irrigation water quality (see Appendix 3: VG15068 Milestone report 103 appendices) and were published as a research article in Scientific Reports (see Appendix 4).

Activity 3: Bench scale assessment of the pre-harvest efficacy of EO water on contaminated produce

This activity assessed whether irrigating with EO water effectively reduced microbial contamination during pre-harvest crop management. A reasonable worst-case scenario was used where leafy vegetables were contaminated through exposure to animal manure with inoculated microorganisms. The inoculum was prepared by sterilising manure (through gamma irradiation) and re-inoculating it with the same model organisms, *E. coli*, *Listeria*, and *Salmonella*, used in Activity 2 above. Two vegetables were tested: lettuce and spinach. Plants inoculated with these pathogens were sprinkler-irrigated using EO water. Four treatments were compared: EO water wash with 50 mg/L of free chlorine, sodium hypochlorite wash with 50 mg/L of free chlorine, tap water wash and an unwashed control. The plants were destructively sampled at 0, 3 and 7 days after the irrigation treatments to assess the potential for regrowth of pathogens after sanitisation. Culturable microorganisms were detected using selective media and total populations were assessed using quantitative PCR. The effect of the treatments on shelf-life post-harvest (8-10 days) was evaluated by sensory analysis. Experiments were performed on two separate occasions to confirm the results observed. Results were presented in Appendix 5 (VG15068 Milestone report 104 appendices) and a research article has recently been accepted for publication (Appendix 6: Ogunniyi et al. 2020).

Activity 4: Effect of sanitised water on soil microbial functions and composition

This activity tested the effect of repeated applications of sanitised waters on the soil microbial community, key soil functions driving nutrient cycles, and soil physicochemical parameters (see Appendix 7: VG15068 Milestone report 105 appendices). Six different soil types from four States (QLD, SA, TAS and VIC) were collected from key vegetable production regions. Intact soil cores were transported to the laboratory where they were irrigated over a period of 14 weeks with three treatments—control (untreated), 5 mg/L of free chlorine from EO water, and 5 mg/L of free chlorine from sodium hypochlorite. At the end of the incubation period, the columns were sectioned at different depths (e.g., 0–2, 2–5, 5–12 and 12–20 cm) and the samples analysed for EC, pH and a number of microbial parameters including:

- Extracellular enzyme activities: Enzymatic activities of enzymes involved in the carbon, nitrogen and phosphorus cycles were measured at the natural soil pH using a published fluorimetric method already established in our laboratory.

- Microbial community structure and diversity: Soil bacterial (16S rRNA), fungal (internal transcribed spacer region, ITS) and protist (18S rRNA) diversity and community structure were analysed by HiSeq Illumina sequencing.

Bioinformatic analysis of the sequence data is ongoing to allow a comparison of the effect of the disinfection treatments on the microbial community structure and diversity.

Activity 5: Greenhouse and field-testing of sanitised irrigation water on lettuce and spinach

This activity had two parts: 1) greenhouse testing of an irrigation water disinfection system on spinach and lettuce and 2) field testing of the irrigation water disinfection system on lettuce (see Appendix 8: VG15068 Milestone report 106). Previous work in this project had been conducted using the solution produced by the Water Disinfection System (WDS) from Ecas4 Australia, but advances in the technology resulted in the testing of an in-line electrolysis system ("Booster" reactor) as a more suitable approach for field application. Preliminary data was collected to demonstrate the efficacy of the system on similar water samples as tested above for the EO water system.

1. A greenhouse trial was conducted growing spinach and lettuce in soil from the same Virginia field site as described below for the field trial. Water was obtained from the farm dam and transported to the greenhouse for use throughout the trial. Spinach and lettuce seedlings were planted in pots. Water was treated to generate 5 and 20 mg/L of free chlorine using a Booster reactor. Microbial counts of coliforms and total heterotrophs were assessed in the treated water. Plants were irrigated through an overhead irrigation system. Total bacterial counts and coliforms on the plant leaves were assessed at fortnightly intervals over 8 weeks.
2. A field trial was conducted at a Virginia field site used for lettuce production. Water was treated with a Booster system (comprising two Booster reactors) to generate a target free chlorine concentration of 5 mg/L. Lettuce plants were grown according to standard practices, with the treated water applied through an overhead sprinkler irrigation system. The control plants were grown using untreated water. Microbial counts in the water and on plant leaves were assessed as above for the greenhouse trial.

Outputs

Activity 1: This activity resulted in the preparation of a comprehensive literature review (Appendix 1: VG15068 Milestone report 102 appendices) and a published review article (Dandie et al. 2019; Appendix 2).

Activity 2: This activity resulted in the preparation of a consolidated dataset showing the boundary conditions of applicability of EO water (Appendix 3: VG15068 Milestone report 103 appendices) and the publication of a research article (Ogunniyi et al. 2019; Appendix 4).

Activity 3: This activity resulted in the preparation of a consolidated dataset showing the ability of EO water sanitisation to treat contaminated produce pre-harvest (Appendix 5: VG15068 Milestone report 104 appendices) and the publication of a research article (Appendix 6: Ogunniyi et al. 2020).

Activity 4: This activity resulted in the preparation of a consolidated dataset showing the effect of EO water sanitisation on soil microorganisms (Appendix 7: VG15068 Milestone report 105 appendices). Preparation of a research article for publication from this work is underway.

Activity 5: This activity resulted in the preparation of a consolidated dataset showing the effectiveness of the Booster system for treatment of microbiologically-compromised irrigation water and the effect of irrigation with treated water on lettuce and spinach in the greenhouse and on lettuce in the field (Appendix 8: VG15068 Milestone report 106 appendices and Appendix 9: Field Trial Images). It is expected that the dataset would generate a research article for peer-reviewed publication.

Two fact sheets have been prepared to summarise the information on the use of EO water for irrigation water disinfection (Appendix 10: Fact Sheet 1) and for implementation of the Booster reactor disinfection system in greenhouse and on-farm applications (Appendix 11: Fact Sheet 2).

Outcomes

This project systematically evaluated the efficacy of electrolysed water to sanitise irrigation water for pre-harvest application to leafy greens. The specific outcomes for each activity are detailed below.

The literature review (Activity 1) resulted in the following conclusions and recommendations:

- In Australia, there is no direct information on the microbial profiling of irrigation water or data on the effects of microbiologically impacted irrigation water on the quality of fresh produce. Therefore, effective post-harvest treatment methods could potentially be exploited for pre-harvest sanitisation of fresh produce.
- EO water technology might hold numerous advantages over other sanitisation methods, and its commercialisation is based on a number of claims in terms of overall safety, cost and environmental impact. Therefore, a rigorous investigation of the veracity of these claims, including comparative efficacy of these sanitisers with existing chlorine-based and other sanitisers, is warranted.
- Direct evidence for the effects of the various irrigation treatment regimens on the viable-but-not-culturable state as well as the dynamics of soil and microbial communities, particularly on high-risk vegetables, is needed.

The results of Activity 2 showed the following:

- EO water treatment of a mixed bacterial suspension of *E. coli*, *L. innocua* 6a and *S.* Enteritidis 11RX resulted in a dose-dependent (less than 1 mg/L of free chlorine), rapid, time-dependent (<2 minutes) and substantial kill (4–6 $\log_{10}$) in water devoid of contaminating organic matter content.
- EO water produced using either potassium chloride or sodium chloride efficiently killed bacteria in a remarkably similar manner.
- The ability of EO water to kill bacteria was not appreciably affected under the range of buffered or unbuffered pH conditions (pH 6.0, pH 7.0, pH 8.4 and pH 9.2) tested.
- The ability of EO water containing 1 mg/L of free chlorine to kill bacteria was substantially reduced in the presence of increasing organic matter content, but not appreciably affected at 5 mg/L of free chlorine level.
- The efficacy of EO water to reduce the microbial load in the absence or presence of natural organic matter compared favourably with that of equivalent concentrations of other chlorine-based sanitisers (NaOCl and ClO_2).

The results of Activity 3 showed that:

- Irrigation of lettuce with 5 mg/L of available chlorine from either EO water or NaOCl in the presence of organic matter was ineffective at reducing inoculant abundance on the lettuce leaf surface, with both treatments providing similar efficacy to that of the tap water control.
- The effect of organic matter on inoculant survival on lettuce in the presence of 20 and 50 mg/L of available chlorine from EO water was significant, with higher inoculant survival in the presence of organic matter.
- Survival of the inoculum was significantly reduced for all three strains tested after irrigation with 20 or 50 mg/L of available chlorine from EO water when compared with the unwashed or tap water controls.
- A comparison of tap water, 50 mg/L of available chlorine as NaOCl and 50 mg/L of available chlorine as EO water was conducted on lettuce and spinach. The effects of NaOCl and EO water were remarkably similar in terms of reduction in inoculant survival; however, there were obvious negative effects of NaOCl at that concentration on the plant leaf appearance, with severe necrotic zones, yellowing and browning of leaves. In contrast, there were no visual effects of irrigation with EO water or tap water on plant leaves.
- Irrigation with 50 mg/L of available chlorine as EO water resulted in reductions in inoculum survival at Day 0 of approx. 1.2 $\log_{10}$ colony-forming units/g for both lettuce and spinach leaves.
- There were no negative effects of pre-harvest irrigation with EO water on post-harvest shelf life, with all leaves showing excellent storage qualities for periods of up to 14 days.

The results of our analyses in Activity 4 showed that:

- Soil pH: There were no significant effects of EO water irrigation on soil pH among the soils tested, except

for QLD soil showing an increase in soil pH at the soil surface (0–2 cm) with EO water irrigation. In comparison, irrigation with NaOCl resulted in significant pH increases for most soils tested at a range of soil depths.

- Soil electrical conductivity (EC): There were no significant effects of EO water irrigation on soil EC for any of the soils tested. There were significant increases in soil EC with NaOCl irrigation for 2 of the soils tested.
- Extracellular enzyme activities: Soil enzyme activities were generally low in the soils tested. There were mixed effects of the EO water and NaOCl irrigation treatments on extracellular enzyme activities in soil, with some soils showing no effects, others showing inhibition of activity and others showing enhanced activity.

This work is ongoing to determine the response of the soil microbial community to the irrigation treatments through analysis of phylogenetic markers and functional genes related to key nutrient cycles.

Activity 5 resulted in the following outcomes:

- During this project, it was determined that for practical implementation on-farm, the use of an alternative electrochemical disinfection technology, the Booster reactor, would be more appropriate than the production and dosing of EO water produced by the Water Disinfection System. The benefits of the Booster reactor include that no additional salts are required for production of the active agent (free chlorine) and that it can be installed in-line on the irrigation system, with a standard power supply needed to generate the current required.
- Preliminary characterisation of the Booster reactor indicated that it successfully reduced the microbial load of contaminated water and was easily adjustable depending on the water properties and power settings. Recirculation through the Booster reactor or increasing the contact time resulted in increased disinfection efficacy.
- Low efficacy was observed for spinach irrigated with Booster-treated water in the greenhouse; however, these results might have been confounded by aphid infestation of these plants.
- Greenhouse and field trials of lettuce irrigated with Booster-treated water were conducted under controlled conditions. The microbial load of both target microorganisms (coliforms) and total heterotrophic microorganisms was substantially reduced in Booster-treated irrigation water with both low (5 mg/L) and high (20 mg/L) concentrations of free chlorine.
- Lettuce plant leaf samples showed reduced microbial load for both coliforms and total heterotrophs in the greenhouse, but only for coliforms in the field. This might be related to the higher variability of field conditions and the season of the field trial, where irrigation was limited because of high natural rainfall during the growth period.

Future field trials of this technology would be valuable to enable further evaluation of the Booster reactor efficacy on a range of irrigation water of varying properties and during multiple growth periods and seasons. Cost-benefit analysis and investigation of any potential for production of disinfection by-products are also warranted.

Monitoring and evaluation

Please see below the Key Evaluation Questions and associated responses.

1. To what extent has the project achieved the project outcomes of increased understanding of the potential application of EO water?

   The project has defined the conditions under which effective application of EO water for disinfection of irrigation water can be achieved.

2. To what extent has the project addressed the needs of levy-paying vegetable growers?

   The project aligns with the following objectives in the Vegetable fund Strategic Investment Plan:
   Improve the use and management of soil and water – critical inputs to commercial vegetable production
   Improve food safety standards
   Increase use of advanced technologies to improve farm productivity and/or reduce input costs for growers

3. Have all outputs been published in the appropriate channels?
   To date, all publishable units have been published in the appropriate, high quality scientific journals, with further manuscripts still in preparation. Several alternative outlets have also published items about the project for a wider audience. Finalisation of this project will see the preparation of a fact sheet to communicate the project outputs with the target grower audience.

4. Has their publication resulted in engagement/interest from vegetable growers?
   The project team has established an ongoing relationship with the hosts of our field trial in Virginia. It would be valuable to leverage this relationship to establish further field trials and use this as a demonstration site of the technology that could be communicated to other growers in the region and across Australia.

5. What efforts did the project make to improve efficiency?
   The original project was targeted towards the Water Disinfection System for production of EO water; however, as detailed above, the Booster reactor technology was ultimately adopted for on-farm application as a more efficient and easily manageable technology. The simple installation and demonstration of this technology provides confidence that it could be widely applied for irrigation water disinfection on-farm.

Recommendations

The main recommendations arising from this project are as follows:

1. EO waters produced using either NaCl or KCl were found to be equally effective, so in situations where EO water is preferred, preparation of EO water with KCl is preferable, to reduce the application of excess Na and for provision of K for plant growth.
2. An effective EO water concentration of 20 mg/L of free chlorine is recommended for complete pathogen removal from contaminated water, even in the presence of high organic matter content.
3. The Booster electrochemical technology may be more suitable for on-farm application, given the ease of installation and operation of this technology.
4. As part of a multi-barrier or hurdle approach to food safety, EO water or Booster technology has the potential to reduce microbial contamination of minimally-processed foods throughout the growth period. In combination with post-harvest treatment, this pre-harvest treatment approach could result in enhanced food safety for leafy greens and other minimally-processed vegetables.

Several key limitations of this study could be addressed in future research:

- EO water or Booster reactor treatment of a wider range of irrigation waters
- Field assessment of EO water or Booster-treated irrigation water over several growth periods and seasons
- Field assessment of the efficacy of irrigation water disinfection processes for a range of different crops, including other leafy greens
- Assessment of the potential for EO water or Booster-treated water to reduce the application of pesticides/fungicides during the plant growth season and/or reduce the incidence of plant pathogens
- Assessment of the potential for EO water or Booster-treated water to reduce biofilm build-up on irrigation pipes and equipment
- Cost analysis of EO water or Booster reactor implementation in the field
- Analysis of the potential production of disinfection by-products by the EO water or Booster reactor processes and for plant uptake in the field from treated irrigation water

Refereed scientific publications

Dandie, C.E., Ogunniyi, A.D., Ferro, S., Hall, B., Drigo, B., Chow, C.W.K., Venter, H., Myers, B., Deo, P., Donner, E. and Lombi, E. (2019) Disinfection options for irrigation water: Reducing the risk of fresh produce contamination with human pathogens, Critical Reviews in Environmental Science and Technology, DOI: 10.1080/10643389.2019.1704172

Ogunniyi, A.D., Dandie, C.E., Ferro, S., Hall, B., Drigo, B., Brunetti, G., Venter, H., Myers, B., Deo, P., Donner, E. and Lombi, E. Comparative antibacterial activities of neutral electrolyzed oxidizing water and other chlorine-based sanitizers. *Scientific Reports* 9, 19955 (2019). DOI: 10.1038/s41598-019-56248-7

Ogunniyi, A.D., Dandie, C.E., Brunetti, G., Drigo, B., Aleer, S., Hall, B., Ferro, S., Deo, P., Venter, H., Myers, B., Donner, E., and Lombi, E. Neutral electrolyzed oxidizing water is effective at decontaminating on-farm fresh produce (2020). Food Microbiology doi.org/10.1016/j.fm.2020.103610.

Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report.

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Project team:

Catherine E. Dandie, Abiodun D. Ogunniyi, Barbara Drigo, Erica Donner and Enzo Lombi, *Future Industries Institute, University of South Australia*

Henrietta Venter, Permal Deo, *Health and Biomedical Innovation, Clinical and Health Sciences, University of South Australia*

Baden Myers, *Australian Flow Management Group and STEM, University of South Australia*

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Appendices

Appendix 1: VG15068 Milestone report 102 appendices

Appendix 2: Dandie et al. 2019

Appendix 3: VG15068 Milestone report 103 appendices

Appendix 4: Ogunniyi et al. 2019

Appendix 5: VG15068 Milestone report 104 appendices

Appendix 6: Ogunniyi et al. 2020

Appendix 7: VG15068 Milestone report 105 appendices

Appendix 8: VG15068 Milestone report 106 appendices

Appendix 9: VG15068 Field Trial Images

Appendix 10: Fact Sheet 1

Appendix 11: Fact Sheet 2

Appendix 1: VG15068 Milestone report 102 appendices

The WEEKLY TIMES

Horticulture

Salty solution: chloride salts are being charged up kill bacteria on vegetable crops

PETER HUNT, The Weekly Times
July 18, 2017 11:30pm

CLEANING vegetables while they are still growing in the field may sound like an odd idea, but South Australian researchers are verifying its value.

The University of SA team is using the same technology used in saltwater swimming pools to create chlorinated irrigation water that can be sprayed onto crops to kill off unwanted microbes.

“One of the biggest flower importers into Australia is using this technique, spraying his flowers to extend their shelf life,” university environmental science and engineering professor Enzo Lombi said.

“It (the treated water) has even been used to extend the shelf life of fish by one or two days.”

The technology involves using the naturally occurring low levels of chloride salts in water, or adding more if needed in the form of potassium chloride, to kill off bacteria on vegetables.

The water is pumped between large planar (large surface area) electrodes, which converts the chloride salts into chlorine.

“Chlorine is a strong oxidiser to which there is no microbial resistance,” Prof Lombi said.

“If you use water that is a little saline, you don’t need to add anything to it. In agriculture we can also use potassium chloride, because sodium can create problems.”

Professor Lombi said that while the simple technique was being used for post-harvest treatment of foods, Hort Innovation Australia was funding his team to see if they could cut the microbial load on standing crops before harvest.

He has already recruited two vegetable growers from SA and NSW to run trials on their farms and may expand his work on electrolysed oxidising water to Victoria, depending on results.

He said the three-year project would examine the value of EO Water as a tool that:

ALLOWS vegetable growers to regularly spray irrigate leafy green crops, such as lettuce, to wash parts of the plant that may be inaccessible once it matures.

CLEAN out biofilms, containing bacteria, from irrigation pipes and lines

GIVES irrigators the ability to use poorer quality water, given they can sanitise it using the EO technique.

Hort Innovation chief executive John Lloyd it could save vegetable growers time and money beyond minimising product losses through food recalls.

“This project has the potential to unlock benefits such as the ability to easily treat irrigation

water from a variety of sources, using a safe and proven method, and the potential

effective removal of sludge and build up in irrigation pipes,” Mr Lloyd said.

Charged irrigation water could boost food safety

By Andrew Spence / 2nd of August, 2017

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TECHNOLOGY that allows vegetables to be cleaned with electricity-charged water before harvest is being trialled in South Australia.

The **University of South Australia (https://www.unisa.edu.au/)** research could also help keep irrigation pipes clean and give growers the ability to irrigate with water previously deemed too poor to be used on vegetables.

Electrolysed Oxidising water techniques (EO) are used to sanitise water used on produce post-harvest to kill bacteria and extend shelf life but using it pre-harvest at large scale is a new concept.

The water is sanitised when it passes electrodes, which convert chloride salts already present or added to the water into chlorine.

The three-year project began in June and is being funded by **Hort Innovation Australia (http://horticulture.com.au/)**. Growers in South Australia and New South Wales have been recruited for on-farm trials.

University of South Australia Chair in Environmental Science and Engineering Enzo Lombi said the technology was scalable and could be used to sanitise thousands of litres of water an hour – enough to service entire farms.

He said the technology would be most effective at treating leafy crops that are consumed fresh such as lettuce, spinach and parsley.

“The technology basically converts the chloride that may be already present in the water or can be added to the water into hypochlorous acid (chlorine), which is a very strong oxidising agent that kills off the micro-organisms,” Professor Lombi said.

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Lombi said the research would mainly look at human pathogens like salmonella and ecoli but will also look out for any positive effects at eliminating crop diseases.

“With more and more demand for food safety now is the right time to test it out and it should be cheap enough so it is affordable for farmers,” he said.

The sanitised water would also help clean biofilms from irrigation pipes and allow farmers to access water sources not previously deemed suitable for irrigation because of high microbial content.

Lombi said the EO water was also certified organic and could play a significant role in organic farming.

“I’m sure there will also be some unexpected things we find along the way and the follow-up might be that we find that it is also useful as an alternative to pesticides in organic farming,” Prof Lombi said.

“We have a pretty heavy laboratory component where we can do some really detailed studies on whether it works for example to reduce the risk of pathogens growing on crops. In the second and third year we will continue with the lab studies while we also have on-farm trials at a least two locations.”

The vegetable industry is one of the Australia’s largest horticultural industries with an annual production of about 3.5 million tonnes and a value of $AU8.7 billion.

Hort Innovation chief executive John Lloyd said the effectiveness of EO water as an irrigation treatment had not yet been fully evaluated.

“This research will fill that knowledge gap and deliver some tangible outcomes that will benefit growers,” he said.

“It has the potential to unlock benefits such as the ability to easily treat irrigation water from a variety of sources, using a safe and proven method, and the potential effective removal of sludge and build up in irrigation pipes.”

Lloyd said Australia had some of the strictest food safety standards in the world.

“Growers want to build on these standards even further by investing in research to stamp out product recalls and maximise consumer confidence. An additional layer of food safety protection can only help achieve that.”

Key contacts

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Reducing the risk of foodborne disease in fresh produce at the source: disinfection options for irrigation water

Prepared for: Horticulture Innovation Australia
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Summary/abstract:

There is increasing interest globally in the development of safe, affordable, effective and environmentally-sustainable technologies to address the growing public health and economic burden of foodborne diseases and illnesses through the reduction or elimination of spoilage and foodborne pathogens. Here we review the scientific knowledge in the area and compare the efficacy of existing and emerging water sanitization technologies in reducing pathogenic microbial load on pre-harvest, high-risk vegetables and other fresh produce. In addition, we examine the impact of current water sanitization strategies on the ecological dynamics of soil microbes and how this could translate into substantial improvement in the overall quality and value of fresh produce, while maintaining environmentally-sustainable irrigation water usage.

This review shows that while a substantial amount of information is available for disinfection of potable water, the peer-reviewed information on treatment of irrigation water is much more limited. Furthermore, the technologies available for drinking water treatment may not be directly transferrable to the treatment of irrigation water. This is a considerable concern given the importance of irrigation water quality in regards to the safety of fresh produce. The review also highlights that there is no direct and comprehensive information on the microbial profiling of irrigation water or data on the effects of microbiologically impacted irrigation water on the quality of fresh produce in Australia.

This review also focuses specifically on electrolysed oxidising (EO) water technologies, describing its functioning and potential (but largely unsubstantiated in the context of irrigation water) advantages over other sanitation methods.

1. Introduction

Water security is an increasing priority for all levels of society and can be defined as "the certainty that society's water needs will be met into the future on an economically, socially and environmentally sustainable basis" (AWA 2017). There are multiple stressors affecting water security in Australia and around the world, including increasing population density, urbanisation and the uncertainty around climate change effects on water resources and availability (Raudales et al 2017).

Crop agro-ecosystems are at the heart of the food–energy–water nexus, accounting for ~70% of total freshwater withdrawal in the world (FAO 2015). In Australia, irrigated agriculture accounted for 58% of all water use in Australia in 2015–16 (ABS 2017), and projected agricultural water demand is set to increase by 50% by 2050 (AWA 2017), leading to increasing pressure on water resources. Global climate change is likely to lead to decreased fresh water availability in important agricultural regions across Australia as rainfall is estimated to decrease or become more uncertain in many areas (AWA 2016), leading to reduced runoff into rivers, lakes and other storage facilities such as reservoirs. Under these conditions, alternative irrigation water sources and the quality and safety of those supplies must be explored to ensure future water and food security. Municipal water, which is treated to potable use standards, would be ideal and safe for use in irrigation, but the cost and volumes required are often prohibitive. Irrigation water can be sourced from a range of other water sources, but the potential for microbiological contamination needs to be carefully considered.

This review focuses on microbiologically-compromised water sources and potential on-farm treatment options for disinfection of human pathogenic microorganisms in irrigation waters, focusing on fresh produce and specific pathogens (enterotoxigenic *Escherichia coli, Salmonella* spp., *Listeria monocytogenes* and *Campylobacter* spp.). Examples of microbiologically-compromised water sources for irrigation include:

- Surface water, which is considered at high risk of contamination, with many potential routes for transmission of pathogens, but is still used in many cases because it is the only economic and feasible choice (Jones et al 2014). Surface water includes the water in rivers, streams, creeks, lakes, dams and reservoirs. There are several potential sources of contamination of surface water, including wildlife or stock intrusion and faecal deposition, sewage or septic discharges and industrial effluents (Steele and Odumeru 2004).
- Groundwater, which is generally considered a high-quality water source, but the overextraction of groundwater and the potential contamination of groundwater resources should be monitored. Contamination of groundwater with human enteric viruses has been documented in several studies (as reviewed in van Haute et al 2015) and multiple enteric bacterial pathogens are known groundwater contaminants (including *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Shigella* spp. and *Yersinia* spp.; Bradford and Harvey 2017).

- Harvested rainwater, which can be a suitable water source for smaller scale irrigation (i.e., at household or small-holder scale) but would not generally be sufficient for large-scale irrigation applications. Harvested rainwater can also be a source of pathogens, with potential for contamination by animal faeces and other organic debris; potential pathogens such as *E. coli*, *Salmonella* spp., *Shigella* spp. and others have been identified in rainwater samples (Dobrowsky et al 2014).
- Recycled wastewater, which has the potential, with sufficient and appropriate treatment, to be used for irrigation; however, the costs of this treatment and supply are also a major consideration. There are multiple potential benefits associated with the reuse of wastewater, but also significant contamination risks from metals, nutrients, chemicals and microbiological contaminants. Recycled water must be "fit for purpose" (Chhipi-Shrestha et al 2017) and provide no (or better low) risks to the environment or public health and safety (NRMMC, EPHC, AHMC 2006). The use of recycled water can increase water security and reduce pressure on fresh water supplies if appropriately managed. Water recycling schemes are well advanced in many nations around the world, especially in countries such as Israel, Singapore, and Cyprus where water scarcity is already a significant national security concern. In Israel, approximately 90% of wastewater is recycled, mainly for agricultural irrigation. In Singapore, 40% of the current water supply is 'NEWater', which is highly treated recycled water of potable quality; current projections intend for recycled water to provide 50% of the total Singapore supply (both domestic and non-domestic) by 2030. Over the past 40 years, Australia has experienced significant changes in attitudes to recycled water, especially after experiencing the most severe drought in recorded history and the impacts of poor wastewater management, including algal blooms and beach closures (Apostolidis et al 2011). As the demands for potable freshwater increase, the identification and implementation of recycled water schemes for other uses have flourished. In addition to reducing the demand on potable water supplies, wastewater reuse provides additional benefits like reducing the disposal of nutrient-rich wastewater to the environment and providing a stable resource for irrigators. However, due to the nature of the final outcome—human consumption of the irrigated produce - the reuse of wastewater for irrigation has particular quality requirements.

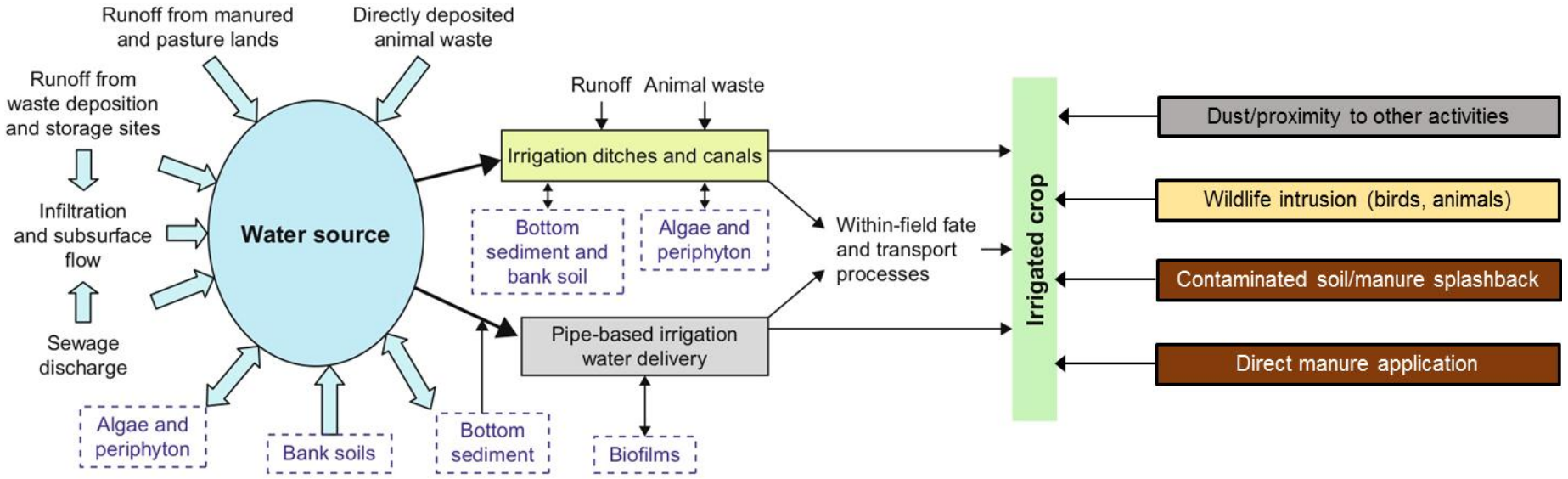

Fig. 1 *Processes affecting the microbial quality of irrigation water and other potential impacts on the microbial safety of fresh produce. Adapted from Pachepsky et al. (2011).*

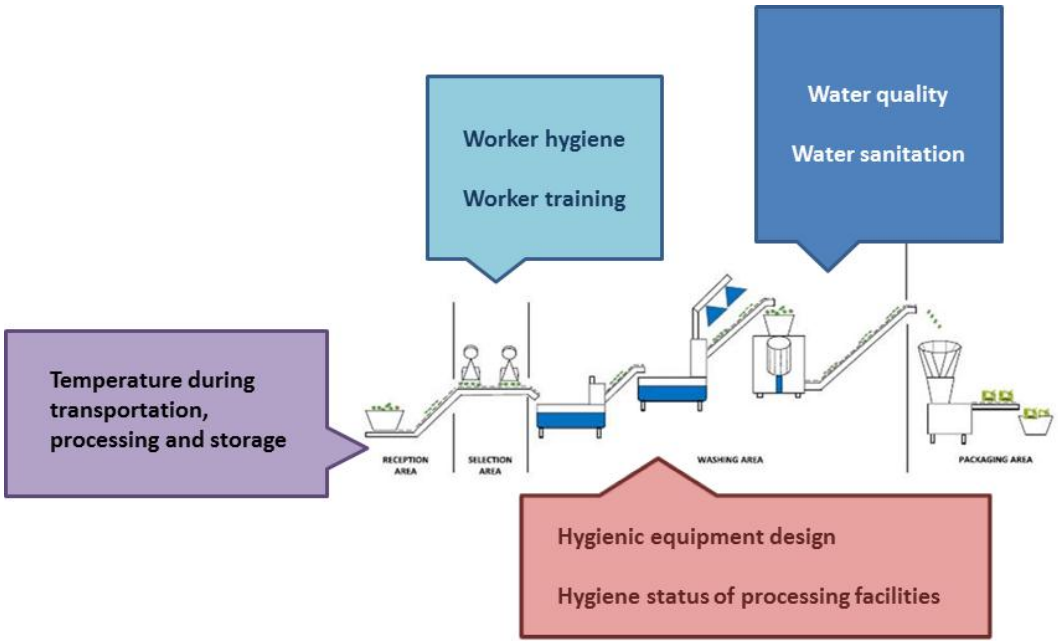

Fig. 2 *Potential pathways of exposure and risk factors for fresh produce during post-harvest handling/processing (adapted from Castro-Ibáñez et al 2017)*

1.1 Minimally processed foods

The use of recycled water for irrigation of edible crops, especially those that are consumed raw or with minimal processing (minimally processed foods: MPFs) is of particular concern for consumers. There have been several outbreaks of human disease linked to microbial contamination of MPFs. In some instances, these outbreaks have been associated with food pathogens that are uncommon in these foods, for instance, *Salmonella* spp. in cantaloupes and pre-packed lettuce leaves (Jasper 2016; Lauder 2016). A recent outbreak of Salmonellosis in Australia was associated with pre-packaged ready-to-eat baby spinach and lettuce leaves, leading to a major recall of these products (FSANZ 2016). Pre-harvest (i.e., irrigation water) or post-harvest water (i.e., washing water) has been identified as the dominant source of product contamination in cases of produce contamination associated with illness (FSANZ 2011) and in these recent outbreaks, investigations have therefore focussed on the quality of water used in processing this produce. However, when investigating the role of water quality, other key aspects to consider are also important, as summarised in Figs. 1 and 2.

The quality of irrigation water is paramount in ensuring the safety of edible produce (Uyttendaele et al 2015). However, many other parameters such as storage conditions, transfer and irrigation method, crop type, and agricultural practices also impact on the transfer of pathogens from irrigation water to produce (Fig. 1)**.** As a result**,** establishing a direct link through epidemiological investigations between contaminated irrigation water and contaminated produce can be difficult. Nevertheless it can generally be concluded that contaminated irrigation water functions as a vector for

the transfer of pathogens to edible produce (Jongman and Korsten 2017; Markland et al 2017). In particular, leafy greens are especially vulnerable to contamination with pathogens because they have large surface areas, are often grown in close proximity to soil, are irrigated intensively, and are mostly consumed raw (De Keuckelaere et al 2015).

1.2 Microbial pathogens associated with disease outbreaks in fresh produce

There are several potential microbiological risks associated with the irrigation of produce with recycled wastewater and other potentially impaired water sources. Most of the risk is associated with human faecal contamination, although contamination with animal waste and industrial waste are also of concern. Bacterial pathogens identified in irrigation waters have included cytotoxin-producing *Escherichia coli*, *Salmonella* spp., *Yersinia enterocolitica*, *Listeria monocytogenes*, *Campylobacter* spp. and others. Key pathogens are covered in more detail below.

1.2.1 *Salmonella*

Salmonella is a ubiquitous enteric pathogen and a leading cause of human intestinal illness worldwide (Levantesi et al 2012). Salmonellae can be found in many different environments, including water, wastewater, sewage, soil, compost, animals (pets, farm animals and wild animals), plants, insects and algae. *Salmonella* infections are often associated with exposure to contaminated drinking water; similarly, contaminated irrigation water has been implicated in the transfer of *Salmonella* contamination to fresh produce (Levantesi et al 2012). Because of the ubiquity of this pathogen, the potential for transfer and proliferation or survival of *Salmonella* from contaminated water sources onto fresh produce is high.

Salmonella outbreaks have been associated with several types of fresh produce in recent years, including tomatoes (Greene et al 2008), serrano peppers (CDC 2008), leafy greens (Vestrheim et al 2016), cantaloupe (Munnoch et al 2009) and others. Among various types of fresh produce, lettuce was identified as having the highest risk of infection by *Salmonella* spp. from treated wastewater irrigation (Amha et al 2015). In fact, the combination of *Salmonella* and leafy greens eaten raw as salad was ranked first among all potential risks of outbreaks of non-animal origin in the EU (Da Silva Felicio et al 2015). In Australia alone, 128 cases of *Salmonella* outbreak associated with Tripod lettuce were reported in Victoria in February 2016 (Australian Food News 2016), 43 hospitalisations linked to *Salmonella* in raw bean sprouts were reported in April 2016 in South Australia (ABC News 2016), and 80 cases of *Salmonella* outbreak associated with rockmelons Australia-wide were documented in August 2016 (Jasper 2016).

1.2.2 *Escherichia coli*

The presence of *Escherichia coli* is associated with faecal contamination of both human and animal origin and for this reason it is one of the main indicator organisms used to monitor water quality.

In fact, microbiological water quality guidelines are often targeted towards monitoring of *E. coli* (i.e., Class A+ recycled water must contain <1 cfu/100 mL in 95% of samples taken over a 12-month period; Public Health Regulation 2005). Specific strains of *E. coli* are of particular interest in terms of foodborne illness, including *E. coli* O157:H7 (or verotoxin producing *E. coli*: VTEC) and Shiga-toxin producing *E. coli* (STEC), which can cause diarrhoea, haemolytic uraemic syndrome and death (Sharapov et al 2016). Although generic *E. coli* or coliforms are generally monitored in water, the relationships between the abundance of generic *E. coli* and specific strains of interest is not clear. Outbreaks of *E. coli* O157:H7 have been associated with spinach (Sharapov et al 2016) and other leafy greens (Cooley et al 2007).

A quantitative microbial risk assessment of *E. coli* in lettuce production found that irrigation water within the recommended guidelines (0–235 CFU *E. coli*/100 mL) did not contribute to increased risk of illness, but low-quality irrigation water (5,000–10,000 CFU *E. coli*/100 mL) was a significant contributor to increased risk of illness from *E. coli* O157:H7 (Pang et al 2017). Transport of *E. coli* O157:H7 from contaminated water or soil/growing medium into internal plant tissues has been demonstrated for lettuce (Solomon et al 2002), indicating that contamination must be reduced throughout the production chain to reduce the incidence of disease associated with this pathogen group.

1.2.3 *Listeria monocytogenes*

Listeria monocytogenes is responsible for the disease listeriosis, a foodborne illness that can be life threatening to people with compromised immune systems and can cause spontaneous abortion in pregnant women (Vivant et al 2013). Outbreaks of listeriosis have been associated with prepackaged salad mix and bean sprouts in the USA (CDC 2016). *L. monocytogenes* is commonly found in soil (Vivant et al 2013) but is also often detected in irrigation water sources. Unlike *E. coli* and *Salmonella* spp. (Gram negative), *L. monocytogenes* is Gram positive, which potentially influences its susceptibility to water sanitizing treatments.

Contaminated irrigation water has been shown to be a significant risk factor associated with pre-harvest contamination of fresh produce with *L. monocytogenes* (Weller et al 2015). The fact that *L. monocytogenes* is difficult to eliminate from plant surfaces once attached is also problematic (Ölmez and Temur 2010), so best practice is to minimise the risk of contamination along the whole farm to fork continuum. *L. monocytogenes* can also grow at low temperatures (down to −0.4°C; Junttila et al 1988), leading to significant risk once food is contaminated, even if food is appropriately stored throughout the supply chain.

1.2.4 *Campylobacter* spp.

Campylobacteriosis leads to acute gastroenteritis worldwide, but most cases are identified as sporadic evidence rather than as parts of recognized outbreaks. Outbreaks of *Campylobacter* illness resulting from contaminated fruits, vegetables and related fresh produce have been reported, however

they are infrequent (for fresh produce) when compared to other enteric pathogens. The prevalence of *Campylobacter* spp. in fresh produce has been a subject of research interest since the late 1990's (Gardner et al 2011). Previous studies have reported *C. jejuni* as the predominant species isolated from spinach, lettuce, radishes, green onions, parsley, peas and potatoes (Park and Sanders 1992; Kumar et al 2001; Gardner et al 2011). Although *Campylobacter* spp. cannot multiply outside a warm-blooded host, they can survive in several environmental reservoirs, including biosolids, manure and various water sources (surface water, groundwater and rainwater; Whiley et al 2013). *C. jejuni* strains inoculated onto spinach survived for up to 7 days at 4°C, indicating the potential risk from this pathogen associated with fresh produce contamination (Guévremont et al 2015).

1.3 Human health effects of contaminated fresh produce and mechanisms of transmission

The direct effects of contaminated water and soil (or its constituents) on human health are through ingestion, inhalation, and absorption, whilst indirect effects arise from the quantity and quality of fresh produce that humans consume (IPCC 2017). Some of these effects can be detrimental to human health, particularly if toxic substances or opportunistic pathogens enter the food chain and are passed to immunocompromised or vulnerable individuals (Schrag 2012).

Aside from potentially harbouring causal agents of life-threatening haemorrhagic colitis and haemolytic uremic syndrome such as *Salmonella enterica* serovar Typhimurium, *Campylobacter* spp., and *E. coli* O157:H7, the vegetable phyllosphere and rhizosphere also provides niches for opportunistic pathogens that can cause enterohaemorrhagic, skin, wound, pulmonary and urinary tract infections (Myers et al 2013; McBratney et al 2014). Over the past decades, the increasingly large-scale production, storage, and distribution of fresh vegetables and the trend of increasing numbers of immunocompromised individuals have been associated with significant numbers of fatalities in Australia, Europe, Asia, and Northern America (Schrag 2012; Myers et al 2013).

Various vegetables have been reported to host opportunistic pathogens and their geographic distribution and disease outbreaks are linked to farming and food processing practices as well as natural hazards including droughts, floods, tropical cyclones, forest fires, and heat waves (Mendes et al 2013). Predicting the conditions under which humans are most vulnerable to pressures from irrigated water and soil derived infectious disease and how these pressures will translate to changes in global health and food security is an important topic in ecological studies (Berg et al 2005, 2013b; van Baarlen et al 2007; Altizer et al 2010).

Many opportunistic human pathogens colonizing fresh produce have an endophytic lifestyle, using vegetables as an alternative host to survive in the environment and as a vehicle to re-colonize human and animal hosts once ingested. This leads not only to intimate interactions with their host, but also to difficulties for decontamination. The mode of transmission/colonisation of microbes in fresh produce will determine the effectiveness of water treatment in reducing the risk of pathogen contamination. Microbes can be transferred directly to plant surfaces from overhead irrigation, or can

infiltrate plants via the root system as endophytes. Pathogen survival on plant surfaces has been demonstrated, especially in biofilms, as has the internalisation of pathogens into plant tissues (Lim et al 2014). Treatment of irrigation water may be effective in reducing the incidence of pathogen contamination through direct transfer of pathogens from irrigation water to plant surfaces, but other best practice agricultural management practices will also be required to reduce the potential for endophytic pathogen colonisation from contaminated soil/manure.

Potential human pathogens in recycled water and soil can be highly competitive for nutrients and may produce antimicrobial metabolites that assist them to colonize and proliferate in the phyllosphere, stem, and rhizosphere in the presence of indigenous microflora (Mendes et al 2013). Indeed, persistent phyllosphere and rhizosphere invasion and inherited seedborne endophytic colonization have been demonstrated for *S.* Typhimurium, *Cronobacter sakazakii*, associated with cases of meningitis and sepsis in neonates, and for the severe human pathogen *Burkholderia pseudomallei* in the rhizosphere of diverse exotic grasses in Europe, Northern America and Australia (Berg et al 2005, 2013a; Tyler and Triplett 2008; Critzer and Doyle 2010; Kaestli et al 2012; Altizer et al 2013; Mendes et al 2013).

The ability of a pathogen to invade human tissues encompasses mechanisms for colonization such as adherence and initial multiplication, production of extracellular substances (invasins) that promote the immediate invasion of tissues, and ability to bypass or overcome host defence mechanisms that facilitate the actual invasive process. Following adherence, the path of plant invasion by the pathogens appears to be largely extracellular, whereby they reside within the fluid-filled apoplastic spaces between plant cells (Holden et al 2013). Infection of plants, whether internalised or not, results in utilization of plant-derived nutrients by pathogens who also avoid or supress the plant immune responses (Paungfoo-Lonhienne et al 2010, 2013, 2014; Berg et al 2013a; Drigo and Kowalchuk 2013). Most studies assessing the modality of plant infections by human pathogens have been carried out using either fluorescently-labelled or bioluminescent bacteria (Berg et al 2005, 2013a, 2013b; van Baarlen et al 2007; Tyler and Triplett 2008; Whipps et al 2008; Holden et al 2009; Teplitski et al 2009, 2011; Critzer and Doyle 2010; Paungfoo-Lonhienne et al 2010, 2013, 2014; Kaestli et al 2012; Drigo and Kowalchuk 2013). A common technique involves sterilizing the external surfaces of the plant tissue, allowing quantification of bacteria that are internalised and protected from the sterilizing agent (Paungfoo-Lonhienne et al 2010, 2013, 2014; Berg et al 2013a; Drigo and Kowalchuk 2013). The majority of these studies have shown that human pathogens preferentially invade plant root tissue rather than foliage (Holden et al 2013). In addition, pathogen invasion has been observed more frequently at lateral root junctions, perhaps because extracellular spaces at these points are large and amenable to pathogen entry (Kaestli et al 2012; Berg et al 2013a; Drigo and Kowalchuk 2013; Holden et al 2013; Paungfoo-Lonhienne et al 2013). Experiments conducted on wheat, sugarcane, rice, maize, sunflower, potato, lettuce, barley and black pepper roots showed that following attachment to the rhizosphere, human pathogens and in particular Enterobacteriaceae infect the root tissue at the point of lateral root

emergence and reside in the apoplastic spaces of the plant host (Altizer et al 2013; Berg et al 2013a; Holden et al 2013; McBratney et al 2014; Mendes et al 2013; Myers et al 2013). Interestingly, the mechanisms involved in the colonization process appear to be similar to the mechanisms involved in virulence and colonisation of human tissues (Berg et al 2005; van Baarlen et al 2007). Once infected plant tissues are ingested, pathogens may colonise the gut of immunocompromised individuals, although proliferation at 37°C is a crucial prerequisite for successful survival and virulence in the human body (Altizer et al 2013; Berg et al 2013a; Wheeler and von Braun 2013).

In addition to the general risks to human health associated with pathogen contamination in the foodchain, the heightened risks posed by antibiotic/antimicrobially-resistant pathogens is also of key relevance here. Antimicrobial resistance is a major concern worldwide, and is recognised by the World Health Organisation as a "global health security emergency". In May 2014, The World Health Assembly adopted a resolution to develop a Global Action Plan (GAP; http://www.who.int/antimicrobial-resistance/global-action-plan/en/) on antimicrobial resistance (AMR). The Australian Government has been an active participant in the GAP development and has also produced 'Australia's First National Antimicrobial Resistance Strategy (2015-2019; Department of Health, Department of Agriculture (2015). A number of areas specifically highlighted as AMR research needs have been documented during associated workshops and discussions and many of them are of direct relevance to food chain water supply (Wuijts, S. et al., 2017). For example, research needs include the identification of treatment technologies that can remove antibiotics and other antimicrobial agents, their metabolites, AMR bacteria and AMR genes in environmental media for which the WHO provides specific guidance (i.e. water). Disinfection is a key component in successfully controlling pathogen populations in water but has also been linked in some studies to the selection of antimicrobially-resistant microorganisms (Rizzo et al., 2013). This potential should be carefully considered in the risk-benefit analysis of any proposed disinfection strategy, particularly where this is linked directly to the human food chain.

1.4 Strategies to reduce contamination of fresh produce

Although there is a substantial industry around post-harvest washing/processing of fresh produce, if we accept the premise that preventing contamination is easier than applying corrective procedures, the best strategy to reduce contamination of fresh produce is to prevent contamination occurring in the first place (Sigge et al 2016). Colonisation of bacteria on plant/leaf surfaces proceeds in two stages: (1) initial attachment, which can occur within seconds and is based on weak, reversible binding processes, and (2) irreversible or 'firm' attachment, which occurs over longer time periods (minutes to hours) and can be influenced by multiple factors including bacterial- or produce-related properties and environmental factors (Yaron and Romling 2014). Banach et al (2017) showed that even given several washes with a chlorine-based sanitizer, pathogens (*E. coli* and *S.* Typhimurium) were difficult to remove from produce surfaces once firmly attached. This means that selection of an appropriate water source and/or degree of treatment for irrigation water is critical (De Keuckelaere et

al 2015) and that irrigation practices and distribution networks must be maintained at the highest possible levels to ensure that the potential for contamination is minimised. As with drinking water treatment, a multiple barrier approach is recommended to ensure that irrigation water quality remains high even in the event of failure or suboptimal performance of individual treatment modules (NHMRC, NRMMC 2011). As part of a multiple barrier approach, on-site treatment of irrigation water is a sound approach to ensure water quality is maintained at an appropriate level for safe irrigation use and to maintain the safety of the on-site distribution network.

2. Treatment technologies for irrigation waters

There are multiple water treatment methods available and they can be applied in different sequences and combinations to ensure that water is of suitable quality or "fit for purpose" for use in irrigation. Generally, the processes can be separated into clarification and disinfection. Clarification processes include physical/mechanical methods like screening, slow sand filters and membrane treatment, as well as biological methods such as biofilters, and chemical methods such as coagulation and flocculation. Disinfection processes commonly involve application of chemicals, such as chlorination, ozone, peracetic acid (PAA), or hydrogen peroxide (H_2O_2); however there are also non-chemical disinfection methods such as ultraviolet (UV) irradiation, membrane filtration, and detention lagoons. Several of these methods are applicable to on-site treatment of microbiologically-impaired irrigation water.

A multitude of factors can affect the choice of treatment technology selected. Selection criteria for treatment technologies can generally be broken down into three categories—technological, managerial, and sustainability (van Haute et al 2015). Technological criteria include quality of the water source (including microbiological load, temperature, pH, suspended solids, organic matter content, etc.), distribution system characteristics, the required water quality (in terms of physical and microbiological parameters), and process parameters of the water treatment technology (i.e., dose and time of treatment required). Managerial criteria include the upfront and operational costs, complexity of operation, monitoring and safety issues (in terms of chemical handling, storage and production of disinfection by-products). Sustainability criteria include maintenance considerations, including the ability to monitor the efficacy of the process, environmental considerations and the associated costs (Fig. 3).

All water purification processes generally benefit from pre-treatment of some sort to remove larger particulates, suspended solids, and associated microbial contaminants. For instance, in the case of waters with significant organic matter loading, ponding/lagooning can be used to substantially decrease biological oxygen demand and suspended solids prior to further treatment processes.

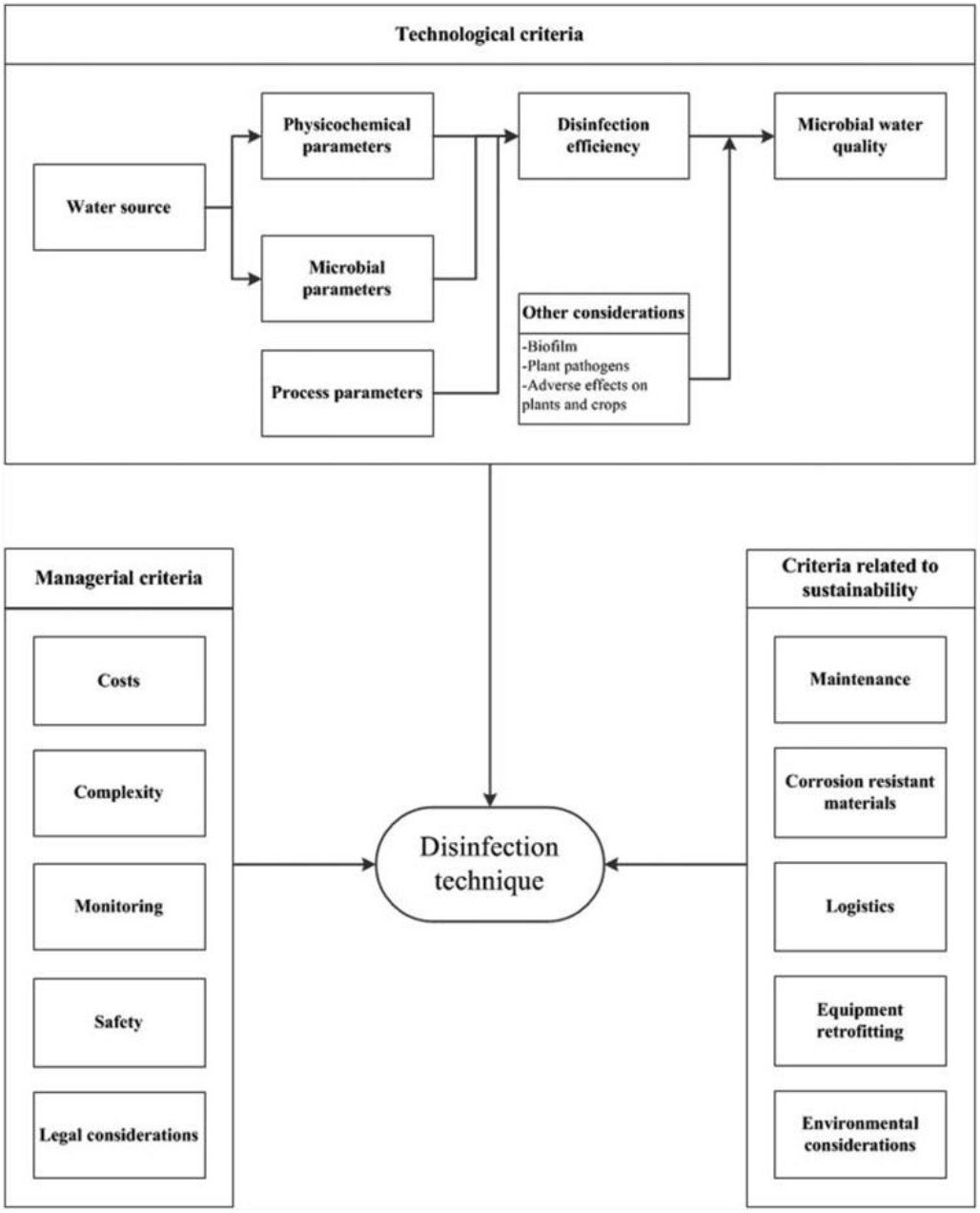

Fig. 3 *Overview of the selection criteria for choosing a water disinfection technique for pre- and postharvest practices. From van Haute et al 2015.*

Several potential treatment technologies for irrigation water are outlined below and the advantages and disadvantages of each process are summarized in Table 1 (with further information in Appendix: Table A1). It should be noted however that the scientific literature regarding on site disinfection of irrigation water is rather limited and this review covers a range of technologies currently used for water disinfections for other purposes and for wastewater treatment.

2.1 Coagulation/flocculation

Coagulation and flocculation are used in municipal water treatment and wastewater treatment to reduce the turbidity of water. Coagulants are generally metal-based (either aluminium or iron), although other compounds are also used. Modern treatment processes use pre-hydrolyzed coagulants, which are effective over a wide range of pH and temperature conditions. Coagulants are used to neutralise the charge of non-settleable colloids and promote the formation of microflocs.

Flocculants are generally polymers that have the ability to destabilize or enhance the aggregation of colloids/microflocs. Flocculants can be natural or synthetic polymers.

Coagulation/flocculation would usually be considered part of an advanced water/wastewater treatment process, requiring substantial expertise and monitoring to ensure optimal treatment processes are applied in any given scenario. There might be some instances where this treatment would be appropriate for application within an on-farm irrigation water treatment system, but this would have to be assessed on a case-by-case basis.

2.2 Slow sand filtration

Slow bed sand filtration has been used for a long time to improve water quality by reducing the load of suspended solids/turbidity and associated microbiological contamination. The process involves the slow passage of water through a porous material, generally sand, with the size of the sand particles and the flow rate determining the effectiveness of the treatment. Contaminants (chemical and microbial) are retarded within the porous material and also interact with a biofilm (or *Schmutzdecke* meaning 'dirt cover') that forms on the surface of the material. Hence slow bed sand filtration is capable of a high degree of water treatment because of both physical processes, such as the slow filtration rate and the fine size of the sand particles, and biological processes, associated with the biofilm layer (Graham and Collins 2014). Pathogens are retained mainly through straining and absorption (physical processes) through the sand bed. Pathogen inactivation occurs as a combination of biotic and abiotic processes, including starvation, predation, lysis and the generation of algal-derived reactive oxygen species (Seeger et al 2016). *E. coli* removal is commonly associated with protozoan grazing and other biological factors in slow bed sand filters (Haig et al 2015; Pfannes et al 2015). Slow bed sand filtration is a low technology, low-cost approach, but the slow filtration time and limitations of microbial removal by this technique mean that it might not be the most suitable approach, at least in isolation, for high-volume treatment of irrigation water to high standards.

2.3 Membrane treatment technologies

Membrane microfiltration (MF) or ultrafiltration (UF) methods can be applied for the production of purified water for potable or other purposes (Hai et al 2014). Water is driven through a membrane by differences in pressure to produce permeate (or filtrate), with contaminants (or retentate) retained on the membrane. The choice of membrane/pore size dictates the conditions (pressure, size of molecules

retained) of the filtration process and the retention of pathogens. MF membranes generally have pore sizes in the range of 0.1–1.0 µm, whereas UF membranes have pore sizes in the range of 5–100 nm, with 10 nm pore size commonly used in membrane bioreactors (Hai et al 2014). Total removal of protozoa is expected with either MF or UF membranes because they range in size from 3–14 µm and bacteria (0.6–1.2 µm diameter, 2–3 µm length) are also expected to be comprehensively removed. Membrane filtration cannot be guaranteed to completely remove viral particles as they are smaller than the pore size of commonly used membranes (Hai et al 2014), thus it is generally not applied in isolation but as part of a series of water treatment processes. Nanofiltration and reverse osmosis processes have the capacity to remove all particles <10 and 1 nm, respectively and dissolved contaminants, including toxins. Substantial expertise is required to install and run membrane filtration facilities, with high capital and ongoing cost, making it an unlikely choice for on-site irrigation water treatment.

2.4 Ozone

Ozone is often used in drinking water treatment, because it is generally regarded as safe for use in the food industry, leaves little or no residual by-products and decomposes to safe products on exposure to oxygen (Martinez et al 2011). Ozone has the highest oxidizing potential (2.07 mV) of all currently used water treatment processes (Markland et al 2017); its mode of action is the decomposition of ozone into free radicals, leading to inactivation of cell membranes and DNA damage (Sigge et al 2016). It is highly effective against a range of bacterial pathogens and viruses, with varying reports of its effectiveness against protozoan parasites (Martinez et al 2011; Sigge et al 2016). Commercially-produced ozone is generated using the corona discharge method (Markland et al 2017). The development of novel ozone generation methods (such as microplasma ozone generation; Dong et al 2017) might open up new markets and applications for ozone at varying scales. Consideration must be given to the destruction of unused or residual ozone, as it is an environmental contaminant, and production systems must be compliant with relevant guidelines (Elmer et al 2014).

In a study of wastewater treatment in Spain, ozone significantly reduced faecal coliforms to levels suitable for irrigation of fresh produce whilst having little observed effect on the nutrient content, meaning that it is a suitable treatment for preserving nutrients in treated wastewater for subsequent application to irrigated crops (Martinez et al 2011). However, ozone reacts with organic and inorganic material in water, limiting its effectiveness in turbid or contaminated waters (Markland et al 2017) and the use of ozone for disinfection of irrigation water has never been widely accepted (Elmer et al 2014). The high cost of implementation might be prohibitive depending on the scale of application (Sigge et al 2016). The formation of disinfection by-products (non-halogenated organic by-products, bromate) has also been observed with ozone treatment of water containing natural organic matter, limiting its potential application to impaired water sources.

2.5 Chlorination

Chlorination is one of the most widely applied forms of water treatment (Sigge et al 2016). Chlorine is available in gaseous form (Cl_2), or as hypochlorite (HOCl), but chlorine in gaseous form is less commonly used because of safety concerns.

2.5.1 Hypochlorite

Hypochlorite is commonly used for water treatment because it is safely available in liquid (sodium hypochlorite) or tablet (calcium hypochlorite) form (Allende and Monaghan 2015). Hypochlorite treatment can be highly effective in reducing concentrations of microorganisms, with reported reductions of up to 4 log CFU/mL. The active agent is hypochlorous acid, so activity of hypochlorite treatments is greatly influenced by pH, with the optimal activity occurring around pH 6 (Fig. 4; Rutala and Weber 1997). Hypochlorite acts against the cell membrane (Fig. 5; Fukuzaki 2006; Venkobachar et al 1977) and affects metabolic cellular processes; uncoupling of the electron chain or enzyme inactivation have been associated with the bactericidal mechanism of chlorine (Virto et al 2005). The main concerns and limitations of chlorine disinfection are around the formation of toxic disinfection by-products and appropriate levels of chlorine residual. *N*-nitrosodimethylamine (NDMA) is the most common disinfection by-product associated with chlorination of treated wastewater, although other *N*-nitrosamines have also been detected in chlorinated wastewater effluents (Garcia-Rodríguez et al 2014). Contamination of produce and build-up of disinfection by-products in irrigated soil are potential problems associated with the use of chlorinated irrigation water. High levels of chlorine residual could also interact with organic material in soil or on plant surfaces, leading to formation of additional by-products during the irrigation process.

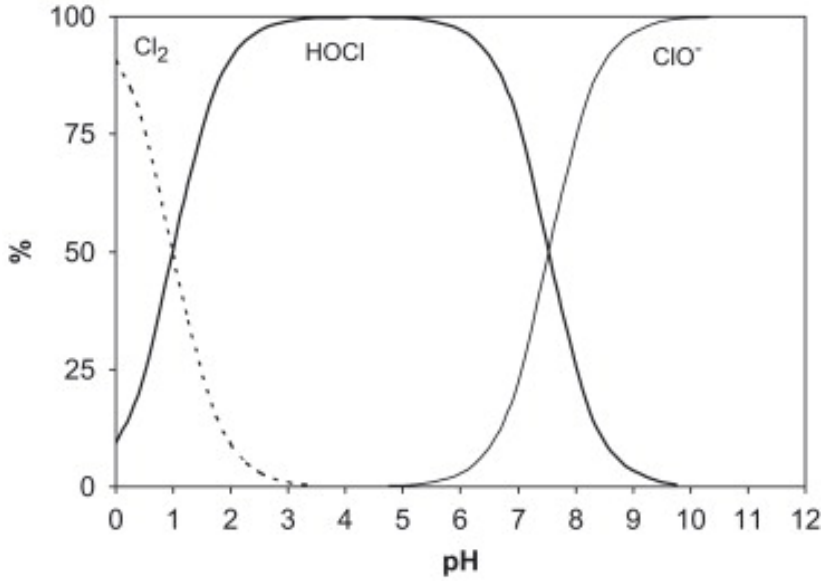

Fig. 4 Relative distribution of the main aqueous chlorine species as a function of pH at 25°C and for a chloride concentration of 5×10^{-3} M (177.5 mg L^{-1}; from Deborde and von Gunten 2008).

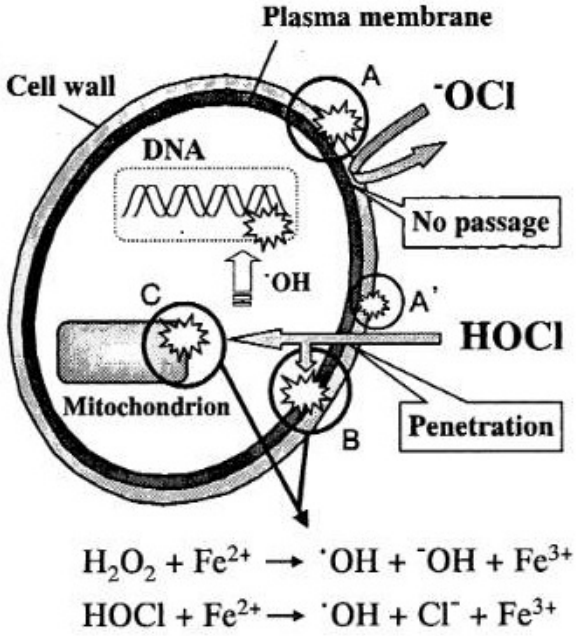

Fig. 5 A model illustrating the mechanisms of the germicidal actions of HOCl and $^{-}$OCl based on their ability to penetrate the membrane into the microbial cell. Ionized $^{-}$OCl has a poor germicidal activity because of its inability to diffuse through microbial plasma membrane, and it exerts an oxidizing action only from outside of the cell (circle A). HOCl can penetrate the lipid bilayer in the plasma membrane by passive diffusion due to its electrical neutrality. HOCl can attack the microbial cell both from the outside (circles A') and inside the cell (circles B and C), which is responsible for the potent germicidal activity of HOCl (from Fukuzaki 2006).

2.6 Chlorine dioxide

Chlorine dioxide (ClO_2) has been used in drinking water, wastewater and irrigation water treatment as it is a strong oxidant with high effectiveness against parasites (Stanfield 2003; Scarlett et al 2016). ClO_2 is reported to be at least 1.2 × more effective than sodium hypochlorite at the same dose and is effective across a wide pH range (4–10; Scarlett et al 2016). ClO_2 treatment efficacy is affected by the organic load and inorganic content of water, with high organic or inorganic content increasing the ClO_2 required to effectively reduce the pathogen load (Raudales et al 2014). The high activity of ClO_2 is most likely attributed to oxidation activity rather than activity associated with chlorine (Banach et al 2015). Stabilised chlorine dioxide solutions have higher oxidation capacity and generate lower amounts of disinfection by-products than sodium hypochlorite (López-Gálvez et al 2017), although inorganic by-products such as chlorate and chlorite have been identified during chlorine dioxide treatment of wastewater (Veschetti et al 2005) and these are regulated in drinking water. ClO_2 can be applied at high 'shock' concentrations (20–50 mg/L) for short periods of time to eliminate biofilms (Raudales et al 2014), or can be supplied continuously at low concentrations for residual control (0.25 mg/L or <1 mg/L; Raudales et al 2014; Scarlett et al 2016). ClO_2 can be supplied as a gas or dissolved in water. Concerns with its use exist around worker safety (ambient monitoring required), transport of precursor chemicals, and instability during on-site generation (Sigge et al 2016); however its activity at a wide range of pH values and its high oxidative capacity make it a feasible option for on-site irrigation water treatment.

2.7 UV light

UV light covers the range of wavelengths from 100–400 nm, but the majority of water treatment applications utilise UV at or covering the wavelength of 254 nm (200–280 nm; UV-C), which is the optimal wavelength for absorption by DNA/RNA and inactivation of microorganisms (Chen et al 2017). UV light at 254 nm causes the production of thymine dimers, which hinder DNA replication and prevent cell division, whereas UV light at other wavelengths can cause cell death by damaging critical proteins required for cell function. There are two main sources of UV light currently used: low-pressure UV lamps that produce UV light of 254 nm, and medium-pressure UV lamps that produce UV light in the range of 200–300 nm (Chahal et al 2016). The UV dose is measured in $mJ.cm^{-2}$ and can be substantially affected by water quality, turbidity and flow rate (see Fig. 6). Particles, measured as suspended solids, are particularly important in determining the efficacy of UV irradiation, as microbes can be shielded by particles or attach in protective flocs, thus reducing the ability of UV to penetrate cells (Fig. 7). Large particles can completely shield pathogens, and smaller particles (particularly silica) can scatter UV light, limiting light penetration through the UV reactor (Chahal et al 2016). Therefore, removal of particles by filtration prior to UV treatment is highly recommended.

A UV dose of ≥40 $mJ.cm^{-2}$ would be considered suitable to remove virtually all pathogenic microorganisms from recycled wastewater (Guo et al 2009), although lower doses can still be effective.

Several log reductions in a range of pathogens (human: *E. coli*, *Salmonella enterica* subsp. *enterica*, *Listeria monocytogenes*, plant: *Pseudomonas syringae*, *Clavibacter michiganensis* subsp. *michiganensis*, *Phytophthora capsici*) were obtained after treatment of unfiltered surface irrigation water with UV light (14 $mJ.cm^{-2}$ using a thin film UV treatment unit; Jones et al 2014). UV has been shown to be effective against a range of other pathogens (protozoan cysts, pathogenic viruses, *Ascaris* eggs); however, high doses of low-pressure UV are required to inactivate adenoviruses (Eischeid et al 2009), indicating that medium-pressure UV might provide a better range of activity against pathogens in water. UV treatment can often be coupled with other treatment technologies in advanced oxidation processes (i.e., UV/H_2O_2, UV/PAA); Sigge et al 2016) to improve the efficacy of treatment. Recent developments in UV-LED technology are promising. These remain relative high cost at present, but there is considerable potential for significant design innovation to facilitate optimised water treatment (Chen et al 2017).

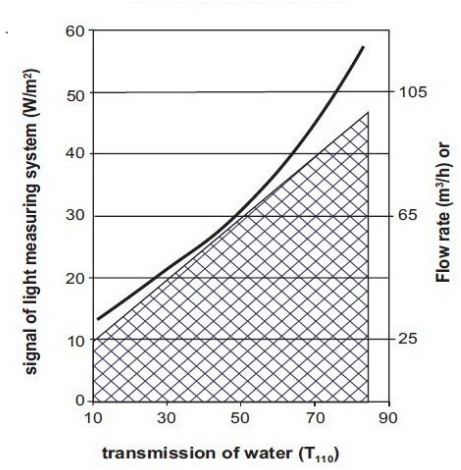

Fig. 6 *Measured UV light intensity as a function of UV transmission of the water (reproduced from Stanfield et al 2003). The dashed area represents combinations of UV transmissions and flow rates, which results in reliable disinfection. The solid curve represents the signal of the light measuring device as a function of the UV transmission of the water. UV transmission (T_{100}) is given as percent transmission using a 100 mm light pass.*

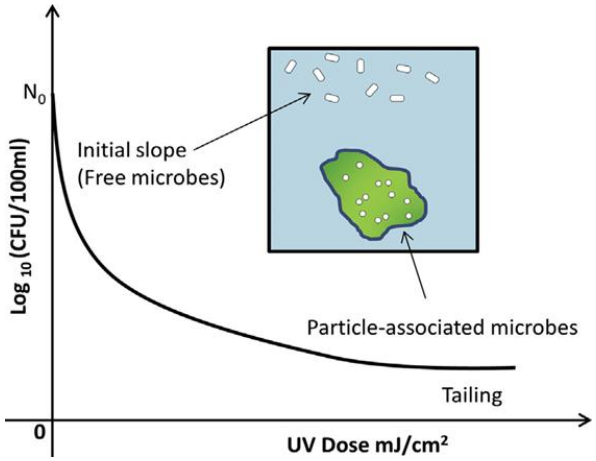

Fig. 7 *Typical UV inactivation curve for microorganisms comparing log inactivation versus UV dose, highlighting the steep inactivation slope representing inactivation of free microorganisms and a shallow slope representing tailing. Reproduced from Farnood (2014).*

2.8 PAA

Peracetic acid is an alternative to chlorine-based water disinfectants that has generated substantial interest, particularly for recycled/wastewater treatment (Kitis 2004). Commercially available PAA consists of a mixture of acetic acid, H_2O_2, PAA, and water, and has strong oxidising properties (Bonetta et al 2017). Commercially available PAA is provided at 10%–15% and is relatively stable (Sigge et al 2016). PAA is thought to work similarly to H_2O_2 via the production of a range of reactive oxygen species, including hydroxyl (HO·), alkoxyl (RO·) and hydroperoxyl (HO_2·) radicals and superoxide ($O_2^{\cdot -}$), resulting in the disruption of cell walls through disruption of sulphydryl and sulphur bonds, reaction with proteins and enzymes, and disruption of DNA molecules through their strong oxidative effect (Karpova et al 2013; Fig. 8). PAA treatment at low doses results in little to no generation of toxic or mutagenic by-products, although low dose PAA treatment (1–2 mg/L) was not able to improve wastewater quality to the level required for irrigation purposes in an Italian wastewater treatment plant (Bonetta et al 2017). PAA treatment of wastewater was insensitive to changes in temperature and showed minimal response to changes in wastewater quality (Bhatt 2016), indicating that it might be a suitable alternative for treatment of irrigation water of varying initial quality.

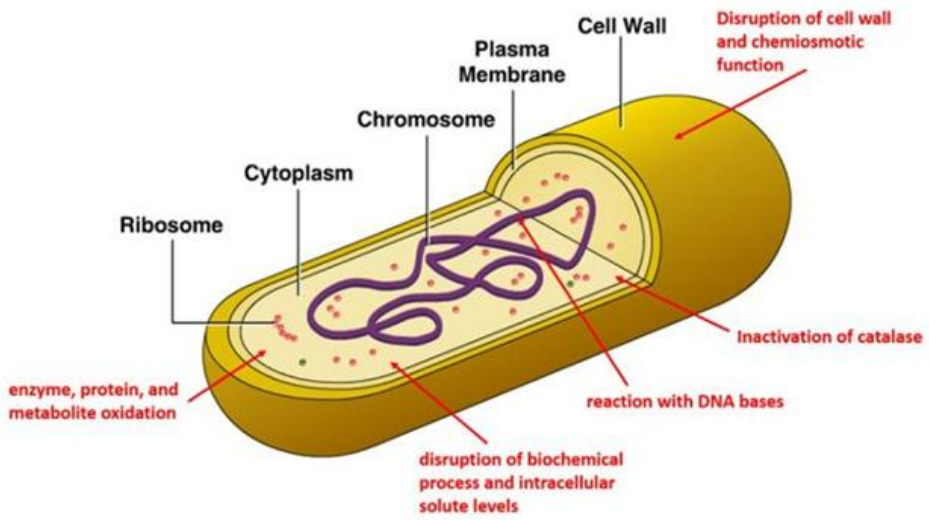

Fig. 8 *Mode of action of PAA. Reproduced from Peroxychem (2017).*

2.9 Electrolysed oxidizing (EO) water

EO water is formed from the electrolysis of water in the presence of a chlorine salt (generally NaCl or KCl) to form hypochlorite and reactive oxygen species (ozone, hydrogen peroxide) that are toxic to microorganisms. The following describes the various types of EO water as summarized in Rahman et al (2016):

Acidic electrolysed oxidizing water (AEW) – pH 2–3 (anolyte)

Slightly acidic electrolysed oxidizing water (SAEW) – pH 5–6.5

Neutral electrolysed oxidizing water (NEW) – pH 7–8 (produced from recombination of anolyte and catholyte after production)

Slightly alkaline electrolysed oxidising water (SAlEW) – pH 8–10

Alkaline electrolysed oxidising water (AlEW) – pH 10–13 (catholyte)

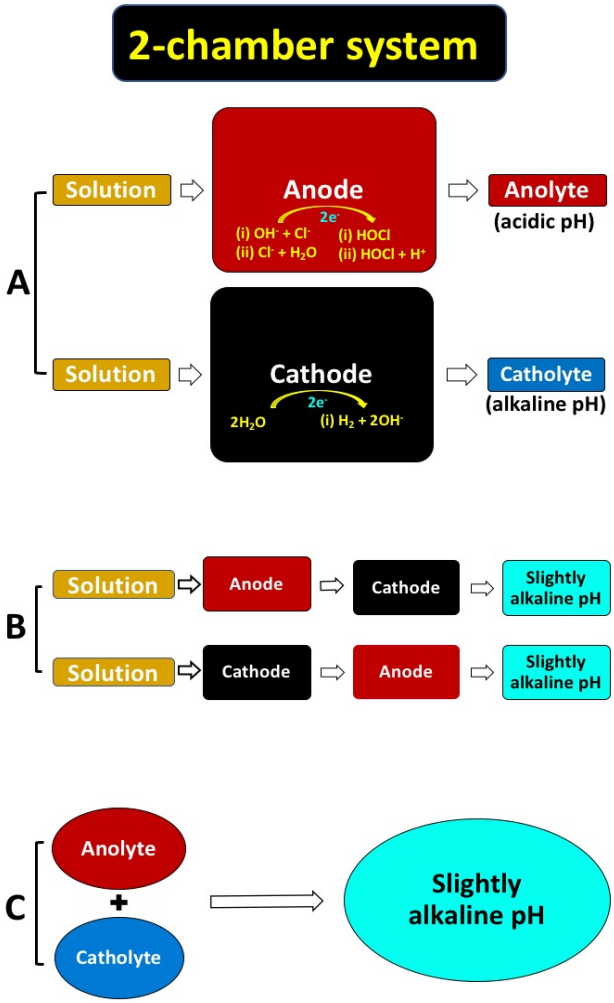

Fig. 9 *Schematic representation of a 2-chamber electrolysed oxidising activated solution system. (A), an acidic anolyte or an alkaline catholyte, if the solution of sodium chloride and water is passed through the anode or cathode, respectively; (B) a slightly alkaline anolyte, if the solution is initially passed through the anode or cathode, and then fed to the other compartment thereafter; or (C), a slightly alkaline anolyte, resulting from a combination of an acidic anolyte and an alkaline catholyte.*

Broadly, the various types of EO water can be generated by electrolysis of a solution of NaCl or KCl and water in either a 2-chamber system or a 4-chamber system. In the 2-chamber system, three outcomes are possible, depending on the system design: (i) if the solution of NaCl or KCl and water is passed through the anode or cathode, this would result in either an acidic anolyte or an alkaline catholyte (Fig.

9A); (ii), if the solution is initially passed through the anode or cathode, and subsequently fed to the other compartment, a slightly alkaline anolyte is produced in both scenarios (Fig. 9B); (iii) However, if an acidic anolyte and an alkaline catholyte are combined, this produces a slightly alkaline anolyte (Fig. 9C).

In a 4-chamber system, the saline solution is initially passed through two cathodic compartments, generating a catholyte which is then passed through two series of anodic compartments, resulting in a pH-neutral anolyte, NEW (Fig. 10).

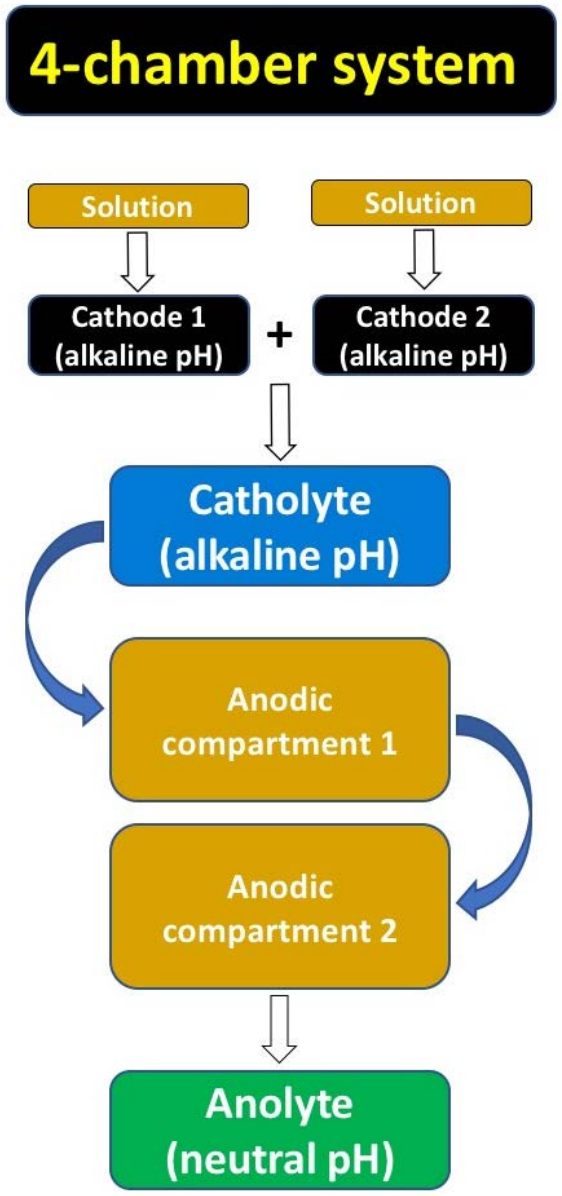

Fig. 10 *Schematic representation of the 4-chamber electrolysed oxidising activated solution system. A catholyte generated from two cathodic compartments is initially passed through an anodic compartment and then passed through a second anodic compartment, resulting in a pH-neutral anolyte.*

There is some debate as to the main active components in the sterilization activity of EO water – with pH, oxidation-reduction potential (ORP), and available chlorine concentration (ACC) considered the key variables (Rahman et al 2016). Similarly to chlorinated treatments, the optimal activity of hypochlorite generated in the electrolysis process will be obtained when the pH of electrolysed water is around 6. AEW can have some negative reactions, including corrosion of distribution systems and deleterious effects if applied direct to fresh produce (i.e., in spray irrigation), therefore this is unlikely to find wide application. AEW was reported to be effective against a range of STEC strains tested and was found to be more effective in eliminating STEC than sodium hypochlorite at the same available chlorine concentration (45 mg/L) (Jadeja et al 2013). EO waters in the range of slightly acidic/neutral/slightly alkaline have the most potential for widespread use in a range of industries.

Of the various types of EO water available, NEW is a fairly recent water sanitization and surface disinfection technology (Fig. 10). In the USA, NEW is now included among the allowed antimicrobial treatment of meat, poultry and egg products by the Department of Agriculture, Food Safety and Inspection Service (FSIS) as "Electrolytically generated hypochlorous acid" (FSIS Directive 7120.1 2017). In Europe, NEW is currently used in the healthcare industry to control *Legionella* in water supplies (Migliarina and Ferro 2014). In Australia trials with NEW have focused mostly on the food industry (Khazandi et al 2017)..

Another example of EO water is SafeWater® (EAU Technologies, USA), which claims to remove biofilms, kill bacteria, yeasts, molds, viruses, spores and other pathogenic microorganisms on crops of fruits and vegetables. The product is also claimed to reduce carbon footprint, wastewater generation and treatment requirements, is suitable for disinfection of all surfaces, and is approved for use by the US Department of Agriculture, US Environmental Protection Agency and the US Food and Drugs Administration. Given the ascribed characteristics of EO water, a rigorous investigation of the veracity of these claims, including comparative efficacy of these sanitizers with existing chlorine-based and other sanitizers, is warranted.

2.9.1 Use of EO water in agriculture

Several studies have described the activity of EO waters against suspensions of target human pathogens (*E. coli*, *Salmonella* spp. and *Listeria* spp.; Appendix: Table A2); the studies found substantial log reductions in viable microorganisms with EO water treatment across a range of conditions of exposure time, pH, temperature, available chlorine and ORP (Hassenberg et al 2017; Jones et al 2014; van Haute et al 2015). However, there are limited published applications of the use of EO water in treating irrigation water, and very few pre-harvest organic methods for sanitization of vegetable products have been described, although more information is available for disinfection of fruit crops. In one instance, Grech and Rijkenberg (1992) found that irrigation with AEW at 40–50 µg/mL active chlorine to the roots of citrus plants using micro-emitters was highly effective at killing water-borne root pathogens such as *Phytophthora* spp., *Fusarium* spp., algae, and 'skin-forming' bacteria.

Importantly, micro-irrigation did not result in chlorine-induced phytotoxicity in field-grown plants. Hirayama et al (2016) applied NEW in an overhead irrigation system to control fungal disease in strawberries and showed that exposure of plants to NEW via irrigation resulted in significant disease suppression. In another study, Bandte et al (2016) applied electrolytically produced potassium hypochlorite (KOCl) to control virus transmission in a recirculating tomato hydroponic system. They reported low levels of dosing and contact time (0.2 mg free chlorine/L, 60 min weekly or 0.5 mg free chlorine, 30 min/weekly) were required to control virus transmission in the recirculating system. Stevenson et al (2004) applied EO water to reduce *E. coli* O157:H7 contamination of water and compared the characteristics of EO waters produced using deionized water, tap water and surface irrigation water. In that study, EO water produced using irrigation water (with pH 8.24, organic carbon 8.9 ppm) had similar ORP to tap water but lower ORP than that of deionized water. However, the efficacy of irrigation water-produced EO water against *E. coli* was not directly tested in the study. EO waters produced using deionized water were extremely effective against *E. coli* O157:H7 at a range of concentrations tested. Spraying of cucumbers with AEW was also shown to be effective at arresting fungal growth and reducing the incidence of powdery mildew on the crops (Schoerner and Yamaki 1999). Tamaki et al (2001) demonstrated the suppressive effects of AEW and AlEW against rice blast disease caused by the fungal pathogen, *Magnaporthe grisea*, when sprayed on rice plants before the pathogen penetrated into plant tissues. In addition, the use of AEW as a foliar spray on a variety of bedding plants grown under greenhouse conditions demonstrated very little to no phytotoxicity to the plants while exhibiting rapid elimination of pathogenic fungi such as powdery mildews and gray molds (Buck et al 2003). Similarly, Bonde et al (1999) observed that treatment of wheat seeds with AEW stimulated germination and prevented contamination by fungi including *Aspergillus*, *Cladosporium*, and *Penicillium* spp, together indicating a potential for AEW as an alternative to using fungicides.

3. Factors affecting choice of treatment method

Treatment of irrigation water needs to be assessed on a case-by-case basis depending on several factors, including the quality of the water source to be treated (in terms of COD, organic matter content, microbial load, pH, temperature, turbidity, etc.), the final use of the water (i.e., for irrigation of MPF crops or other irrigation applications with less stringent requirements), the volume of water required, the cost of the proposed treatment (including up-front vs. ongoing costs), the safety of the process, and expertise required to run and maintain the treatment facilities (Fig. 3). Compatibility with current water distribution/treatment systems is also an important factor to consider (Raudales et al 2014).

Variation in water quality is a significant factor in the efficacy of different treatments. UV treatment is particularly vulnerable to turbidity, although in a study by Jones et al (2014) different surface irrigation water sources with turbidities ranging up to 20 nephelometric turbidity units (NTU) showed 6–10 log reductions in pathogens using a UV treatment unit specifically designed for use with unfiltered apple cider. Organic matter content can substantially affect the efficacy of chlorine-based

treatments, with high organic matter content increasing the chemical demand and reducing the efficacy of chlorine-based treatments against pathogens (Hassenberg et al 2017). Hypochlorite treatment is optimal at pH 6, whereas chlorine dioxide has a much wider working range (3–9) and PAA is also effective over a wide pH range (5.5–8.2; van Haute et al 2015). Temperature can influence the efficacy of water treatment by affecting the decomposition rate of active ingredients (i.e., ozone) and their solubility; high temperatures might lead to high microbial loads from increased growth rates and increase the fouling rate of membranes or UV treatment units.

3.1 Costs

The cost of the initial water source itself is a factor in the economics of water treatment (i.e., municipal water sources can be quite expensive relative to lower quality water sources, but require little or no further treatment for unrestricted use). The costs of implementing water treatment technologies can be divided into direct costs, such as equipment costs, chemical costs, labour and maintenance costs, and indirect costs, such as those related to crop losses from plant disease, product recalls of contaminated produce and maintenance of clogged irrigation equipment (Raudales et al 2017). The expertise required to optimise and maintain water treatment technologies and the up-front and ongoing costs are very important in the decision making process for adoption of a particular treatment technology. The perception of actual or potential risk is also important as the costs of non-adoption (product recalls, crop loss, labour costs, ability to use lower quality water) need to be assessed to identify the full benefits of a particular treatment (Raudales et al 2017).

3.2 Monitoring and persistence of disinfection

The monitoring of reclaimed water quality immediately after treatment does not provide a true representation of quality at the point of use (Jjemba et al 2014). Water quality deterioration during storage and distribution is a major challenge for the industry, with residence time a major factor in water quality deterioration (Ajibode et al 2013). Deterioration is mainly associated with decline in the disinfectant residual, where there is one. Growth or regrowth can occur from viable but non-culturable (VBNC) microorganisms, treatment-resistant microorganisms (i.e., chlorine-resistant microorganisms (Ajibode et al 2013), dark repair or photoreactivation after UV treatment (Guo et al 2009), or from microorganisms located in biofilms on the surface of distribution network pipes and storage facilities (Pachepsky et al 2012; Shelton et al 2013). The choice and location of water treatment therefore has potential impacts on the lifetime of treatment technologies and regrowth/reinoculation of pathogens in irrigation water from biofilms in the distribution system. Treatment at the point of use might be described as a “booster” disinfection station (Fig. 11), to reduce the total amount of chemicals required and the requirement for residuals to be maintained throughout the distribution system (Jjemba et al 2014). As such, the booster station will allow for flexibility through the use of multiple disinfection

doses to physically separate antimicrobial efficiency requirements from the need to maintain disinfectant residual in the distribution system unique to each site (Tryby et al 1999).

Water quality should be monitored at the point of use (i.e., at the field) because of the potential degradation of water quality through transport in the distribution system (Pachepsky et al 2012). For instance, although wastewater might be treated to very high quality at the WWTP, there is high probability that water quality will be impacted by the time it is transported and distributed at the field/point of use (Garner et al 2016; Fig. 11). Regulations for water reuse are generally tested and applied to the point of discharge from the WWTP and there is no requirement for monitoring throughout the distribution system (Weinrich et al 2010).

There is limited or no information on methods to achieve biostability of recycled water throughout distribution systems. Biologically available organic carbon (assimilable organic carbon, AOC or biodegradable dissolved organic carbon, BDOC) is related to regrowth of bacteria in distribution systems or storage reservoirs and some disinfection processes (i.e., chlorination) can increase AOC, leading to reduced efficacy of disinfectant residuals (Weinrich et al 2010). Specific and cost-effective methods for controlling regrowth or treatment at the point of use are required to ensure the safety of the applied water.

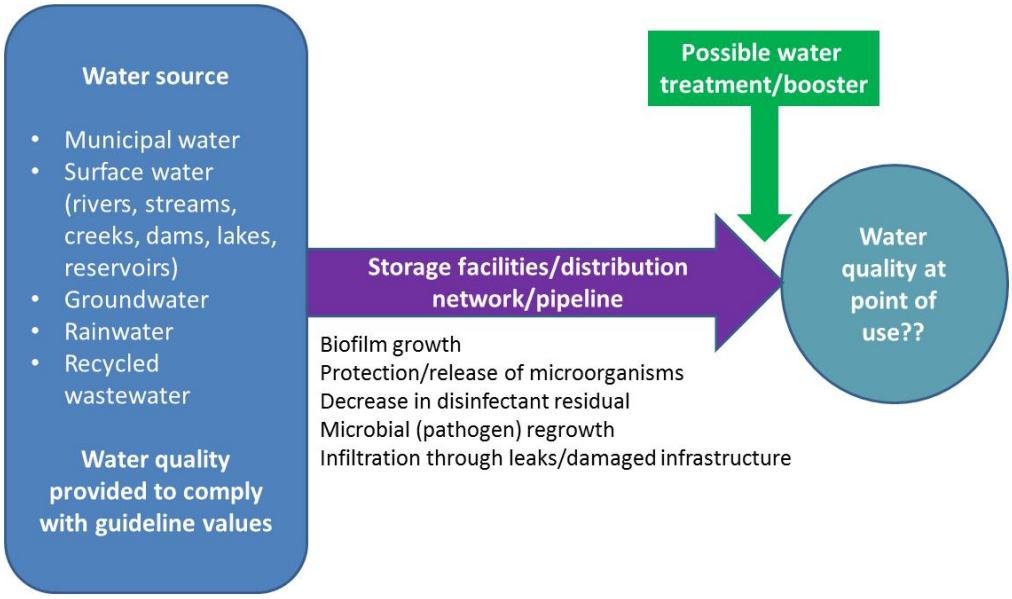

Fig. 11 *Factors affecting water quality at the point of use through water quality degradation throughout storage facilities/distribution network/pipelines.*

4. Effects of pre-treated irrigation waters on soil and food microbial communities

Soil-borne microbes constitute a major proportion of the organisms identified on fruit and vegetable products, and contribute significantly to the maintenance of an ecological equilibrium in most agricultural systems. The vast majority of the resident microbes (the "microbiome") on vegetables and fruits are not responsible for spoilage but rather act as a "natural biological barrier" against spoilage organisms, which are often a smaller subset of the entire soil microbial population (Andrews and Harris 2000; Janisiewicz and Korsten 2002; Barth et al 2009). Indeed, it has been shown that an inverse relationship exists between soil microbial diversity and the survival of an invading pathogen (van Elsas et al 2012). In general, the main factors that substantially contribute to changes in the microbial ecology in soil, vegetables and fruits after treatment include irrigation water quality, soil type, harvest season, harvest techniques, pre- and post-harvest sanitation practices, nature and relative abundance of resident microflora in the rhizosphere, and treatment regimens (Barth et al 2009; Berg and Smalla 2009; Cluff et al 2014; Frenk et al 2014; Allende and Monaghan 2015; Becerra-Castro et al 2015; Zheng et al 2017; Fig. 2). Therefore, it is critically important that good agricultural practices are implemented before, during and after harvest to maintain good soil quality and promote a balanced and functioning microbial ecosystem. These practices are defined in the Codex General Principles on Food Hygiene (Codex Alimentarius Commission 2003) and aim at maximizing the quality of the crop harvested. Moreover, a thorough understanding of the unique microbial ecosystem in the soil and on the surface of each produce type pre- and post-harvest will be important for informing better treatment strategies to minimise microbial contamination.

A search through the literature reveals very few original manuscripts and/or reviews pertaining to changes in the microbial ecology of soil and foliar tissues after irrigation with treated irrigation water. In general, drinking water is considered the irrigation water source with the lowest risk; ground water is also less likely to have microbial contaminants than surface water, which has a number of potential contamination sources including livestock, sewage treatments wildlife and other watershed activities (Tyrrel et al 2006; Delbeke et al 2015; Uyttendaele et al 2015). Mañas et al (2009) reported significant increases in faecal streptococci, *Salmonella* spp, sulphite-reducing *Clostridium* spp as well as total and faecal coliform counts in lettuce irrigated with treated wastewater (using trickling filters), relative to control plants receiving drinking water (groundwater). These findings indicate potential deleterious effects of microbiologically impacted irrigation water on fresh produce. However, the use of tertiary water treatment regimes such as final disinfection using UV light, chlorination and/or ultrasound have been shown to effectively remove indicator microorganisms and pathogens to below limits of detection at the point of discharge (Pachepsky et al 2011; Villanueva et al 2015). Chevremont et al (2013) documented the changes in microbiological properties of soils irrigated with UV-LED treated wastewaters over a one-year period. When compared with watering with untreated wastewater, watering with the UV-LED treated wastewater resulted in decreased occurrence of faecal coliforms, and showed no deleterious effects on overall microbial diversity and function.

In Australia, there is no direct information on the microbial profiling of irrigation water or data on the effects of microbiologically impacted irrigation water on fresh produce. However, a report by Premier (2013) evaluating different post-harvest washing chemical treatments available to commercial vegetable growers in Australia concluded that (i) PAA-based sanitizers are more effective for treatment of post-harvest leafy vegetables than organic-based sanitizers, but are more expensive and result in lower shelf-life of the vegetables; (ii), emerging technologies such as EO water are safe, inexpensive and demonstrate superior efficacy over other sanitization methods, and resulted in increased shelf lives of the vegetable products. In a review by Cheng et al (2012), EO water was reported to be highly effective in reducing the levels of human pathogens such as *E. coli* 0157:H7, *L. monocytogenes*, and *Salmonella* Enteritidis (the main pathogens of concern in vegetable and fruit products). Given these observations, it is tempting to speculate that some post-harvest treatment methods could potentially be exploited for pre-harvest sanitation of fresh produce, and are likely to produce similar outcomes. Cost-effectiveness and relative efficacy would need to be determined.

While there are many publications and reports on cost savings, safety and bactericidal activity of EO water (e.g., Al-Haq et al 2005; Cheng et al 2012; Colangelo et al 2015), direct reports on the use of EO water in changing the dynamics of microbial populations are scant. A recent investigation by Thorn et al (2017) compared the efficacy of EO water fogging with that of no treatment or RO water fogging on fresh, post-harvest tomato, rocket, broccoli and cucumber produce artificially contaminated with *Pseudomonas syringae*, *E. coli* or *Penicillium expansum*. The results showed that EO water fogging significantly reduced total viable counts as well as specific pathogen and spoilage organism levels on

all the produce tested after 5 days of storage, when compared with no treatment and RO water fogging. Consistent with these findings, we have also recently observed significant reduction in total viable counts as well as significantly lower counts on fresh post-harvest spinach leaves contaminated with *E. coli* ATCC25922, *L. innocua 6a* (ATCC33090), and *S. Enteritidis* 11RX after treatment with pH-neutral EO water at either 45 ppm or 75 ppm free available chlorine, as compared with untreated leaves, for up to 10 days after treatment (Ogunniyi et al, manuscript in preparation). Results from those experiments also suggest a change in the dynamics of the microbial communities in the EO water treated groups when compared with untreated controls.

4.1 VBNC microbial populations

One important consideration in the evaluation of various water treatment technologies is the existence of certain microbial populations in a VBNC state, a survival strategy used by many Gram-positive and Gram-negative bacteria in response to adverse environmental conditions (Ramamurthy et al 2014). VBNC microbes have lipid-rich membranes, tend to be smaller, exhibit reduced metabolic activity, and display altered cellular changes including cell leakage, depletion of energy pools, as well as altered gene expression and DNA replication (Trevors et al 2012). Furthermore, VBNC organisms do not even grow upon plating on culture media that would normally support their growth *in vitro*, rendering them difficult to detect by conventional means. Interestingly, under favourable conditions (such as through expression of resuscitation-promoting factor), these organisms can be revived. For example, it has been shown that *L. monocytogenes* treated with distilled water entered into the VBNC state and became virulent after resuscitation using embryonated eggs (Cappelier et al 2007). Similarly, laboratory-induced VBNC *E. coli* O157:H7 cells produced Shiga-like toxins in a vero-cell microplate cytotoxicity assay, demonstrating a potential health hazard (Liu et al 2010). It has also been shown that the VBNC state can be induced in bacteria by many factors, including H_2O_2 (Arana 1992), antibiotic pressure (Pasquaroli et al 2013), chlorination (Oliver et al 2005), high/low temperature (Patrone et al 2013; Pawlowski et al 2011), UV-irradiation (Zhang et al 2015), peroxide-based disinfectants such as peracetic acids (Park et al 2014) and high-pressure CO_2 (Zhao et al 2013). The VBNC state in *S. epidermidis* contributes to the formation and persistence of biofilms, resulting in tolerance to multiple antimicrobials and immune evasion (Cerca et al 2011). Thus, it is critical to investigate whether the various water treatment regimens induce the VBNC state in a microbial community.

Taken together, there is a paucity of information on the direct consequence of irrigation with different water treatment technologies on soil and food microbial communities. Therefore, specific on-field experiments and advanced molecular and cellular techniques geared towards obtaining direct evidence for the effects of the various irrigation treatment regimens on the VBNC state as well as the dynamics of soil and microbial communities, particularly on high-risk vegetables, is warranted and paramount.

5. Conclusions and Recommendations

- In Australia, there is no direct information on the microbial profiling of irrigation water or data on the effects of microbiologically impacted irrigation water on the quality of fresh produce. Therefore effective post-harvest treatment methods could potentially be exploited for pre-harvest sanitation of fresh produce.
- EO water technologies might hold numerous advantages over other sanitation methods, and their commercialisation is based on a number of claims in terms of overall safety, cost and environmental impact. Therefore, a rigorous investigation of the veracity of these claims, including comparative efficacy of these sanitizers with existing chlorine-based and other sanitizers, is warranted.
- Direct evidence for the effects of the various irrigation treatment regimens on the VBNC state as well as the dynamics of soil and microbial communities, particularly on high-risk vegetables, is needed.

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Table 1: Advantages and disadvantages of different water treatment processes for on-farm irrigation water disinfection

Water treatment process	Mode of action	Advantages	Disadvantages
Membrane filtration	Size exclusion – pore sizes of 10 nm – 1 µm (microfiltration), 3–10 nm (ultrafiltration), 2–5 nm (nanofiltration)	Effective in reducing microbiological load, no by-products, no chemicals required	Expensive, requires expertise to run, clogging leads to reduced effectiveness, removal of microorganisms/viruses limited by membrane pore size, slow, retentate treatment required prior to disposal
Slow bed sand filtration	Size exclusion and biological activity of *Schmutzdecke*	No chemicals required, easily built, low cost	Slow, not suitable for large volumes required for irrigation, high water quality required to avoid clogging
Ultraviolet irradiation	DNA damage	Short contact time, very effective, no by-products (odour or toxic compounds)	Expensive up front but low ongoing maintenance, efficacy reduced by low quality water (turbid, high organic content), no residual, some organisms resistant (dark repair)
Ozone	Oxidizing	Very effective with short contact time, no residual, wide spectrum of activity, 'generally regarded as safe' for use in the food industry, can be used in organic production systems, low or no by-products	High instability, lack of residual, specialist expertise required, potential exposure of workers, high cost
Peracetic acid	Oxidizing	Low or no by-products, broad pH and temperature range of activity, low cost of implementation (can be retrofitted to other treatment systems)	Organoleptic effects on produce, high cost of chemical, pre-treatment to remove organic matter required, corrosive
Hypochlorite	Oxidation/chlorination	Effective in reducing a range of pathogens, widely used, cost-effective, residual prevents re-colonisation	By-products formation (THMs, HAAs), not effective against Cryptosporidium, residual, effects on soil, effectiveness highly related to pH (7.4–7.6 required for most effective treatment), turbidity and organic content, less effective on G+ than G– microorganisms
Chlorine dioxide	Oxidizing	Safe stabilised solutions available, wide pH range, less affected by organic matter than hypochlorite	Inorganic by-products (chlorate, chlorite), low THMs/HAAs
Electrolyzed oxidising water	Oxidizing	Safe, no transport of chemicals, on-site production, wide spectrum of activity, low ongoing costs/maintenance, short treatment time	High initial investment, limited residual activity, efficacy influenced by organic matter content, water hardness and other pollutants

THMs: trihalomethanes; HAAs: haloacetic acids

Appendix:

Table A1: Characteristics and pros/cons of different water treatment processes for irrigation water

Process	Form	Mode of action	Specificity	Log removals	Safety	Cost (upfront /ongoing)	DBP formation/toxicity	Active range/inhibitors/ problems?	Refs
Sand filtration	Physical	Filtration and biological activity	Non-specific, depends on sand grain size and activity of biological layer	2–4 log removal of bacteria, cysts, viruses, oocysts, algae and parasite and nematode eggs	No problems	Low/low	No	Can clog if turbidity is high	Graham and Collins 2014
Membrane filtration	Physical	Filtration	Depends on filter size range, hydrophobicity; viruses might not be completely removed	5-6 log removal of coliform bacteria	No problems	High / medium	No	Membrane fouling, long treatment time (up to 20 h)	Hai et al 2014
Ultra violet irradiation	Irradiation	DNA damage	Non-specific, some organisms can show resistance	4–8 log reduction in vegetative bacteria, 3–6 log inactivation of viruses	No problems	High/low	No	Turbidity	Stanfield et al 2003
Ozone	Gas	Oxidising	Gram + more resistant to ozone than Gram −, spores more resistant than vegetative cells	3–4 log removal of faecal coliforms	Must be monitored	High/high?	No/limited	No residual; 10–15 mg O_3 L^{-1}	Martinez et al 2011
Peracetic acid	Chemical	Oxidising	Efficiency in the order of: bacteria > viruses > spores > cysts	Up to 3.5 log removal of *E. coli*	No problems	Med/Med	No/limited	Wide temperature range, unaffected by pH in the range 5–7.5, decreased efficacy at alkaline pH	Bhatt 2016; Sigge et al 2016
Hypochlorite	Chemical	Oxidising	Bacteria > viruses Cysts resistant	Up to 4 log	Safest form of	Med/Low	Yes: trihalomethanes	Optimal pH around 7–7.5;	Allende and Monaghan

			Gram + more resistant than Gram −	removal of bacteria	chlorine		(THMs), haloacetic acids (HAAs)	organic matter interference	2015; Sigge et al 2016
Chlorine dioxide	Chemical/Gas	Oxidising	Highly effective against parasites	Up to 4 log removal of bacteria	If supplied in gas form, must be monitored ; stability of precursors , transport	High/Med	Yes: THMs, HAAs, lower levels than hypochlorite; inorganic byproducts (chlorite, chlorate)	More effective at alkaline pH, wide pH range (4–10); less affected by organic matter than hypochlorite	Stanfield 2003; Raudales et al 2014; Scarlett et al 2016
Electrolyzed oxidising water	Chemical	Oxidising	Similar to hypochlorite	Up to 8 log removal of bacteria	No problems	Med-High/low	Similar to hypochlorite?	Organic matter interference	Rahman et al 2016

Table A2: Efficacy of electrolysed oxidising water treatments on specific pathogens in suspension

Pathogen	Matrix/Active agent	Dose/contact time	Log reduction	Reference
Escherichia coli	PBS/ SAEW (20:1)	5–10 min; 60 ppm ACC; ORP +910 mV; pH 6.4; volume ratio 20:1	~8 log CFU/mL	Ye et al 2017
E. coli	TSB/ NEW (0.1/9.9 mL)	10 min; 20–100 ppm total residual chlorine; ORP +800-900 mV; pH 6.3–6.5; 25°C	6.1–6.7 log CFU/mL	Guentzel et al 2008
E. coli	0.85% NaCl/ LcEW (1/9 mL)	1 min; 5–10 mg/L ACC; ORP +660–700 mV; pH 6.8–7.4	4.9–5.3 log CFU/mL	Rahman et al 2012
E. coli O157:H7	[a]Culture/sterile water/NEW (1/1/8 mL)	5 min; 89 mg/L ACC; pH 7.99–8.19; ORP +745–771 mV; 23°C	>6 log CFU/mL	Deza et al 2003
E. coli (range of strains)	NECAW	30 s; 100 ppm FAC; ORP +864 mV; pH 7.0	>5 log CFU/mL	Yang et al 2013
Salmonella (range of strains)	NECAW	30 s; 100 ppm FAC; ORP +864; pH 7.0	>5 log CFU/mL	Yang et al 2013
Salmonella enteritidis	Culture/sterile water/NEW (1/1/8 mL)	5 min; 89 mg/L ACC; pH 7.99–8.19; ORP +745–771 mV; 23°C	>6 log CFU/mL	Deza et al 2003
Listeria monocytogenes	Culture/sterile water/NEW (1/1/8 mL)	5 min; 89 mg/L ACC; pH 7.99–8.19; ORP +745–771 mV; 23°C	>6 log CFU/mL	Deza et al 2003
Listeria monocytogenes (range of strains)	PW/NECAW (1/99 mL)	30 s; 50–100 ppm FAC; ORP +824–864; pH 7.0	>5 log CFU/mL	Yang et al 2013
Listeria innocua	Cells resuspended in NEW	10 min; 150 ppm ACC; ORP +840 mV; pH 6.9; 23°C	2.7 log CFU/mL	Feliciano et al 2012
Listeria innocua	Cells resuspended in AEW	10 min; 150 ppm ACC; ORP +1100 mV; pH 2.7; 23°C	4.7 log CFU/mL	Feliciano et al 2012
Listeria monocytogenes	TSB/ NEW (0.1/9.9 mL)	10 min; 20–100 ppm total residual chlorine; ORP +800–900 mV; pH 6.3–6.5; 25°C	6.1–6.7 log CFU/mL	Guentzel et al 2008
Listeria monocytogenes	0.85% NaCl/ LcEW (1/9 mL)	1 min; 5–10 mg/L ACC; ORP +660–700 mV; pH 6.8–7.4	5.2–5.6 log CFU/mL	Rahman et al 2012
Listeria monocytogenes	0.85% NaCl/NEW (1/9 mL)	30 s; 20 ppm total chlorine concentration; ORP +1100 mV; pH 7.0; 30°C	≥5 log CFU/mL	Arevalos-Sanchez et al 2012

NEW: neutral electrolysed water; AEW: acidic electrolysed water; SAEW: slightly acidic electrolysed water; CFU: colony forming unit; ACC: available chlorine concentrations; NECAW: neutral electrochemically activated water; LcEW: low concentration electrolysed water; ORP: oxidation-reduction potential; PBS: phosphate buffered saline; TSB: trypticase soy broth; PW: peptone water

[a] details of culture medium not provided.

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Disinfection options for irrigation water: Reducing the risk of fresh produce contamination with human pathogens

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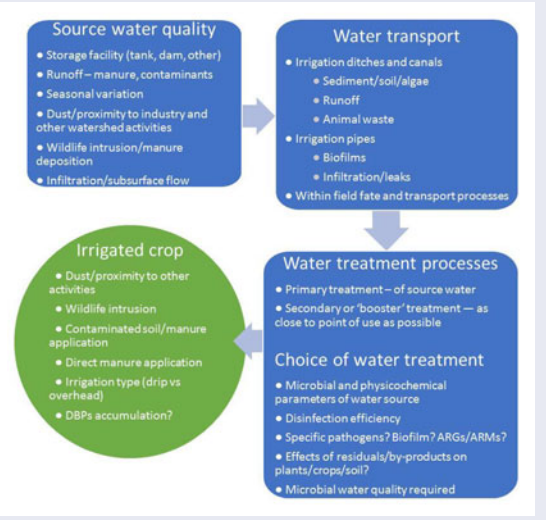
ABSTRACT

The growing health and economic burden posed by foodborne pathogens has stimulated global interest in the development of safe, affordable, effective and environmentally-sustainable irrigation water treatment technologies. This review critically compares the potential of existing and emerging methods for disinfection of irrigation water to reduce pathogenic microbial loads on high-risk vegetables and minimally processed fresh produce. We explore electrochemical disinfection and electrolyzed oxidizing water as alternatives to traditional chlorination, and identify hydrodynamic cavitation as an emerging disinfection strategy worthy of further investigation in this context. In addition, we assess the state of the knowledge regarding the impact of current water sanitation strategies on the ecological dynamics of plant and soil microbes and the potential induction of viable but nonculturable cells. Increased research in these areas could translate into substantial improvement in the overall quality and value of fresh produce, while maintaining environmentally-sustainable irrigation water usage.



KEYWORDS Foodborne pathogens; irrigation water disinfection; viable-but-non-culturable

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1. Introduction

Crop agro-ecosystems are at the heart of the food–energy–water nexus, accounting for ~70% of total freshwater withdrawal in the world (Food and Agriculture Organization of the United Nations (FAO), 2015). As an example, irrigated agriculture accounted for 58% of all water use in Australia in 2015–16 (ABS, 2017), and projected agricultural water demand is set to increase by 50% by 2050 (AWA, 2017). The increasing demand for water to support food production is a global trend that is significantly exacerbating pressure on water resources. Thus, alternative irrigation water sources are increasingly sought, and the quality and safety of those supplies must be ensured to safeguard future water and food safety.

1.1. Microbiological contamination of irrigation water and pathogen transfer to food

Irrigation water can be obtained from a range of water sources and the potential for microbiological contamination needs to be carefully considered. Table 1 lists the range of available water sources for irrigation and their relative risk of microbial contamination. In the case of irrigated food crops, particularly minimally processed foods such as lettuce, spinach, parsley and other leafy greens, opportunistic and human pathogens are of particular concern. Despite increasing efforts to improve sanitation, outbreaks linked to microbial contamination of minimally processed foods continue to occur around the world. In some instances, these outbreaks have been associated with pathogens that are uncommon in these foods, for example, *Salmonella* spp. and *Listeria monocytogenes* in cantaloupes, prepacked lettuce, and baby spinach leaves (FSANZ, 2016; Zhu, Gooneratne, & Hussain, 2017). Pre-harvest water supplies (i.e., irrigation water) and postharvest water (i.e., washing water) have previously been identified as the main sources of contamination in produce associated with illness (FSANZ, 2011), and the growing use of whole genome sequencing in outbreak investigations is providing increasing evidence for the role of contaminated irrigation water in pathogen outbreaks (Hoelzer, Switt, Wiedmann, & Boor, 2018). It is clear that contaminated irrigation water can transfer pathogens to edible produce (Jongman & Korsten, 2017; Markland, Ingram, Kniel, & Sharma, 2017) and leafy greens are especially vulnerable to contamination with opportunistic human pathogens because they have large surface areas, are often grown in close proximity to soil, are irrigated intensively, and are mostly consumed raw (De Keuckelaere et al., 2015).

Given the above, it is evident that in some settings effective sanitation of irrigation water is paramount in ensuring the safety of edible produce. Guideline values for pathogens in irrigation water have historically been

Table 1. Irrigation water sources and their potential for microbial contamination.

Water source	Potential for microbial contamination	References
Municipal/potable water	Low risk (treated to potable use standards), but cost and volumes required might be prohibitive	Uyttendaele et al., 2015
Surface water (incl. rivers, streams, creeks, lakes, dams and reservoirs)	High risk, with many potential sources of contamination, incl. wildlife or stock intrusion and fecal deposition, sewage or septic discharges and industrial effluents; high turbidity from suspended solids	Jones, Worobo, & Smart, 2014; Steele & Odumeru, 2004
Groundwater	Generally considered low risk, but overextraction of groundwater and contamination of shallow aquifers contributes to higher potential risk	Bradford & Harvey, 2017; Van Haute et al., 2015
Harvested rainwater	High risk of contamination by animal feces and organic debris; volumes required might be prohibitive except for small-scale applications	Dobrowsky, De Kwaadsteniet, Cloete, & Khan, 2014
Recycled wastewater	High initial microbial content but generally low risk with sufficient and appropriate treatment	Allende & Monaghan, 2015
Untreated wastewater/indirect wastewater reuse	High risk; prevalent because of insufficient wastewater treatment infrastructure in expanding urban areas	Thebo, Drechsel, Lambin, & Nelson, 2017

framed around fecal contamination and associated indicators (i.e., fecal coliforms), with the WHO guideline value of ≤1,000 colony forming units of fecal coliforms per 100 ml in wastewater for irrigation (World Health Organization (WHO), 1989). Other guidelines might be more specific and restrictive, specifying *E. coli* rather than coliforms (i.e., <1 *E. coli* per 100 ml of recycled wastewater; E.P.H.C., 2006) or targeting other pathogens (absence of *Salmonella* required in 100% of samples in recent EU legislation; European Commission, 2019). The impetus or trigger for irrigation water treatment should be derived from relevant local guideline values and microbial risk assessment of the potential pathogen exposure from contaminated crops (Uyttendaele et al., 2015).

The intention of this review is to critically assess the literature relating to existing methods for disinfection of irrigation water for food crops. The information presented is mainly focused on bacterial pathogens, whilst acknowledging that there are substantial disease burdens associated with other pathogens such as viruses, protozoa and helminths (Ramírez-Castillo et al., 2015). Where there is limited information on the application of treatments specifically to irrigation water, we have drawn on literature assessing the application of sanitation technologies in other scenarios and their potential for adoption for irrigation water treatment.

1.2. Human health effects of contaminated fresh produce and mechanisms of pathogen contamination

There are substantial human health effects of contaminated fresh produce—for instance, between 2004 and 2013, over one third of foodborne illnesses in the USA were from the consumption of contaminated fresh produce (Fischer, Bourne, & Plunkett, 2015). Considering that the World Health Organization (WHO) reported a global burden of 600 million cases of foodborne illness in 2010 (420,000 resulting in death), the importance of water sanitation during the pre- and postproduction of fresh produce should not be ignored (World Health Organization (WHO), 2015b).

Pathogen survival on plant surfaces has been clearly demonstrated, especially in biofilms, as has the internalization of pathogens into plant tissues – i.e., endophytes (Berg, Eberl, & Hartmann, 2005; Berg et al., 2013; Hardoim et al., 2015; Lim, Lee, & Heu, 2014). In fact, many opportunistic human pathogens colonizing fresh produce have an endophytic lifestyle, using vegetables as an alternative host to survive in the environment and as a vehicle to colonize human and animal hosts once ingested (Mendes, Garbeva, & Raaijmakers, 2013). Critically, the endophytic interaction leads to difficulties for postharvest decontamination of fresh produce (Berger et al., 2010). Therefore, while treatment of irrigation water might be

effective in reducing the incidence of pathogen contamination through direct transfer of pathogens from irrigation water to plant surfaces and soil, changes in agricultural management practices might also be required to reduce the potential for endophytic pathogen colonization from contaminated soil and/or manure-based fertilizers.

In addition to the general risks to human health associated with pathogen contamination in food, the heightened risks posed by antimicrobial resistant microorganisms and antimicrobial resistance genes, particularly when associated with pathogenic microorganisms, is also of key relevance (Thanner, Drissner, & Walsh, 2016). Antimicrobial resistance is a major concern worldwide and is recognized by the WHO as a "global health security emergency", prompting the World Health Assembly to develop a Global Action Plan on antimicrobial resistance (World Health Organization (WHO), 2015a). A number of areas specifically highlighted as antimicrobial resistance research needs have been documented and many of them are directly relevant to food irrigation water supply (Wuijts et al., 2017), e.g., the identification of treatment technologies that can remove antibiotics and other antimicrobial agents, their metabolites, antimicrobial resistant microorganisms and antimicrobial resistance genes in water.

1.3. Strategies to reduce contamination of fresh produce

To reduce the potential for pathogen contamination of fresh produce, selection of an appropriate water source and/or pretreatment of irrigation water is critical (De Keuckelaere et al., 2015). Irrigation practices and distribution networks must be maintained to the highest possible standards to ensure that the potential for contamination is minimized. As with drinking water treatment, a multiple barrier approach is recommended to ensure that irrigation water quality remains high even in the event of failure or suboptimal performance of individual treatment modules (NHMRC & NRMMC, 2011). On-site treatment of irrigation water could represent an important component of a multiple barrier approach, especially in the context of irrigation with recycled water.

2. Treatment technologies for irrigation water

Water treatment for potable use and wastewater treatment for reuse or discharge draw on a range of different treatment technologies, many of which are potentially applicable to irrigation water treatment. A multitude of factors can affect the choice of irrigation water treatment technology (Figure 1). Selection criteria for treatment technologies can generally be broken down into three categories—technological, managerial, and sustainability

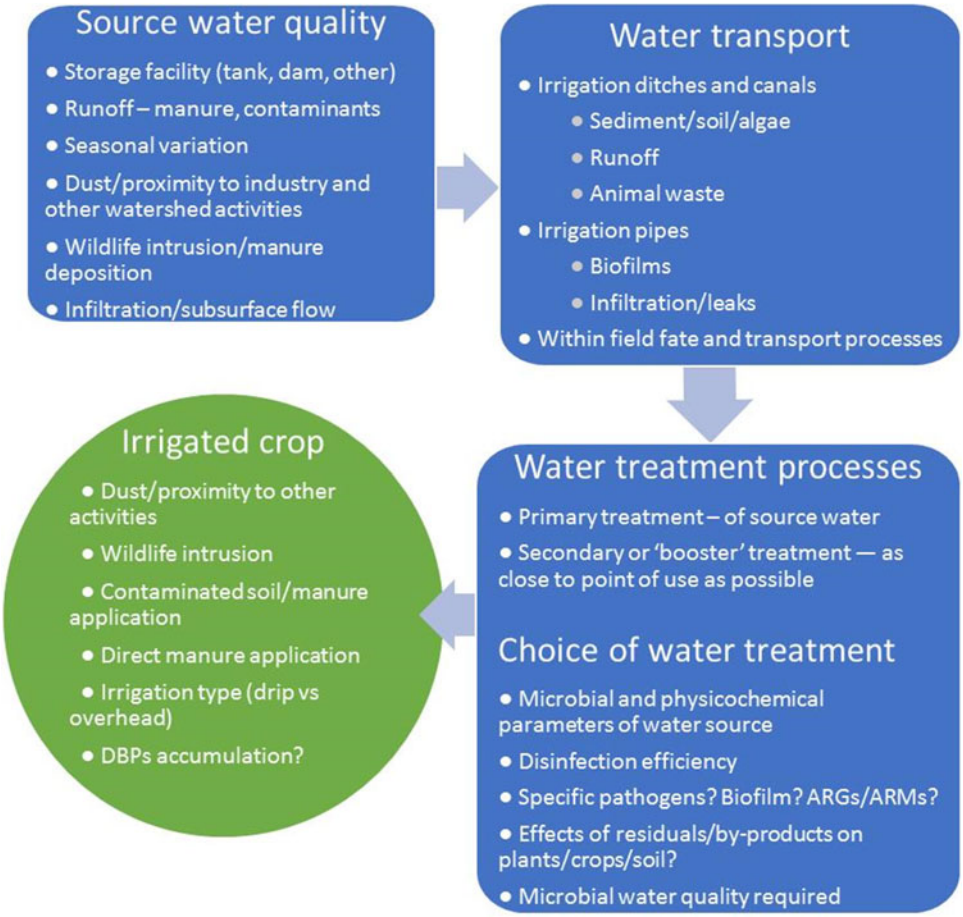

Figure 1. Factors affecting irrigation water quality and selection of water treatment processes to improve the microbial safety of fresh produce. ARGs: antimicrobial resistance genes; ARMs: antimicrobial resistant microorganisms; DBPs: disinfection byproducts.

related (Van Haute, Sampers, Jacxsens, & Uyttendaele, 2015). Technological criteria include the quality of the water source (e.g., microbiological load, temperature, pH, turbidity, suspended solids, organic matter content), distribution system characteristics, required water quality (in terms of physical and microbiological parameters), and water treatment parameters (i.e., treatment time and dose). Managerial criteria include the upfront and operational costs, complexity of operation, monitoring, and safety issues (in terms of chemical handling, storage, production of disinfection by-products (DBPs) and DBP accumulation in plants). Sustainability criteria cover maintenance, monitoring, environmental considerations and associated costs.

Generally, treatment approaches can be separated into clarification and disinfection processes. Clarification processes can be classified as follows: physical/mechanical methods, like screening, slow sand filters and

membrane filtration treatment; biological methods, such as biofilters; and chemical methods, such as coagulation and flocculation. Disinfection processes can involve the application of chemicals, such as chlorine, ozone (O_3), peroxyacetic acid (PAA), or hydrogen peroxide (H_2O_2), or might be based on non-chemical disinfection methods like ultraviolet (UV) irradiation.

Traditional treatment technologies and potential but largely untested treatment technologies for irrigation water are outlined below and the advantages and disadvantages of each process are summarized in Tables 2 and 3. As the scientific literature about on-site disinfection of irrigation water is rather limited and generally targeted toward plant pathogens rather than human pathogens (Raudales, Parke, Guy, & Fisher, 2014), this review also draws on parallel literature and examples from other applications such as potable water and wastewater treatment when necessary.

2.1. Traditional water treatment technologies

The advantages and disadvantages of traditional water treatment technologies are summarized in Table 2 and there have been several recent reviews covering many of these technologies in detail (Chahal et al., 2016; Hai, Riley, Shawkat, Magram, & Yamamoto, 2014; Hoslett et al., 2018; Jhaveri & Murthy, 2016; Kitis, 2004; Majsztrik et al., 2017; Martínez, Pérez-Parra, & Suay, 2011; Raudales et al., 2014; Scarlett et al., 2016; Yang, Li, Huang, Yang, & Li, 2016). Chlorination and UV irradiation are widely applied, mostly because of their low relative cost and convenient application.

Chlorination can be applied in gaseous form (Cl_2) or as hypochlorite (OCl^-) in either liquid or tablet form; it is well characterized, economical and effective against a broad range of pathogens. Optimum treatment conditions occur at pH 6, where the active form of undissociated hypochlorous acid is most prevalent. Hypochlorite treatment is relatively easy to implement for irrigation systems and has been widely applied in large-scale irrigation water treatment (Allende & Monaghan, 2015; Gil et al., 2015; Suslow, 2010). The disadvantages of chlorine treatment are mostly associated with the formation of DBPs, whose formation could be greater in irrigation water with high organic matter content, which would also have a high chlorine demand.

UV treatment efficacy can be substantially affected by water quality, turbidity and flow rate. Turbidity can reduce the penetration of UV irradiation, thus prefiltration or the use of thin films is required. Because of the lack of residual, there is significant potential for regrowth of pathogens after UV treatment, via photoreactivation mechanisms. UV is certified for

Table 2. Traditional water treatment technologies.

Treatment type	Mechanism	Target organism(s)/contaminants	Advantages	Disadvantages
Physical/Mechanical treatment technologies				
Coagulation/flocculation	Inorganic metal coagulants (i.e., aluminum sulfate or ferric chloride) Polymeric coagulants Organic polyelectrolytes Composite inorganic–organic coagulants Natural compounds (i.e., chitosan)	Turbidity/suspended solids Organic matter Phosphorus Cryptosporidium Giardia Viruses Bacteria	Targets larger pathogens that are not targeted by or susceptible to many other disinfection processes Nutrient removal reduces nutrient source for pathogen growth/survival	Must be optimized for specific water source in terms of pH, dosage, temperature, ionic strength and treatment and settling times Insufficient log removal of pathogens for unrestricted irrigation Substantial expertise required Generates sludge that must be disposed of appropriately No residual
Slow bed sand filtration	Pore size and depth of sand bed (straining and adsorption) Biofilm (or *Schmutzdecke*) (predation, starvation, lysis, reactive oxygen species)	Turbidity/suspended solids Bacteria Fungi (spores)	Low cost, low technology approach to provide substantial improvement in water quality Limited expertise required to implement and maintain	Large footprint Clogging requires regular maintenance and filter downtime Insufficient log removal of pathogens for unrestricted irrigation May not provide sufficient throughput for large scale irrigation No residual
Membrane filtration	Membrane pore size Microfiltration (MF: 0.1–1.0 μm) Ultrafiltration (UF: 5–100 nm) Nanofiltration (NF: 1–10 nm) Reverse osmosis (RO: ~0.1 nm) Pressure	Particles (MF/UF) Dissolved contaminants (NF/RO) Protozoa (MF) Bacteria (MF/UF) Viruses (UF/NF/RO) ARGs? (NF/RO?)	Removal of all contaminants possible Potential for contaminant-specific applications	High expertise required to run and maintain Fouling/clogging requires backwashing, regular maintenance and membrane replacement Membrane failure can be catastrophic and hard to detect Costly to install and maintain No residual
Ultraviolet (UV) irradiation	Broad spectrum (i.e., UV-C 200–280 nm) or specific wavelength (i.e., 254 nm) DNA/RNA adsorption, production of thymine dimers, inhibition of replication, protein damage	Bacteria Viruses Protozoa Fungi	Widely used in drinking and wastewater treatment Effective against a wide range of pathogens Easy to install and maintain No DBPs UV-LED technology should lead to treatment innovation Can be combined with advanced oxidation processes	No residual Potential for pathogen regrowth, limiting opportunity to store treated water Effectiveness limited in turbid water

Disinfection processes				
Ozone (O_3)	Ozone (O_3) Free radicals	Bacteria Viruses Protozoa?	Generally regarded as safe for food industry use High oxidizing potential Decomposes upon exposure to oxygen	Can generate DBPs in reaction with organic matter (non-halogenated organic products, bromate) High cost of installation Residual O_3 must be removed from excess water and disposed of safely Potential phytotoxicity
Peroxyacetic acid (PAA) ($C_2H_4O_3$)	Acetic acid (CH_3COOH) Hydrogen peroxide (H_2O_2)	Bacteria Biofilms Fungi Spores Viruses Protozoan cysts	Easy to use and common for post-harvest treatment Broad-spectrum of activity and effective at low concentrations Relatively insensitive to organic loading, total suspended solids, ammonia, nitrite and phosphates No quenching (dechlorination) requirement Low formation of DBPs such as THMs and HAAs relative to chlorination Small dependence on pH; effective under a wide temperature range Strong oxidizing properties; requires short contact time	Potential microbial regrowth from higher organic content in effluent (acetic acid in PAA formulation and as breakdown product) High concentrations required to inactivate bacterial spores and protozoan cysts High initial purchase cost due to limited production capacity Formation of iodo-DBPs possible, which are highly cyto- and genotoxic Limited research into pre-harvest applications
Chlorine dioxide (ClO_2)	Chloride (Cl^-) Chlorite (ClO_2^-) Chlorate (ClO_3^-)	Multidrug-resistant bacteria Mycobacteria Protozoa Biofilms Fungi Bacterial and fungal spores Viruses	Potent across a wide pH range High oxidation capacity Does not generate DBPs such as THMs, HAAs, dioxins, furans, but can generate chlorates Does not leave odor or taste nuisance	Concerns around worker safety Efficacy is affected by high organic load and inorganic water content Concerns over transport of precursor chemicals (risk of explosion and instability) Unstable during on-site generation Sensitive to light and high temperatures Chlorate DBP generation
Chlorination/Hypochlorite	HOCl ClO^-	Bacteria Biofilms Fungi Algae Viruses	Widely used, well characterized and easy to implement Less hazardous than chlorine gas (Cl_2) Can be generated on site, reducing need for transport and handling of hazardous chemicals	Formation of DBPs (THMs, HAAs) in reaction with organic matter or during production (chlorate) Conc. solution (12%–15%) hazardous to workers – high pH, corrosive, burns Unstable On-site generation can produce hydrogen, an explosion hazard Potential phytotoxicity Inactivation in the presence of high organic matter

DBPs: disinfection byproducts; THMs: trihalomethanes; HAAs: haloacetic acids; LED: light emitting diode.

use in organic treatment regimens and largely used in conventional closed greenhouse systems (Dorais et al., 2016).

All currently available technologies have several advantages and disadvantages (Table 2), such that it is difficult to provide generalized recommendations. The main advantages of the physical/mechanical treatments is that they do not form DBPs; disadvantages include the lack of residual disinfectant, and the requirement for pretreatment to reduce the potential for clogging with filtration and increase the efficacy of UV treatment. The advantages of chemical sanitation treatments are that, in some cases, residual disinfection can be maintained throughout the distribution system, thus reducing the risk of pathogen regrowth. The main disadvantages of chemical sanitation treatments are 1) the formation of DBPs, which are generally formed during the reaction of oxidants with organic matter; 2) the maintenance of residual disinfection during storage; 3) the handling and transport of dangerous chemicals, and 4) the expertise required to run and maintain complex water treatment technologies. The application of chemical disinfectants requires careful monitoring and process control to ensure suitable residual disinfectant concentration and avoid phytotoxicity (Allende & Monaghan, 2015). In addition, depending on the source, irrigation water might have high organic matter content, meaning that the formation of DBPs and their potential for plant accumulation should be carefully considered when selecting treatment technologies.

Given the above, the identification and investigation of treatment technologies that 1) generate no or minimal DBPs; 2) provide some residual disinfection without resulting in phytotoxicity or increasing the risk of antimicrobial resistance; and 3) are simple to implement with no additional chemicals required, is a high priority in a world with increasing regulation of irrigation water and food production. The potential for point-of-use water treatment is also appealing, so that storage/transfer time is minimized and the water is of the highest quality directly prior to crop application in the field.

2.2. Potential irrigation water treatment technologies

We have identified several treatment technologies (Table 3) that have the potential to address some of the concerns outlined above, whilst also acknowledging that in many cases multiple treatment technologies in combination will likely be the best scenario for effective irrigation water treatment. The common feature through all of the methods outlined below is that they have an element of advanced oxidation processes, because of the generation of reactive oxygen species (ROS), particularly hydroxyl radicals ($HO^{\bullet}$), for the degradation/oxidative attack on organic material including

Table 3. Potential irrigation water treatment technologies.

Treatment type	Active constituent(s)	Target organism(s)	Advantages	Disadvantages
Hydrodynamic cavitation	Mechanical generation and subsequent collapse of vapor bubbles, causing aggressive physico-chemical environments, under high temperature and pressure	Bacteria and viruses Cyanobacterial cell disruption Removal of pharmaceuticals and pesticides Degradation of organic pollutants (e.g. textile dyes)	Can be combined with UV treatment and other AOPs Reactor design is simple, scalable and easy to operate High energy efficiency No chemicals required	Most effective under acidic conditions which favors generation of hydroxyl radicals Requires optimization of configuration for each application No residual No available research for irrigation water use
Electrolyzed oxidizing (EO) water	Hypochlorous acid (HOCl) Hypochlorite (ClO^-) Hydrogen peroxide (H_2O_2) Ozone (O_3)	Bacteria Biofilms Fungi Algae Viruses	Wide applicability in health, food, agriculture Scalability for small, medium and large applications Some EO water technologies such as NEW are pH neutral and require no hazardous chemical usage, therefore non-corrosive and non-hazardous NEW is nontoxic, the electrodes do not contain ruthenium	High initial set-up cost Inactivation in the presence of high organic matter, which might require more brine and increase cost Potential different kill rates/ concentration against a variety of micro-organisms Formation of DBPs (THMs, HAAs) in reaction with organic matter
Electrochemical treatment	Hypochlorous acid (HOCl) Hypochlorite (ClO^-) Hydrogen peroxide (H_2O_2) $^{\bullet}OH$ Ozone (O_3)	Bacteria Biofilms Fungi Algae Viruses	Easy set up Requires no hazardous chemical usage Transport, storage or dosage with chemicals is not required Disinfection strength can be adjusted according to on-site demand	High initial reactor cost Efficacy dependent on water pH, temperature, suspended solids, microbiological load, organic matter The amount of chloride ions needed will vary depending on the water quality Potential for DBPs untested Level of on-going maintenance uncertain

DBPs: disinfection byproducts; THMs: trihalomethanes; HAAs: haloacetic acids; NEW: neutral electrolyzed water; AOP: advanced oxidation process; UV: ultraviolet.

pathogenic microorganisms. The $HO^{\bullet}$ molecule has the highest oxidizing potential of all oxidizing agents used in water treatment (Deng & Zhao, 2015).

2.2.1. Hydrodynamic cavitation

Hydrodynamic cavitation (HC) is a technique with a range of potential applications in water treatment and environmental remediation (Zupanc et al., 2019). First characterized in the 19th century, research has shown that HC treatment can generate localized high temperature and pressure hot spots under nearly ambient 'bulk' conditions. Previously, ultrasound was the main method used for producing cavitation but the adoption by industry has been poor because of the cost and extensive expertise required to operate the equipment successfully. HC is a cheaper and simpler alternative than the ultrasound-based process; the cavitation is produced by the rapid constriction and subsequent expansion of a liquid through a Venturi or orifice plates under controlled conditions (Ciriminna, Albanese, Meneguzzo, & Pagliaro, 2016; Dular et al., 2016). As the fluid flows through the constriction, HC occurs in regions where the (hydro)static pressure drops below the vapor pressure of water, causing evaporation and the formation of vapor bubbles (Figure 2). On return to regions of normal static pressure, vapor re-condenses and cavitation bubbles collapse, leading to the formation of very short lived (μs) but also very aggressive physico-chemical microenvironments characterized by very high temperature (>1,500 °C), pressure (>69 MPa), and turbulence (100 m s^{-1} micro jets; Tao, Cai, Huai, Liu, & Guo, 2016), all while the bulk water environment remains at ambient conditions. Reactive oxygen species (ROS; including $HO^{\bullet}$ and $HO_2^{\bullet}$ radicals), while generated during cavitation, can also be added (H_2O_2, O_3) to further enhance organics removal during water treatment applications (Jusoh, Aris, & Talib, 2016; Raut-Jadhav et al., 2016; Tao et al., 2016).

Research has shown the potential beneficial uses of HC for remediation of contaminated waters, with applications including: elimination of refractory organic pollutants (Petkovšek et al., 2013; Tao et al., 2016); disinfection and pathogen destruction (Dular et al., 2016; Li, Song, & Yu, 2014; Tao et al., 2016; Torabi Angaji & Ghiaee, 2015); removal of oxyanions (As, Se) and pharmaceuticals (Zupanc et al., 2013, 2014); and recovery of base/precious metals from mine waters (Kirpalani, Singla, Lotfi, & Mohapatra, 2016).

HC can be used as a stand-alone process or in conjunction with UV (Zupanc et al., 2013), and H_2O_2 treatments (Rajoriya, Carpenter, Saharan Virendra, & Pandit Aniruddha, 2016). The main drawback of this treatment technology is the lack of residual disinfection, which might mean that it is best used in combination with another form of disinfection, or

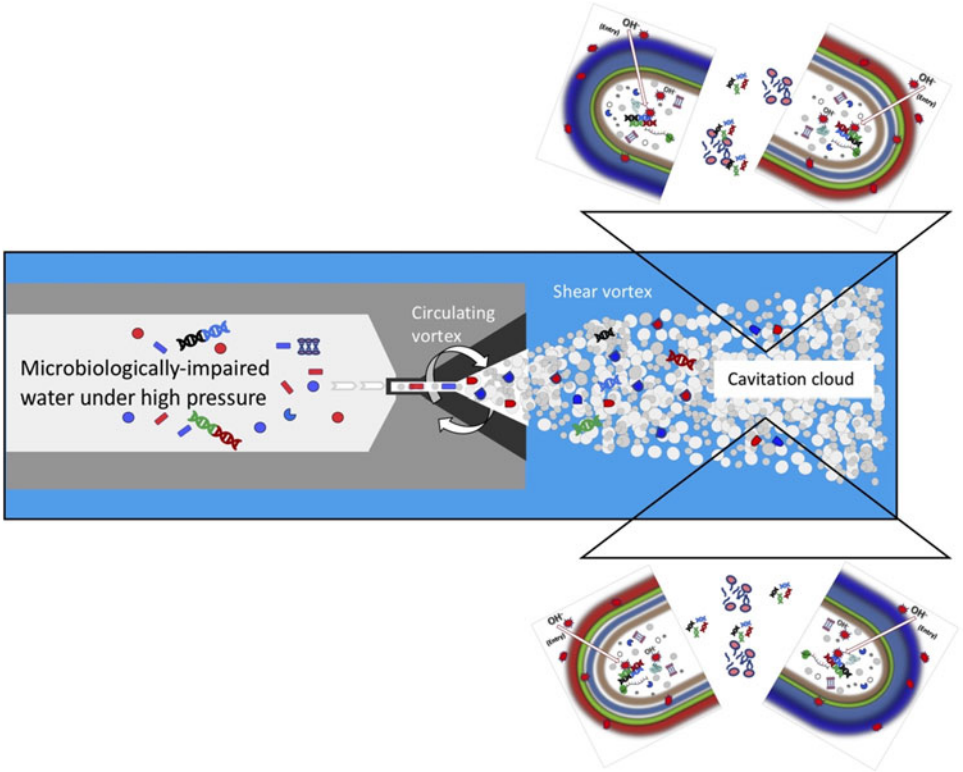

Figure 2. Principles of hydrodynamic cavitation. Formation and collapse of vapor bubbles from liquids in orifices or Venturi occur rapidly under very high temperature and high pressure changes, resulting in very high energy densities and generating hydroxyl radicals, leading to pathogen destruction.

implemented as a point-of-use water treatment. Also, given the paucity of reports in the literature, various issues such as the potential for clogging at the constriction point and the durability of the cavitation chamber need to be considered. On the other hand, the simple reactor design, easy operation, high energy efficiency and scalability have made this technology attractive for deployment (Tao et al., 2016). The review by Zupanc et al. (2019) summarized recent research on the effects of cavitation on a range of organisms, including bacteria (both Gram negative and Gram positive), cyanobacteria, algae, fungi, yeast and viruses, whilst also highlighting the many limitations of research in this area. Despite the potential of this technology, much research is required to optimize HC treatment for application to irrigation water and ensure optimal pathogen inactivation.

2.2.2. Electrolyzed oxidizing (EO) water

EO water is obtained through the electrolytic treatment of brine (water containing NaCl or KCl salts; Bakhir, 1985). In the presence of chloride,

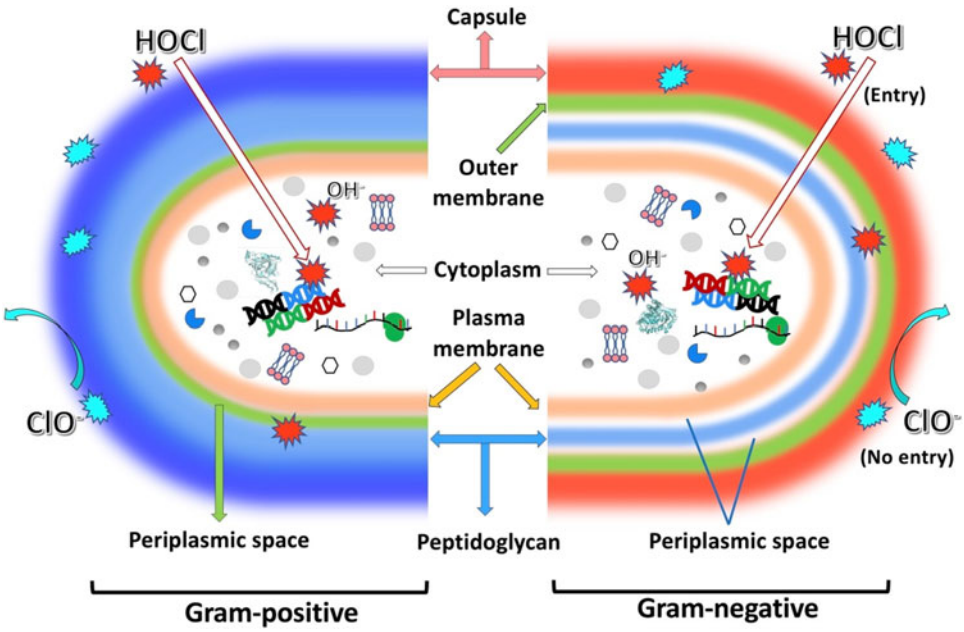

Figure 3. Schematic representation of the abilities of hypochlorous acid (HOCl) and hypochlorite (ClO^-) to kill Gram-positive and Gram-negative bacteria. The potent activity of HOCl is due to its dual cidal action on bacterial cells: HOCl is electrically neutral and can passively diffuse through the cell wall and plasma membrane into the cytoplasm where it attacks constituents including nucleic acids, proteins and lipids. HOCl is also able to directly destroy the cell wall and plasma membrane through its oxidizing action. However, ClO^- is unable to penetrate the cell and only exerts its cidal action on the bacterial cell surface.

active chlorine (sodium hypochlorite or hypochlorous acid) and ROS (O_3, H_2O_2) are formed, which are toxic to microorganisms (Figure 3). The resulting concentrated solution (300–500 mg l^{-1} active chlorine) can then be diluted into water for disinfection treatment. Electrolysis in a 2-chamber system generally results in both an acidic anolyte and an alkaline catholyte, while a 4-chamber system produces a pH-neutral anolyte, NEW (Bohnstedt, Surbeck, & Bartsch, 2009; Ferro, 2015; Migliarina & Ferro, 2014; Quadrelli & Ferro, 2010).

The main active component in the disinfection activity of EO water is free chlorine. ROS are also produced but their action is limited by their short half-life. The EO water activity will largely depend on the pH, oxidation reduction potential (ORP) and available chlorine concentration (Rahman, Khan, & Oh, 2016). Similar to traditional chlorination treatments, the optimal activity of free chlorine generated in the electrolytic process occurs when the pH of the EO water is around 6. Of the various types of EO water available, NEW (pH 6.5–7.5) is arguably the most promising as it contains predominantly HOCl. This compound is uncharged and poorly solvated by water molecules and as such it is able to penetrate

bacterial cell walls and oxidize polysaccharides (Bonfatti et al., 2000). EO waters with extremes of pH are likely to damage infrastructure and cause phytotoxicity and are therefore less suitable for agricultural applications.

Several studies have described the activity of EO waters against suspensions of target human pathogens (*E. coli*, *Salmonella* spp. and *Listeria* spp.; Supplementary information Table S1) where substantial log reductions in viable microorganisms were obtained with treatment under a range of conditions of exposure time, pH, temperature, available chlorine and ORP (Rahman et al., 2016). However, there are limited published applications of the use of EO technology in treating irrigation water. Grech and Rijkenberg (1992) found that micro-emitter-based irrigation to treat citrus root pathogens with acidic EO water at 40–50 μg ml^{-1} active chlorine did not result in chlorine-induced phytotoxicity in field-grown plants. Similarly, the use of acidic EO water as a foliar spray (free chlorine of 54–71 mg l^{-1}) on a variety of bedding plants grown under greenhouse conditions demonstrated very little to no phytotoxicity to the plants while exhibiting rapid killing of pathogenic fungi such as powdery mildews and gray molds (Buck, van Iersel, Oetting, & Hung, 2003). Zarattini, De Bastiani, Bernacchia, Ferro, and De Battisti (2015) reported that the use of NEW at up to 500 mg l^{-1} on tobacco plants and apple trees produced no phytotoxic effects but unexpectedly triggered the molecular defenses of plants. NEW was effective at inactivating norovirus, showing >5-log reduction in suspension with NEW at 250 mg/l free chlorine, but increasing organic load or reduced NEW concentrations were less effective at reducing the viral load (Moorman, Montazeri, & Jaykus, 2017).

Similar to other chlorination treatments, organic matter has a detrimental effect on the efficacy of EO water (Jo, Tango, & Oh, 2018; Stevenson, Cook, Bach, & McAllister, 2004) and can result in the formation of DBPs, although few studies have investigated this in detail (López-Gálvez, Andujar, et al., 2018). Chlorates can also be produced during the electrolysis process itself; this can be controlled by the choice of electrode material, electrolyte composition, applied current, pH and temperature (López-Gálvez, Andujar, et al., 2018). As an alternative to traditional chlorination treatments, the technology is easy to implement and safe to use, with no dangerous chemicals required; however, the production of DBPs is still a concern and further research is required to determine the type and levels of DBPs produced and their potential accumulation in plants.

2.2.3. Electrochemical disinfection

Electrochemical disinfection is achieved by passing an electric current through the water under treatment, using suitable electrodes, without the addition of exogenous salts (Kraft, 2008). At the phase boundary between

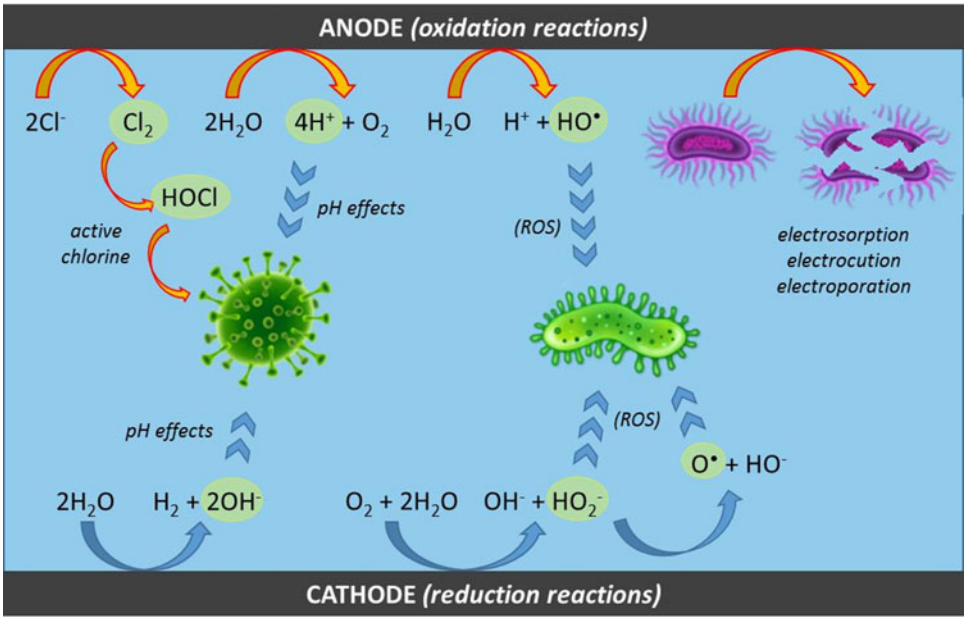

Figure 4. Reactions that occur at the anode and cathode during electrochemical disinfection of water. ROS: reactive oxygen species.

the electrodes and the water, the electric current leads to the electrochemical production of disinfecting species from the water itself (for example, ROS) or from species dissolved in the water (most notably chloride is oxidized to free chlorine; Figure 4; Kerwick, Reddy, Chamberlain, & Holt, 2005). Sufficient free chlorine can be produced to efficiently disinfect water even at low chloride concentrations (less than 100 mg l^{-1}; Kraft, 2008). The disinfection efficacy of the electrochemical approach is thought to be higher than that of chlorination due to the formation of ROS such as hydroxyl radicals ($^{\bullet}OH$), atomic oxygen ($^{\bullet}O$), H_2O_2, and O_3 (Delaedt et al., 2008; Diao, Li, Gu, Shi, & Xie, 2004). Yet, the short lifetime of most of the ROS in solution means that they are only active inside the electrochemical reactor. While most disinfecting agents are produced at the anode, H_2O_2 may also be produced at the cathode, as a product of oxygen reduction (Stoner, Cahen, Sachyani, & Gileadi, 1982).

The inactivation efficacy of electrochemical disinfection systems depends on several factors, including the electrochemical cell configuration, electrode material, water composition, the nature of the target microorganism, flow rate and current density (Jeong, Kim, & Yoon, 2009; Martínez-Huitle & Brillas, 2008). The main process leading to electrochemical water disinfection relies on the electrosynthesis of disinfecting agents, however other phenomena such as the electrosorption of bacteria on the electrode surface (with consequent direct interaction), electrocution, and electroporation might play a role in the process (Matsunaga, Nakasono, Kitajima, &

Horiguchi, 1994; Matsunaga, Okochi, & Nakasono, 1995; Nakasono, Nakamura, Sode, & Matsunaga, 1992). After electrosorption, inactivation of microorganisms can result from the direct electrochemical oxidation of intracellular coenzyme-A, leading to decreased respiration and consequent cell death (Matsunaga et al., 1992). Electrochemical treatment was shown to result in oxidation of viral capsid proteins, leading to loss in structural integrity and viral inactivation (Shionoiri, Nogariya, Tanaka, Matsunaga, & Tanaka, 2015).

An interesting feature of the electrochemical disinfection approach is that the local concentration of the active agents (i.e., within the diffusion layer that forms at each electrode surface) can exceed the average concentration found in the water leaving the reactor by one or two orders of magnitude (Stoner et al., 1982). Consequently, the local concentration can be high enough to destroy highly resistant microorganisms, even if the concentration of active species in the treated water is kept at a low level. When compared with chemical disinfection methods, electrochemical water disinfection has the advantage that no transport, storage or dosage with disinfectants is required. In addition, the disinfection strength can be adjusted according to the on-site demand by adjusting the current. The technology is easy to install and could be integrated into irrigation systems where required. While electrochemical water disinfection has great potential for point-of-use irrigation water treatment, the amount of chloride ions needed, the effect of the water pH, temperature, presence of suspended solids, microbiological load, high organic matter, nature of the electrode material and the potential to produce DBPs need to be carefully evaluated. De Battisti, Formaglio, Ferro, Al Aukidy, and Verlicchi (2018) observed the formation of chlorate and perchlorate during electrochemical disinfection of groundwater, but that the concentrations of these DBPs was lower than the appropriate guideline values.

3. Other considerations in the choice of irrigation water treatment methods

There are many other issues that should be considered when choosing an appropriate irrigation water treatment method. These include potential health risks such as antimicrobial resistance and DBP accumulation, and application concerns such as cost, water quality and application methods.

The treatments described in this review have generally focused on bacterial pathogens, however, the control and treatment of other pathogen types is important. Viral pathogens such as hepatitis A and norovirus have been associated with several recent outbreaks on fresh or frozen berries and other fresh produce such as leafy greens and salads (Chatziprodromidou,

Bellou, Vantarakis, & Vantarakis, 2018). Viral pathogens can be introduced to fresh produce during preharvest operations (from contaminated irrigation water) or during postharvest manipulation (from infectious food handlers or contaminated process/washing water). Thus the role of irrigation water in virus transmission and the efficacy of the disinfection treatments investigated in this review against viral pathogens should be an important focus of future research (Hedberg, 2016).

Disinfection is a key component in successfully controlling pathogen populations in water but has also been linked in some studies to the selection of antimicrobial resistance (Rizzo et al., 2013) and reduced efficacy/increased resistance over time. Recent studies have implicated both DBPs and residual disinfectants in the induction of antimicrobial resistance and horizontal transfer of antimicrobial resistance genes (Li & Gu, 2019). Multidrug resistant opportunistic and human pathogens are an emerging worldwide threat to human health that can be transmitted through a variety of sources, including as foodborne pathogens (Baker, Thomson, Weill, & Holt, 2018). These risks should be carefully considered in the risk–benefit analysis of any proposed disinfection strategy, particularly where this is linked directly to human food.

The accumulation of DBPs in plants and potential health effects also need to be carefully considered (Dannehl, Schuch, Gao, Cordiner, & Schmidt, 2016; López-Gálvez, Andujar, et al., 2018) and this is an area that would benefit from more research. Dannehl et al. (2016) found that using potassium hypochlorite as the disinfectant in a recirculating hydroponic system, resulted in higher chlorate content in the tomatoes being grown than the current European maximum residue limit. Similarly, overhead irrigation with EO treated water resulted in accumulation of chlorates in lettuce to above the maximum residue limit (López-Gálvez, Andujar et al., 2018).

Cost is obviously an important factor in the decision-making process (Raudales, Fisher, & Hall, 2017; Van Haute et al., 2015), but because of its variability at local, national and international scale, it is difficult to draw broad conclusions. Significant research gaps also exist in terms of the practical application of water treatment to irrigation water and potential impacts in the field and beyond. The variability of irrigation water quality and quantity, crops and scale of production also makes it difficult to identify an optimal treatment arrangement that will be suitable for all potential users. For each water source and treatment configuration, the efficacy (in terms of pathogen reduction) and safety (in terms of DBPs production and/or accumulation in plants) should be independently verified to ensure compliance with the relevant guidelines. For instance, water with high turbidity might not be suitable for UV treatment; and water with high

dissolved organic matter content could be problematic for chlorine treatment because of the potential for DBPs and high chlorine demand, resulting in reduced disinfection efficacy. The irrigation method (i.e., drip *vs.* overhead) might also considerably affect the risk of pathogen or DBP uptake from treated water.

Below we provide some perspective on two important considerations for both human and plant health, which are induction of the viable but non-culturable (VBNC) state in microbial populations, and the potential effect of treated waters on soil and plant microbial communities.

3.1. Induction of VBNC microorganisms

Microbial populations can exist in a VBNC state, a survival strategy used by many Gram-positive and Gram-negative bacteria in response to adverse environmental conditions (Ferro, Amorico, & Deo, 2018; Ramamurthy, Ghosh, Pazhani, & Shinoda, 2014). There have recently been several works published investigating the potential for induction of VBNC cells during water disinfection processes (Lin, Li, Gu, Zeng, & He, 2016; López-Gálvez, Gil, Meireles, Truchado, & Allende, 2018; Zhang, Ye, Lin, Lv, & Yu, 2015). This might be of particular concern in low-quality irrigation waters, where disinfection efficacy is compromised by organic matter content or other factors. Hence, investigation of irrigation water disinfection using only conventional microbial culturing techniques might overestimate the efficacy of the disinfection treatment if VBNC organisms are not specifically considered. This is because VBNC organisms do not grow when plated on culture media that would normally support their growth *in vitro*, rendering them difficult to detect by conventional means.

VBNC microbes have lipid-rich membranes, tend to be smaller than their non-VBNC counterparts, exhibit reduced metabolic activity, and display altered cellular changes including cell leakage, depletion of energy pools, and altered gene expression and DNA replication (Arzanlou, Chai, & Venter, 2017; Trevors, Bej, Mojib, van Elsas, & Van Overbeek, 2012). Importantly, under favorable conditions (such as through expression of a resuscitation-promoting factor), these organisms can be revived. For example, it has been shown that *L. monocytogenes* treated with distilled water entered into the VBNC state and became virulent after resuscitation using embryonated eggs (Cappelier, Besnard, Roche, Velge, & Federighi, 2007). It therefore cannot be excluded that VBNC pathogens may be present in treated irrigation water and that they may become virulent again at a later stage. Furthermore, several studies have shown that pathogens may still exert detrimental effects even when in a VBNC state. For instance, laboratory-induced VBNC *E. coli* O157:H7 cells produced Shiga-like toxins

in a vero-cell microplate cytotoxicity assay, demonstrating a potential health hazard (Liu, Wang, Tyrrell, & Li, 2010). It has also been demonstrated that the VBNC state in *S. epidermidis* contributes to the formation and persistence of biofilms, resulting in tolerance to multiple antimicrobials and immune evasion (Cerca et al., 2011).

VBNC bacterial cells can be induced by many factors, including water sanitation treatments with H_2O_2 (Arana, Muela, Iriberri, Egea, & Barcina, 1992), chlorination (Oliver, Dagher, & Linden, 2005), high/low temperature (Patrone et al., 2013; Pawlowski et al., 2011), UV irradiation (Zhang et al., 2015), peroxide-based disinfectants such as PAA (Park, Lee, Bisesi, & Lee, 2014) and high-pressure CO_2 (Zhao, Bi, Hao, & Liao, 2013). A recent study showed that *E. coli* O157:H7 treated with acidic (pH 2.7–2.9 or pH 5.6–6.3) EO water could become VBNC and be resuscitated at available chlorine concentrations that resulted in no viable counts (30 mg l^{-1}; Zhang, Chen, Xia, Li, & Hung, 2018). Much higher concentrations of available chlorine (50 mg l^{-1}) were required to remove all VBNC cells. Green fluorescent protein-tagged *L. monocytogenes* and *S. enterica* Thompson became VBNC upon exposure to 12 mg l^{-1} and 3 mg l^{-1} chlorine, respectively (Highmore, Warner, Rothwell, Wilks, & Keevil, 2018). Thus, it is critical to investigate whether and under which conditions the various water treatment regimens induce VBNC cells in a microbial community and whether these organisms can become active again on crops or fresh produce postharvest. To fully characterize the induction of VBNC status by the various water treatment technologies, a combination of macromolecular and cellular techniques such as real-time PCR (DNA), transcriptomic (RNA) metabolic activity (protein, lipid, luminescence) measurements, fluorescence-based imaging flow cytometry, as well as morphometric analyses by transmission and scanning electron microscopy will be essential.

3.2. Effects of treated irrigation waters on soil and plant microbial communities

Soil-borne microbes constitute a major proportion of the resident organisms (the "microbiome") identified on fruit and vegetables. The vast majority of these are not responsible for spoilage but rather act as a "natural biological barrier" against plant opportunistic pathogens, which are often a smaller subset of the entire soil microbial community (Andrews & Harris, 2000; Barth, Hankinson, Zhuang, & Breidt, 2009; Janisiewicz & Korsten, 2002). Indeed, it has been shown that an inverse relationship exists between soil microbial diversity and the survival of an invading pathogen (van Elsas et al., 2012). Hence, it is important that the irrigation with treated water does not negatively alter the microbial ecology of soils as this could directly

influence the plant microbiome (by altering the plant endophytic and phyllosphere microbial community) or indirectly by compromising organisms important for soil health (fertility and biocontrol) and thereby decreasing the health status of plants.

Many factors contribute to changes in the microbial ecology of soil, vegetables and fruits, including soil characteristics, climatic conditions and agronomic practices (Allende & Monaghan, 2015; Barth et al., 2009; Becerra-Castro et al., 2015; Berg & Smalla, 2009; Cluff, Hartsock, MacRae, Carter, & Mouser, 2014; Frenk, Hadar, & Minz, 2014; Zheng et al., 2017). Irrigation water quality also contributes to changes in microbial communities in soil and plants, especially in copiotrophic environments/ecosystems. For instance, Mañas, Castro, and de Las Heras (2009) reported significant increases in fecal streptococci, *Salmonella* spp., sulfite-reducing *Clostridium* spp. as well as total and fecal coliform counts in lettuce irrigated with minimally treated wastewater (using trickling filters), relative to control plants receiving potable water (groundwater). These findings indicate the potential deleterious effects of microbiologically impacted irrigation water on fresh produce. However, the use of tertiary water treatment regimes, such as final disinfection using UV light, chlorination and/or ultrasound, have been shown to effectively remove indicator microorganisms and pathogens to below limits of detection at the point of discharge (Pachepsky, Shelton, McLain, Patel, & Mandrell, 2011; Villanueva, Luna, Gil, & Allende, 2015). Therefore, it is critically important that good agricultural practices are implemented before, during and after harvest to maintain soil health and promote a balanced and functioning microbial community. These practices are defined in the Codex General Principles on Food Hygiene (Codex Alimentarius Commission, 2003) and aim at maximizing the quality of the crop harvested. However, a search through the literature reveals very few original manuscripts and/or reviews pertaining to changes in the microbial ecology of soil and foliar tissues after irrigation with treated irrigation water. Chevremont, Boudenne, Coulomb, and Farnet (2013) documented the changes in microbiological properties of soils irrigated with UV-LED treated wastewaters over a one-year period. When compared with watering with untreated wastewater, watering with the UV-LED treated wastewater resulted in decreased occurrence of fecal coliforms, and showed no deleterious effects on overall microbial diversity and function. Truchado, Gil, Suslow, and Allende (2018) recently investigated the effect of a low residual ClO_2 concentration (approx. 0.25 mg l^{-1}) in irrigation water on the soil microbiome and baby spinach phyllosphere bacterial community. Next generation sequencing demonstrated that while the composition of these microbiomes was not significantly altered, the relative abundance of specific bacterial genera was influenced. In particular, the relative abundance of

Pseudomonaceae and *Enterobacteriaceae* significantly decreased when the water was treated with ClO_2.

Our overall knowledge of how the microbial ecosystems in the soil and on the surface of each produce type are influenced by the treatment of irrigation water, especially when disinfectant residues are present, is still very limited. Considering the importance of the soil and plant microbiomes to directly and indirectly control the occurrence of both human and plant pathogens, more research effort is needed in this regard.

4. Conclusions

The need to utilize water bodies and sources with sub-optimal microbiological characteristics is anticipated to increase in line with increased demand for water by the agricultural sector and society in general. In the case of fresh produce, it is of paramount importance that the microbiological quality of the water is optimized to minimize the potential for pathogen outbreaks. A significant number of treatment technologies are available for the treatment of irrigation water and they include both physical and chemical treatments. At present, the use of sodium hypochlorite and UV disinfection are widely applied because of both cost and convenience. However, other treatments such as EO water and electrochemical water disinfection (which do not require addition of chemicals) could provide interesting alternatives. Hydrodynamic cavitation should also be considered and further investigated as, in addition to not requiring chemicals due to it being a "mechanical treatment process," it may also mitigate disinfection-induced selection of resistant bacteria (which are often pathogenic), particularly if it is proven to also destroy resistance genes and not induce the VBNC state. As noted above, however, it is generally advisable that multiple treatments are used in conjunction in high-risk settings (e.g., salad crop production), in order to ensure continuity of high water quality even in the event of total or partial failure of individual treatment barriers. We propose the concept of multistep irrigation water treatment that could be implemented for on-farm sanitation, which could vary depending on the physico-chemical parameters of the water to be treated, level of contamination and the size and cost implications of the approach to be adopted.

While there is a significant body of work on the relative efficacy of various water treatments for production of clean water, there is little direct information on the microbial profiling of irrigation water. More critically, there is little data on the effects of microbiologically impacted irrigation water on the quality of fresh produce or its effects on soil microbial communities. Direct evidence, via specific in-field experiments and advanced molecular and cellular techniques, showing the effects of the various

irrigation treatment regimens on the VBNC state as well as their effects on the dynamics of soil and microbial communities, particularly on high-risk vegetables, is warranted and paramount. Equally important is a thorough evaluation of the long-term effects and benefits of the irrigation treatment methods on soil sustainability, produce quality and overall farm productivity. Moreover, judicious implementation of environmentally-friendly treatment technologies that can effectively remove antibiotics and other antimicrobial agents, their metabolites, antimicrobial resistant microorganisms and antimicrobial resistance genes in irrigation water will improve the overall safety and value of minimally-processed foods.

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HIA Project VG15068 Milestone Report 103: Appendices

Materials and method development

Bacterial strains, growth conditions and preparation of inocula. The bacterial strains used in this activity report were *Escherichia coli* (ATCC 25922), *Listeria innocua* 6a (ATCC 33090) and *Salmonella enterica* serovar Enteritidis 11RX. Glycerol stock cultures were maintained at –80°C and were streaked onto Luria Bertani (LB) agar (Oxoid) to obtain isolated colonies. Single colonies were streaked onto the following selective agar plates (ThermoFisher Scientific) to confirm purity: Eosin Methylene Blue (EMB) agar (PP2169) for *E. coli*; *Listeria* Selective Agar Oxford (OXF) agar (PP2141) for *L. innocua* 6a, and Xylose Lysine Deoxycholate (XLD) agar (PP2004) for *S.* Enteritidis 11RX.

For experiments, single colonies from selective agar plates were emulsified in LB broth and grown overnight at 37°C with aeration at 150 rpm on a digital platform mixer (Ratek Instruments). Thereafter, bacteria were subcultured at a 1:10 dilution into fresh LB broth and incubated further at 180 rpm for 2–3 h until A_{600}=1.0 (for *E. coli* and *S.* Enteritidis 11RX) or A_{600}=0.5 (for *L. innocua* 6a) was reached (equivalent to approx. 1×10^9 colony-forming units (CFU)/ml for *E. coli* and *S.* Enteritidis 11RX or approx. 5×10^8 CFU/ml for *L. innocua* 6a). Bacteria were then harvested and washed extensively (3×) in autoclave-sterilised Milli-Q water (PURELAB Classic, ELGA) to remove residual culture medium and then resuspended in sterile Milli-Q water to approx. 1×10^9 CFU/ml for each strain.

Reagents, solutions and instruments. Electrolysed oxidising (EO) water (anolyte) was provided by ECAS4 Australia (Unit 8 / 1 London Road, Mile End South, SA 5031) at 300–350 mg/L free chlorine. Sodium hypochlorite (NaOCl) was obtained as a 125 ml/L solution (UN No 1791) from Chemwell Pty Ltd (3 Clive St, Springvale, VIC 3171. Chlorine dioxide (ClO_2) was obtained as TwinOxide Tabs® Part No 121710 (TwinOxide®) and was prepared as a 1,000 ppm chlorine solution according to the manufacturer's instructions. Suwannee River natural organic matter (SRNOM) was used as the source of natural organic matter and was resuspended to the equivalent of 200 mg/L dissolved organic carbon.

Milli-Q water at pH 6.0, 7.0, or 8.4 was prepared using 1 mM $NaNO_3$ as background electrolyte, while water at pH 9.2 was prepared in 0.01 M carbonate-bicarbonate buffer (9.1 mM sodium bicarbonate and 0.9 mM anhydrous sodium carbonate) and verified on a Eutech Instruments PC 700 pH meter. Anolytes of different estimated free chlorine concentrations were prepared in these buffers. The amount of free chlorine in anolyte and NaClO was measured using the free chlorine and chlorine ultra-high range portable photometer (HI 96771C; Hanna Instruments) according to the manufacturer's recommendations, while the HACH DR/890 Colorimeter was used to measure the amount of free chlorine and ClO_2 (method 10126 for water) according to the manufacturer's instructions. All figures were drawn and statistical analyses performed using Prism v7 software.

EO anolyte time-kill and dose response analysis

Initially, the anolyte (sodium-based) was prepared to provide 0, 3, 30 and 150 ppm free chlorine in Milli-Q water (pH 7.0) to each of which approx. 2×10^8 CFU *E. coli*, *L. innocua* 6a or *S.* Enteritidis 11RX was added. Please note that throughout the values referred to as *x* ppm of a treatment indicates the concentration of free chlorine (in mg/L) in that treatment at the beginning of the experiment. Aliquots were then withdrawn immediately and at 5, 30, 60, 120, 300 and 600 s thereafter and added to 0.05% (vol/vol) final concentration of sodium thiosulphate ($Na_2S_2O_3$) to inactivate the anolyte. These experiments were repeated at least twice for each bacterial strain. Results of these initial experiments showed that the anolyte at 3 ppm consistently killed the vast majority of the bacteria at 120 s post-exposure (Figure 1). Subsequently, experiments were performed by incubating a mixed suspension of *E. coli*, *L. innocua* 6a and *S.* Enteritidis 11RX (approx. 1×10^8 CFU/ml of each strain) to the anolyte at 0, 0.15, 0.3, 0.6, 1.2, 1.8, 2.4 and 3.0 ppm, for 120 s after which $Na_2S_2O_3$ was added as before. This time was chosen as it is a reasonably worst-case scenario of a short contact time under field conditions. The suspension was serially diluted 10-fold and aliquots plated on LB agar (for total bacterial enumeration), EMB agar (for selective and differential enumeration of *E. coli* and *S.* Enteritidis 11RX), XLD agar (for selective enumeration of *S.* Enteritidis 11RX) and OXF agar (for selective enumeration of *L. innocua* 6a). The experiments were performed on 4 different occasions. Non-linear regression analysis showed that the EO treatment of the mixed bacterial suspension resulted in a dose-dependent (less than 1 ppm free chlorine) and substantial kill (4-6 $\log_{10}$) of all the bacteria (**Figure 1**).

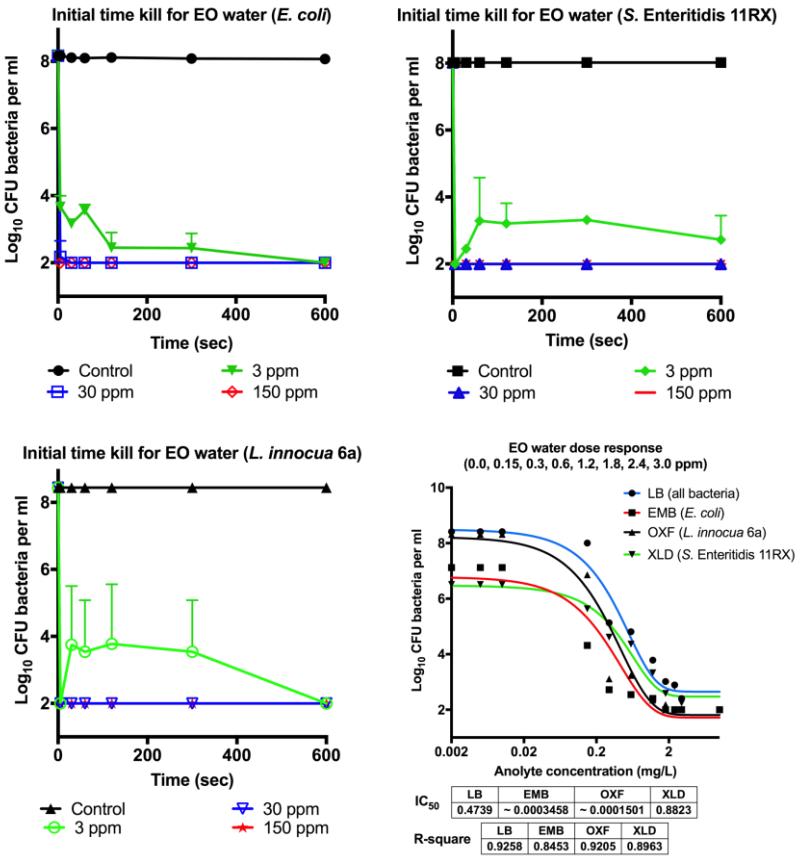

Figure 1. Time and dose-dependent bactericidal activity of EO water against individual and mixed bacterial suspensions of *E. coli*, *L. innocua* 6a and *S.* Enteritidis 11RX).

Comparison of the kill kinetics of sodium (Na)-based and potassium (K)-based anolytes

The ability of Na-based and K-based anolytes to kill bacteria was compared side-by side to verify if there are differences in their efficacy, using the time and dose-dependent kill kinetics of the Na-based anolyte established above. Our results showed that the kill kinetics of Na-based and K-based anolytes were remarkably similar (**Figure 2**).

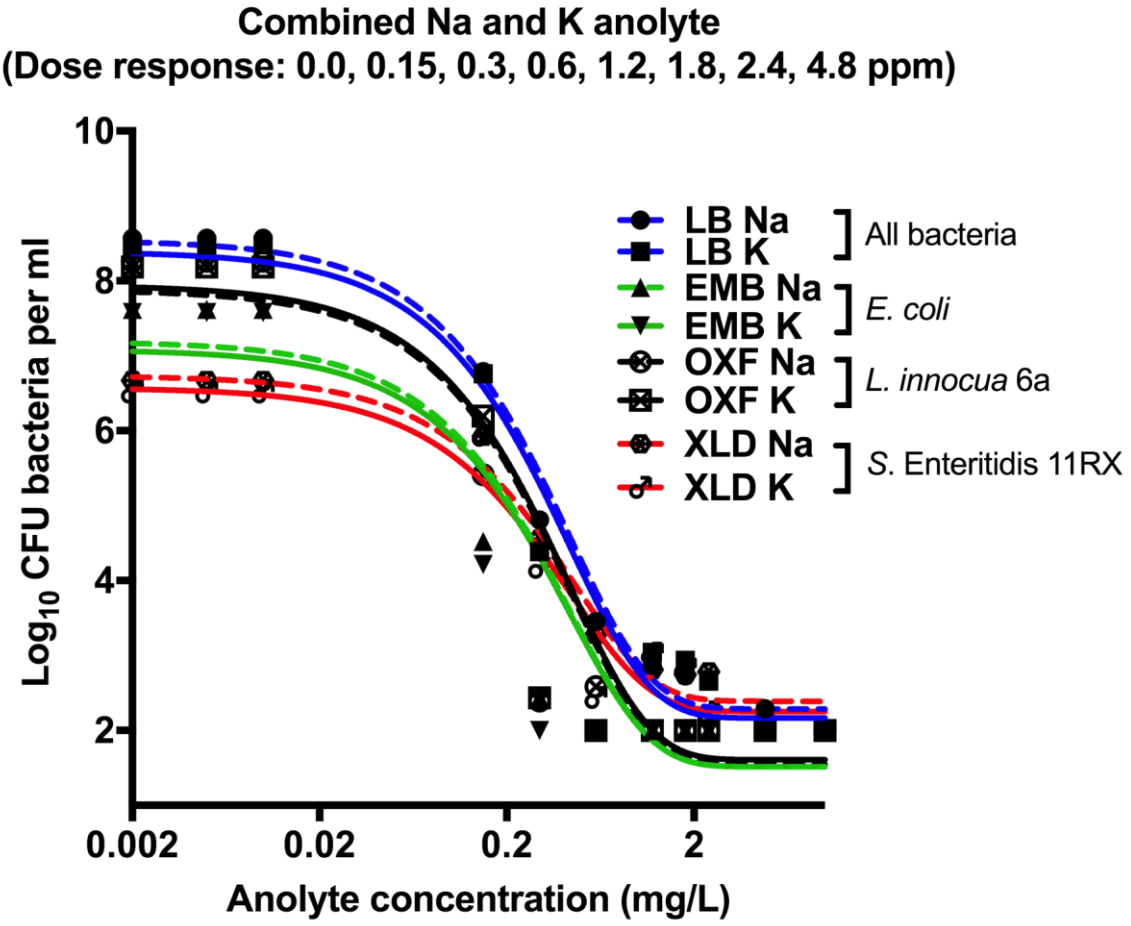

Figure 2. Comparison of the kill kinetics of sodium-based and potassium-based anolytes.

Bactericidal activity of EO water under a range of unbuffered and buffered pH conditions.

The efficacy of the anolyte to kill the mixed bacterial populations at pH 6.0, 7.0, or 8.4 (using 1 mM $NaNO_3$ as background electrolyte) was tested under the range of free chlorine concentrations described above. We found that the $NaNO_3$ electrolyte did not produce effective buffered solutions, with the pH decreasing to near neutral for the pH 8.4 buffer upon addition of increasing concentration of the anolyte. Therefore, water at pH 9.2 was prepared in 0.01 M carbonate-bicarbonate buffer, which was stable in the presence of increasing concentration of the anolyte. Nevertheless, our results showed that the bactericidal activity and kill kinetics of the anolyte was not appreciably affected under the range of unbuffered (**Figure 3**) and buffered (**Figure 4**) pH conditions (pH 6.0, pH 7.0, pH 8.4 and pH 9.2) tested.

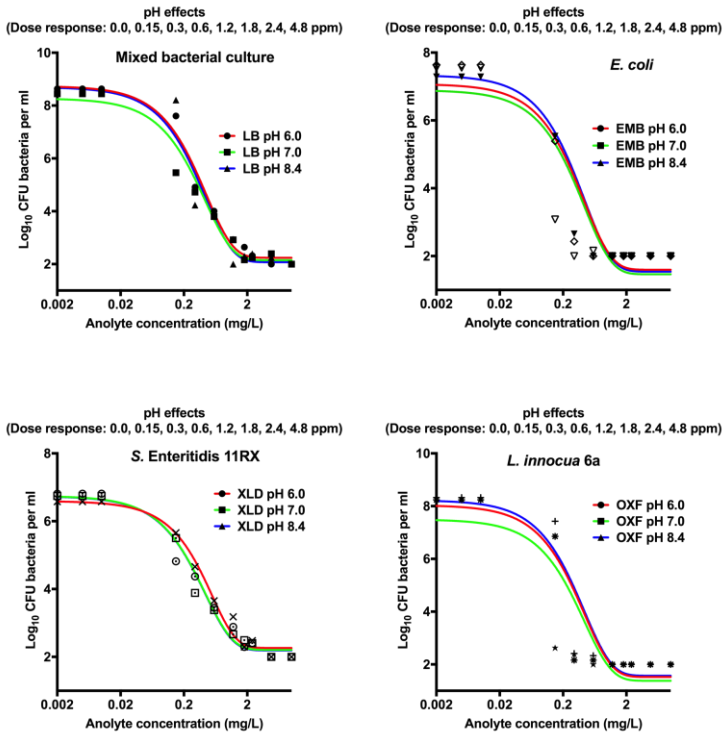

Figure 3. Bactericidal activity of the anolyte under a range of unbuffered pH conditions.

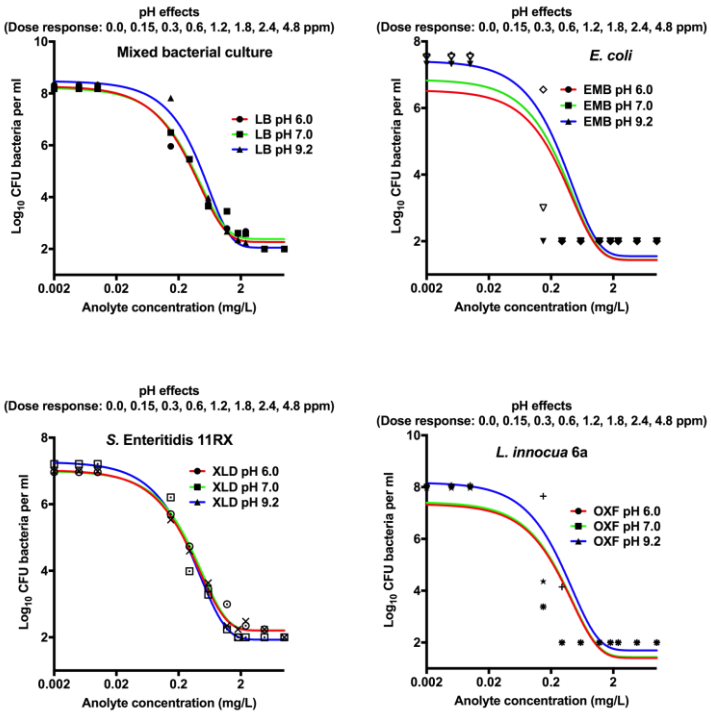

Figure 4. Bactericidal activity of the anolyte under a range of buffered pH conditions.

Bactericidal activity of EO water in the presence of increasing organic matter content.

We tested the ability of increasing concentrations of dissolved organic carbon (using SRNOM) to reduce the amount of free chlorine present in EO water preparation by assessing its quenching at 1 ppm or 5 ppm in the presence of 0, 2.5, 5, 10, 15, 20, 30 and 40 mg/L SRNOM. We found a dose-dependent reduction in the amount of free chlorine in both preparations, with only 0.43 ppm of free chlorine left in the 1 ppm EO water preparation in the presence of 2.5 mg/L SRNOM, while 0.85 ppm of free chlorine was still available in the 5 ppm initial free chlorine preparation in the presence 30 mg/L SRNOM (**Figure 5, left panel**). We then tested the hypothesis that efficacy of the anolyte to reduce the microbial loading of irrigated water will be reduced in the presence of organic matter by assessing the bactericidal activity of EO water at 1 ppm and 5 ppm in the presence of 0, 2.5, 5, 10, 15, 20, 30 and 40 mg/L SRNOM. The mixed bacterial populations were added to each preparation for 120 sec, and the reaction quenched using $Na_2S_2O_3$ as described above. As expected, we found that the ability of the anolyte containing 1 ppm free chlorine to kill the bacterial population was substantially reduced in the presence of increasing organic matter content, but its efficacy was not appreciably affected at 5 ppm free chlorine level (**Figure 5, right panel**).

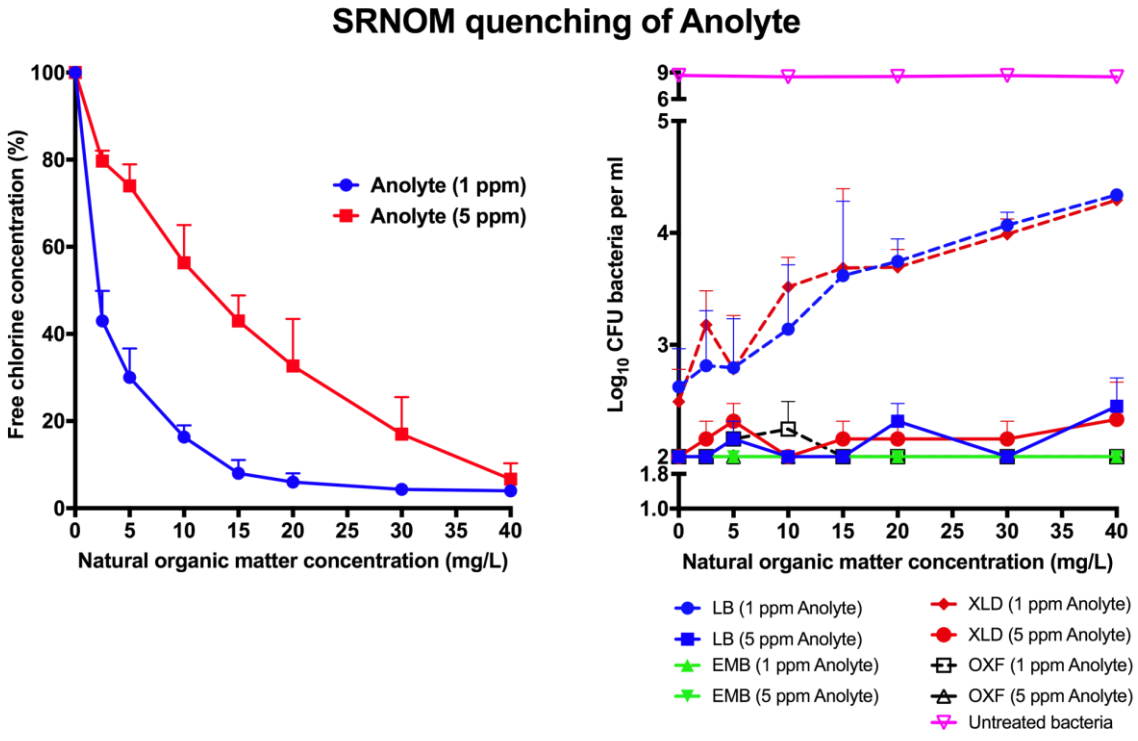

Figure 5. Bactericidal activity of the anolyte in the presence of increasing organic matter content. LB = All bacteria (*E. coli*, *S.* Enteritidis 11RX and *L. innocua* 6a); EMB = *E. coli*; XLD = *S.* Enteritidis 11RX and OXF = *L. innocua* 6a.

Comparison of EO water bactericidal activity with equivalent concentrations of other chlorine-based sanitisers NaOCl and ClO_2

We also tested the hypothesis that the efficacy of the EO water to reduce the microbial load is (at least) comparable to that of chlorine-based sanitisers by comparing its efficacy to equivalent free chlorine concentrations of other chlorine-based sanitisers (NaOCl and ClO_2) in the absence or presence of increasing organic matter content, essentially as described above. In the absence of organic matter, the efficacy of the anolyte to reduce the microbial load compares favourably with that of equivalent concentrations of NaOCl and ClO_2 (**Figure 6**). The NaOCl and EO water treatments gave the same results with ClO2 not performing as well in the case of mixed bacterial culture and *L. innocua*.

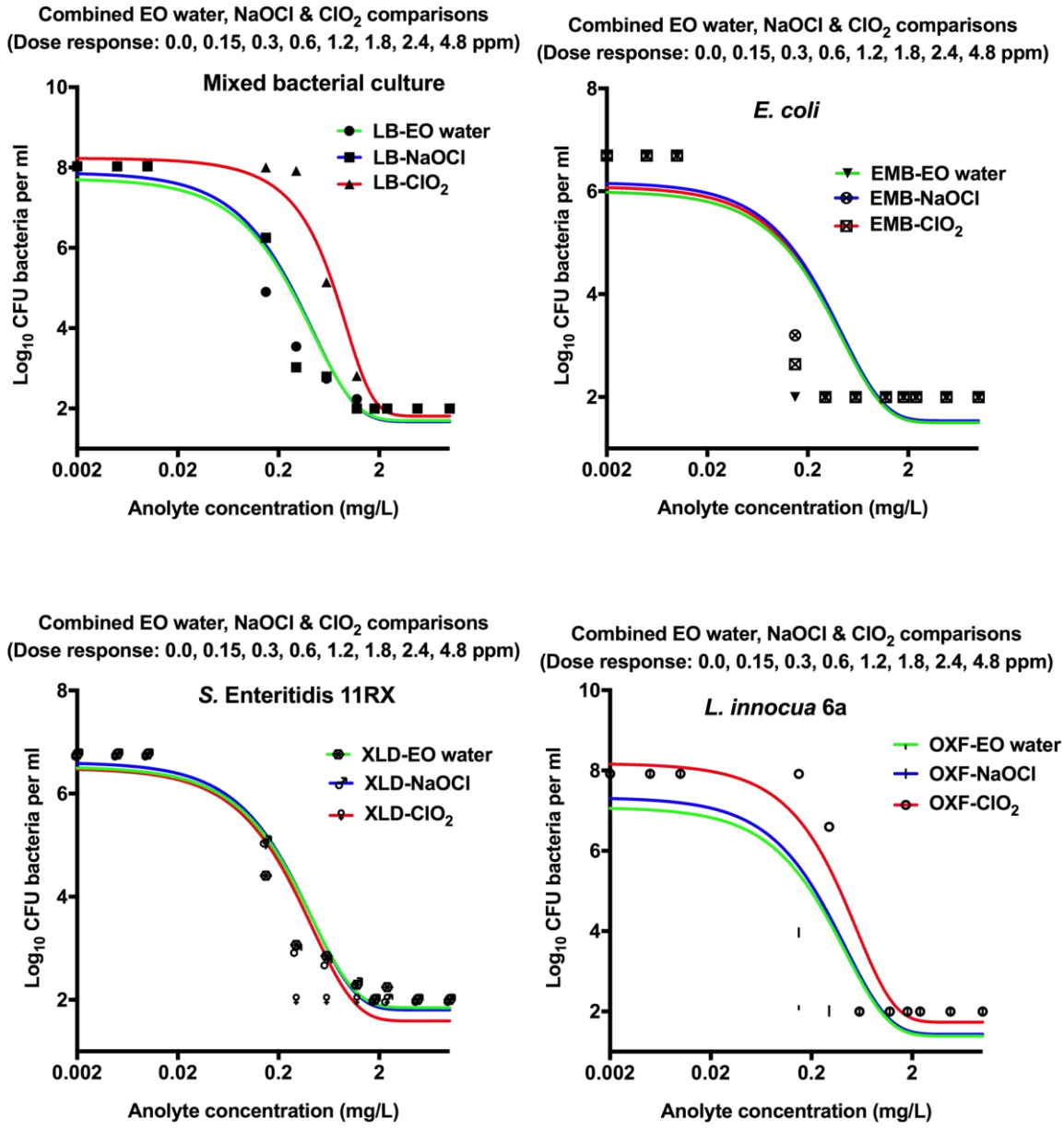

Figure 6. Comparison of EO water bactericidal activity with equivalent concentrations of other chlorine-based sanitisers NaOCl and ClO_2.

We also compared the bactericidal activities of 1 ppm and 5 ppm EO water and NaOCl in the presence of increasing organic matter content. Our results indicate that in the presence of increasing organic matter, there was a progressive inactivation of 1 ppm of either anolyte or NaOCl (**Figure 7, left panel**) as observed earlier for the anolyte previously (**Figure 5**). However, both EO water and NaOCl at 5 ppm were still strongly bactericidal in the presence of increasing organic matter (**Figure 7, right panel**). Together, these results strongly indicate that the efficacy of the anolyte to reduce the microbial load is (at least) comparable to that of chlorine-based sanitisers.

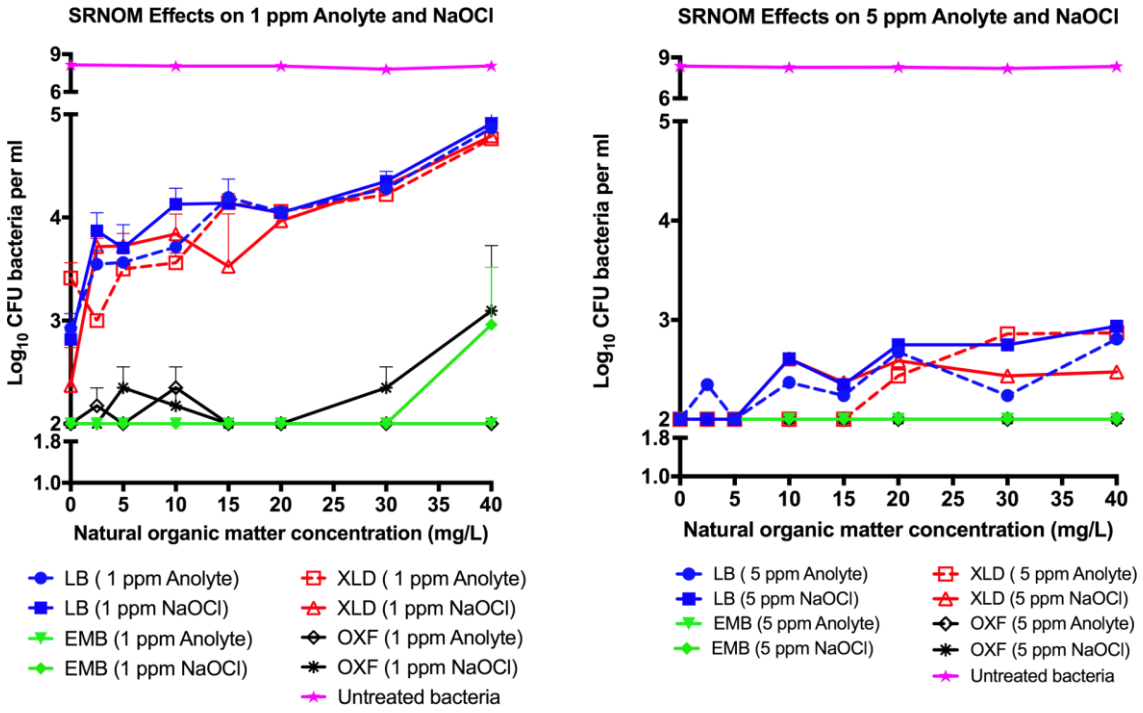

Figure 7. Comparison of bactericidal activities of EO water and NaOCl in the presence of increasing organic matter content. LB = All bacteria (*E. coli*, *S.* Enteritidis 11RX and *L. innocua* 6a); EMB = *E. coli*; XLD = *S.* Enteritidis 11RX and OXF = *L. innocua* 6a.

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Comparative antibacterial activities of neutral electrolyzed oxidizing water and other chlorine-based sanitizers

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There is increasing demand for safe and effective sanitizers for irrigation water disinfection to prevent transmission of foodborne pathogens to fresh produce. Here we compared the efficacy of pH-neutral electrolyzed oxidizing water (EOW), sodium hypochlorite (NaClO) and chlorine dioxide (ClO_2) against single and mixed populations of *E. coli*, *Listeria* and *Salmonella* under a range of pH and organic matter content. EOW treatment of the mixed bacterial suspension resulted in a dose-dependent (<1 mg/L free chlorine), rapid (<2 min) and effective (4–6 Log_{10}) reduction of the microbial load in water devoid of organic matter under the range of pH conditions tested (pH, 6.0, 7.0, 8.4 and 9.2). The efficacy of EOW containing 5 mg/L free chlorine was unaffected by increasing organic matter, and compared favourably with equivalent concentrations of NaClO and ClO_2. EOW at 20 mg/L free chlorine was more effective than NaClO and ClO_2 in reducing bacterial populations in the presence of high (20–100 mg/L) dissolved organic carbon, and no regrowth or metabolic activity was observed for EOW-treated bacteria at this concentration upon reculturing in rich media. Thus, EOW is as effective or more effective than other common chlorine-based sanitizers for pathogen reduction in contaminated water. EOW's other characteristics, such as neutral pH and ease of handling, indicate its suitability for fresh produce sanitation.

Microbial contamination of fresh produce such as spinach, lettuce, parsley and other leafy greens by opportunistic and human pathogens continues to be a major source of foodborne illnesses and disease outbreaks worldwide[1]. In most instances, pre-harvest water such as irrigation water and post-harvest washing water have been identified as the main sources of contamination associated with human illness[2,3]. Current water disinfection processes involve either the use of chemicals (such as chlorine, ozone, peracetic acid, or hydrogen peroxide), or non-chemical disinfection methods such as ultraviolet irradiation and membrane filtration[3–9]. However, these treatment technologies all have shortcomings in terms of efficacy and/or safety concerns. Consequently, there is a growing global focus on the deployment of safe, effective and environmentally-sustainable irrigation water and post-harvest sanitation technologies. One approach being explored is the use of electrolyzed oxidizing water (EOW), which is generated through the electrolysis of chloride-containing water (generally in the form of sodium or potassium chloride (NaCl/KCl) to form hypochlorous acid and reactive oxygen species ($^{\cdot}OH$, O_3, H_2O_2) that are toxic to microorganisms[10,11]. The various types of EOW described in the literature include acidic EOW (pH 2–3), slightly acidic EOW (pH 5–6.5), alkaline EOW (pH 10–13), slightly alkaline EOW (pH 8–10), and neutral EOW (pH 7–8)[12].

Studies investigating EOW treatment of aqueous human pathogen suspensions under varying conditions of exposure time, pH, temperature, available chlorine, and redox potential have consistently shown substantial log reductions in viable microorganisms[13–16]. Of the various types of EOW, neutral EOW has been considered the

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most promising as it contains predominantly HOCl, which is more effective than ClO^- for microbial cell wall penetration and oxidative attack[12,17]. However, there are limited published applications of neutral EOW use in the irrigation and washing of fresh vegetables[18,19] or fruit[20,21], with publications to date mainly focussing on its use in the seafood[22] and meat[17,23,24] industries. The use of Na-based salts rather than K for generation of the EOW might be of concern in the context of vegetable production, because of the potential problems associated with Na accumulation in soil, in contrast to the potential benefit of K supplementation for crop growth.

In this study, we aimed to establish boundary conditions (in terms of pH and organic matter content) for the effective use of neutral EOW prepared using either Na or K salts. Organic matter was introduced in the form of purified natural organic matter, γ-sterilized manure, and in filter-sterilized secondary and tertiary treated wastewater. We also compared EOW efficacy against surrogate foodborne pathogens with that of other chlorine-based sanitizers (sodium hypochlorite and chlorine dioxide). We hypothesized that EOW treatment could significantly reduce the microbial load in contaminated water, thereby expanding the range of safe source water options for fresh produce irrigation. We further hypothesized that the efficacy of EOW to reduce the microbial load would be at least comparable to that of the other chlorine-based sanitizers and that its efficacy would not be diminished under a range of pH conditions but might be under conditions of high organic matter content. Finally, we investigated the efficacy of EOW and other chlorine-based sanitizers in relation to their potential to induce viable but nonculturable (VBNC) cells. This is a significant concern associated with disinfection processes, especially in terms of the potential dissemination of VBNC pathogens via treated irrigation or post-harvest wash water. The VBNC state is a survival strategy used by many bacteria in response to adverse environmental conditions[25,26] and multiple works have described the potential for induction of the VBNC state during water disinfection processes[27–29]. This is of importance for improving the quality and safety of fresh produce and preventing future outbreaks, thereby increasing consumer confidence in consumption, particularly by vulnerable individuals.

Methods

Bacterial strains, growth conditions and inocula preparation. The bacterial strains used in this study were *Escherichia coli* (ATCC 25922), bioluminescent *E. coli* WS2572 (Xen14), *Listeria innocua* 6a (ATCC 33090) and *Salmonella enterica* serovar Enteritidis 11RX[30,31]. Glycerol stock cultures were maintained at −80 °C and were streaked onto Luria Bertani (LB) agar (Oxoid; Thermo Fisher Scientific, Scoresby, VIC, Australia) to obtain isolated colonies. Single colonies were streaked onto the following selective agar plates (Thermo Fisher Scientific) for presumptive identification: Eosin Methylene Blue agar (EMB; PP2169) for *E. coli*, Oxford *Listeria* Selective agar (OXF; PP2141) for *L. innocua* 6a, and Xylose Lysine Deoxycholate agar (XLD; PP2004) for *S.* Enteritidis 11RX.

For experiments, single colonies from selective agar plates were emulsified in LB broth and grown overnight at 37 °C with aeration at 150 rpm on a digital platform mixer (Ratek Instruments, Boronia, VIC, Australia). Thereafter, bacteria were subcultured at a 1:10 dilution into fresh LB broth and incubated further at 180 rpm until the optical density at 600 nm (OD_{600}) = 1.0 (for *E. coli* and *S.* Enteritidis 11RX) or OD_{600} = 0.5 (for *L. innocua* 6a) was reached (equivalent to approx. 1×10^9 colony-forming units (CFU)/mL for each strain). Bacteria were then harvested and washed extensively (3×) in autoclave-sterilized Milli-Q water (PURELAB Classic, ELGA; High Wycombe, UK) to remove residual culture medium and then resuspended in sterile Milli-Q water to approx. 1×10^9 CFU/mL for each strain. Where mixed bacterial suspensions were tested, the bacteria were mixed immediately prior to use at approximately equal concentrations to ensure that there was no substantial change in the relationship among the bacteria in the time between mixing and application of the disinfection treatments. The initial concentration of bacteria was high (~2×10^8 CFU/mL) to simulate the worst-case scenario of high bacterial load.

Reagents, solutions and instruments. Neutral EOW was provided by Ecas4 Australia Pty Ltd (8/1 London Road, Mile End South, SA, Australia) at 300–350 mg/L free chlorine. Sodium hypochlorite (NaClO; UN No 1791) was obtained as a 12.5% solution from Chemwell Pty Ltd (3 Clive St, Springvale, VIC, Australia). Chlorine dioxide (ClO_2) was obtained as TwinOxide Tabs® Part No 121710 (TwinOxide®; supplied by Integra Water, Regency Park, SA, Australia) and was prepared as a 1,000 mg/L chlorine solution according to the manufacturer's instructions. Several different treatment solutions were established to test the efficacy of EOW, NaClO and ClO_2. Suwannee River natural organic matter (SRNOM; 2R101N, International Humic Substances Society, St Paul, MN, USA) was used as the source of natural organic matter and was resuspended to the equivalent of 200 mg/L dissolved organic carbon (DOC). Cow manure was γ-sterilized at Steritech (Melbourne, VIC, Australia), dried in an oven at 37 °C and ground to a fine powder using an analytical mill (IKA, Selangor, Malaysia) and then resuspended to the equivalent of 10 g/L in sterile Milli-Q water (pH 7.0). Finally, secondary treated wastewater and tertiary treated effluent from a wastewater treatment plant in Adelaide, South Australia were filter sterilized to produce test solutions with realistic background chemistry. The DOC contents in the SRNOM suspension, cow manure suspension and secondary and tertiary treated effluents were measured on a Shimadzu TOC-L total organic carbon analyzer (Shimadzu Australasia, Rydalmere, NSW, Australia).

Milli-Q water at pH 6.0, 7.0, or 8.4 was prepared using 1 mM $NaNO_3$ as background electrolyte, while Milli-Q water at pH 9.2 was prepared in 0.01 M carbonate-bicarbonate buffer (9.1 mM sodium bicarbonate and 0.9 mM anhydrous sodium carbonate) and verified on a Eutech Instruments PC 700 pH meter (Thermo Fisher Scientific). Dilutions of disinfectants with different estimated free chlorine concentrations were prepared in these buffers. The amount of free chlorine in EOW and NaClO was measured using a free chlorine and chlorine ultra-high range portable photometer (HI 96771 C; Hanna Instruments, Keysborough, VIC, Australia) according to the manufacturer's recommendations, while the concentration of ClO_2 was measured using method 10126 for water on a HACH DR/890 Colorimeter (Hach Pacific, Dandenong South, VIC, Australia) according to the manufacturer's instructions.

Time-kill and dose response analysis of EOW. *Time kill.* A time-kill experiment was conducted to determine the appropriate length of time required for bacterial inactivation by EOW. Na-based EOW was prepared to provide 0, 3, 30 and 150 mg/L free chlorine in Milli-Q water (pH 7.0), to each of which approx. 2×10^8 CFU *E. coli*, *L. innocua* 6a or *S.* Enteritidis 11RX was added. Aliquots were then withdrawn at 0, 5, 30, 60, 120, 300 and 600 s and disinfectant activity was neutralized with a 0.05% (v/v) final concentration of sodium thiosulphate ($Na_2S_2O_3$). Viable counts were obtained by serial 10-fold dilution in phosphate-buffered saline (PBS) and plating onto selective (OXF for *L. innocua*, EMB for *E. coli* and XLD for *S.* Enteritidis) and non-selective (LB for all strains) media followed by incubation overnight at 37 °C. The limit of detection for viable counts was set at 100 CFU/mL in all experiments. All experiments were independently repeated four times.

Comparison of Na- and K-based EOW and dose-response assessments. From the time-kill experiment, a time point of 120 s was chosen for all subsequent experiments as a reasonable worst-case scenario of a short contact time under field conditions. A comparison of the efficacy of Na- or K-based EOW was conducted at a range of chlorine concentrations up to 4.8 mg/L followed by a dose-response experiment with the mixed bacterial suspension using the Na-based EOW. Disinfectant inactivation, dilution and plating were conducted as described above.

Effect of pH on EOW efficacy and comparison with other sanitizers (NaClO and ClO_2). The efficacy of EOW was tested at pH 6.0, 7.0 and 8.4 (unbuffered) or at pH 6.0, 7.0 and 9.2 (buffered) at free chlorine concentrations of up to 4.8 mg/L for the mixed culture as described above. Based on the above experiment, the efficacy of EOW was compared with that of NaClO and ClO_2 at equivalent free chlorine concentrations of up to 4.8 mg/L at pH 7.0. Disinfectant inactivation, dilution and plating were conducted as described above.

Effect of organic matter content on EOW efficacy and comparison with NaClO. The effect of increasing concentrations of DOC on the amount of free chlorine present in EOW that had been prepared with initial concentrations of 1 and 5 mg/L free chlorine was determined using SRNOM concentrations of up to 40 mg/L DOC. The abilities of EOW and NaClO to reduce microbial loading in the presence of organic matter were compared using EOW and NaClO at 1 and 5 mg/L free chlorine concentration in the presence of SRNOM at concentrations of up to 40 mg/L DOC. The viability assays were conducted on the mixed bacterial culture as described above. The cells were added to organic matter solutions prior to initiation of the timed assay by the addition of the sanitizer solution.

Comparative assessment of the bactericidal action of EOW, NaClO, and ClO_2. To investigate the potential for induction of the viable but nonculturable (VBNC) state by the different sanitizers, a combination of metabolic activity measurements and molecular approaches were used.

For metabolic activity measurements, ~5×10^7 CFU of bioluminescent *E. coli* Xen14 (PerkinElmer Inc, MA, USA) was added to EOW, NaClO or ClO_2 prepared at 0, 1, 5, 20 and 50 mg/L free chlorine in the presence of either 40 mg/L DOC from SRNOM or 100 mg/L DOC from cow manure for 120 s at room temperature, before stopping the reaction using $Na_2S_2O_3$. Untreated bacteria resuspended in sterile RO water, 40 mg/L DOC from SRNOM or 100 mg/L DOC from cow manure were used as controls. Samples were then serially diluted in PBS and plated on LB agar for bacterial enumeration. To measure bioluminescence, ~1×10^6 CFU of each treatment was added to 200 µL sterile LB broth in a Nunc™ F96 MicroWell™ Black plate (Thermo Scientific, 237105) which was then incubated at 37 °C in a Cytation 5 Cell Imaging Multi-Mode Reader (BioTek; Winooski, VT, USA). Total luminescent signals (relative light units) and optical density measurements (A_{600nm}) were collected over a 40-h incubation period. In another set of experiments, sterile RO water, tertiary treated wastewater (containing 5.6 mg/L DOC) or secondary treated wastewater (containing 19.2 mg/L DOC) were treated as described above. Each experiment was performed on two separate occasions. On one occasion, 20 µL samples from the experiment using the secondary and tertiary treated effluents after 40 h incubation were re-inoculated into 180 µL sterile LB broth in a Nunc™ F96 MicroWell™ Black plate and incubated at 37 °C for an additional 40 h in the Cytation 5 Cell Imaging Multi-Mode Reader to examine any potential regrowth or metabolic activity.

For the molecular analysis of the luminescent *E. coli* (Xen14) cell populations treated above, the propidium monoazide (PMAxx™) real-time PCR bacterial viability protocol (Biotium, USA; Cat No 31050-X) was used, following the manufacturer's recommendations, essentially as described recently[32] using the PMA Enhancer solution for Gram-negative bacteria and PMA-Lite™ LED Photolysis Device for photoactivation. Genomic DNA from bacteria treated above was extracted using the QiIAamp DNA Mini kit (Cat No: 51304; QIAGEN) following the protocol for DNA extraction from Gram-negative bacteria. Quantitative PCR was performed on a LC480 II instrument (Roche Diagnostics) using 16 S rRNA gene primers F: 5′-TCCTACGGGAGGCAGCAGT-3′ and R: 5′-ATTACCGCGGCTGCTGG–3′ and associated fast cycling parameters in Cat No 31050-X (Biotium).

Results and Discussion

Neutral EOW elicits a rapid, dose-dependent and substantial reduction in viable counts of single or mixed bacterial suspensions in water. Results of preliminary experiments showed that EOW at 3 mg/L consistently inhibited the growth of the tested bacteria at 120 s post-exposure (not shown). Non-linear regression analysis indicated that the EOW treatment of the mixed bacterial suspension at low doses (<1 mg/L free chlorine) resulted in substantial (4–6 Log_{10}) reduction in viable counts of all the bacteria tested, comparable to that reported for similar sanitizers by other researchers (reviewed by Rahman *et al.*[12]).

Sodium (Na)- and potassium (K)-based EOW elicit similar efficacy profiles. The ability of Na- and K-based EOW to inhibit bacterial growth was compared to determine whether there are differences in their efficacy, using the time and dose-dependent kill kinetics of the Na-based EOW established above. Our results

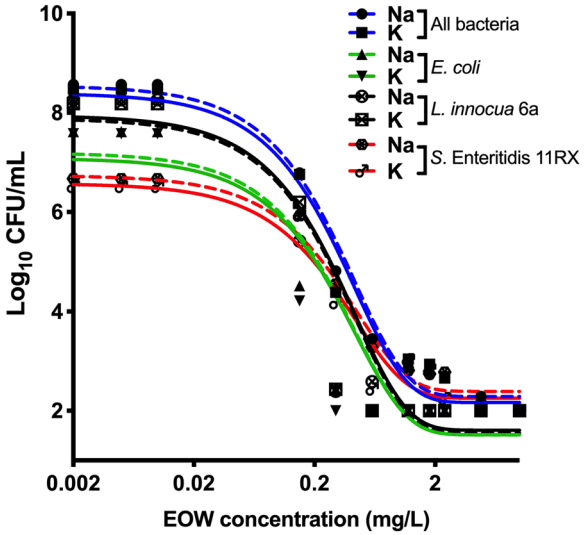

Figure 1. Comparison of sodium (Na)- and potassium (K)-based EOW against a mixed bacterial suspension of *Escherichia coli*, *Listeria innocua* 6a and *Salmonella* Enteritidis 11RX. EOW: electrolyzed oxidizing water in mg/L of free chlorine; CFU: colony forming units. Figures were generated using Prism v8 (GraphPad Software, San Diego, CA, USA).

showed that the efficacy kinetics of Na- and K-based EOW were remarkably similar (Fig. 1). This could be valuable information for growers who might be concerned about Na levels in irrigation water and would prefer to use K-based EOW instead.

EOW is effective under a range of pH conditions. The efficacy of EOW was tested against the mixed bacterial population at pH 6.0, 7.0, or 8.4 (using 1 mM $NaNO_3$ as background electrolyte). We found that the $NaNO_3$ electrolyte did not function as an effective buffer, with the pH decreasing to near neutral for the pH 8.4 solution on addition of increasing concentrations of EOW. Thereafter, water at pH 9.2 was prepared in 0.01 M carbonate-bicarbonate buffer, which was stable in the presence of increasing EOW concentration, and the experiment was repeated. Overall, our results showed that the activity and efficacy of the EOW was not appreciably affected under the range of unbuffered and buffered pH conditions (pH 6.0, pH 7.0, pH 8.4 and pH 9.2) tested (Fig. 2). The efficacy of EOW at pH 9.2 was somehow unexpected, as Pangloli and Hung[33] found that the bactericidal efficacy of EOW against *E. coli* O157:H7 at pH values in the range of 5–8 was unaffected, but that there was a significant decrease in efficacy at pH 8 against *L. monocytogenes*. Similarly, Rahman *et al.*[15] found that the ability of EOW to inactivate all organisms was diminished at pH 9.0. It is unclear which factors contributed to the high activity of the neutral EOW used in this study at high pH. However, it is possible that the consistent free chlorine content and high oxidation-reduction potential of EOW in our study might have contributed synergistically to its efficacy regardless of pH, as suggested by some studies[34,35].

The activity of EOW compares favourably with equivalent concentrations of other chlorine-based sanitizers (NaClO and ClO_2). We tested the hypothesis that the efficacy of EOW to reduce the microbial load is (at least) comparable to equivalent free chlorine concentrations of other chlorine-based sanitizers (NaClO and ClO_2). Our analysis showed that the efficacy of EOW in reducing the microbial load compared favourably with that of equivalent concentrations of NaClO and ClO_2 (Fig. 3). Our analysis confirmed the hypothesis, as NaClO and EOW treatments gave the same results, whereas ClO_2 did not perform as well in the case of *L. innocua* and the mixed bacterial culture (Fig. 3).

Activity of EOW in the presence of increasing organic matter content. We tested the effects of increasing concentrations of DOC (using SRNOM) on the amount of free chlorine present in EOW at starting concentrations of 1 or 5 mg/L free chlorine. We found a dose-dependent reduction in the amount of free chlorine at both concentrations, with only 0.43 mg/L of free chlorine residual in the 1 mg/L EOW in the presence of 2.5 mg/L SRNOM, and 0.85 mg/L of free chlorine in the 5 mg/L EOW in the presence of 30 mg/L SRNOM (Fig. 4a).

We then tested the hypothesis that the efficacy of EOW to reduce the microbial loading of irrigation water will be reduced in the presence of organic matter by assessing the bactericidal activity of EOW in the presence of SRNOM. As expected, we found that the ability of EOW containing 1 mg/L free chlorine to reduce the bacterial population was substantially reduced in the presence of increasing organic matter content, but its efficacy was not appreciably affected when the SRNOM was added to EOW containing 5 mg/L free chlorine (Fig. 4b).

Efficacy of EOW and NaClO are similar in the presence of organic matter. We compared the efficacy of 1 and 5 mg/L EOW and NaClO in the presence of increasing organic matter content. The efficacy of ClO_2 was not assessed on this occasion as it did not perform as well as EOW and NaClO in our earlier evaluation. Our

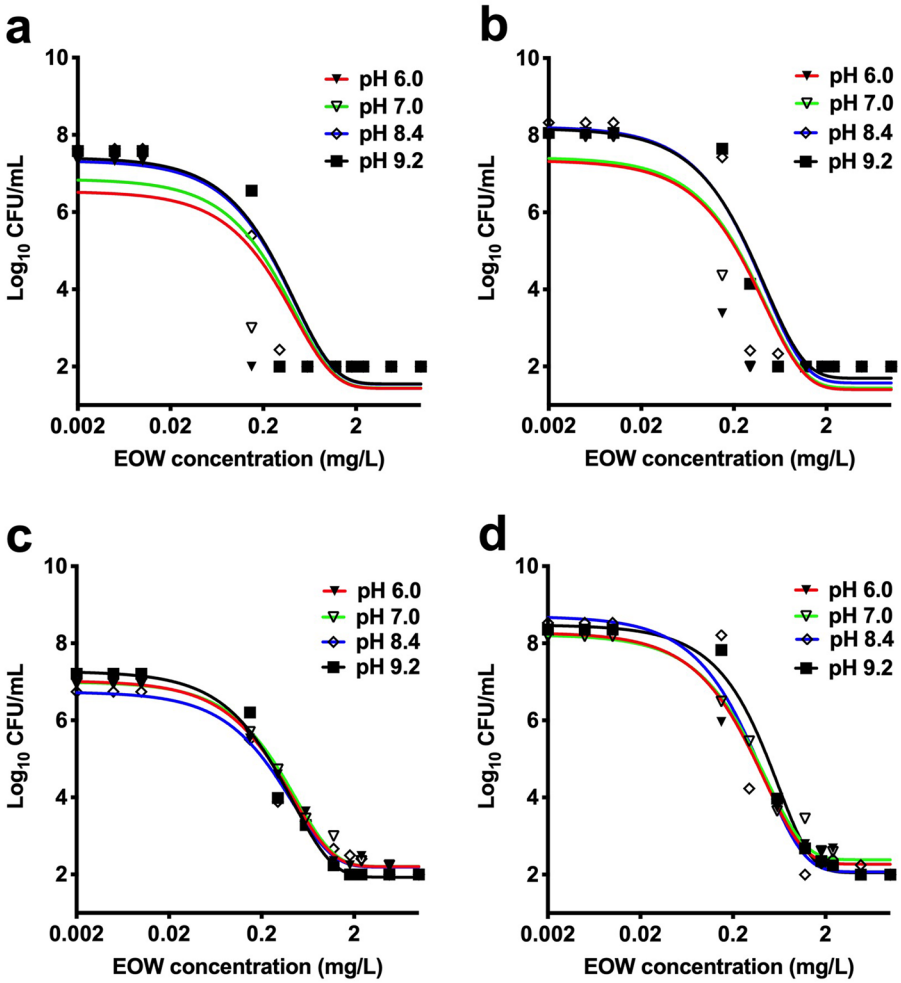

Figure 2. Bactericidal activity of electrolyzed oxidizing water (EOW) under a range of pH conditions. (**a**) *Escherichia coli*, (**b**) *Listeria innocua* 6a, (**c**) *Salmonella* Enteritidis 11RX, and (**d**) mixed bacterial culture. CFU: colony forming units; EOW concentration refers to mg/L of free chlorine. Figures were generated using Prism v8 (GraphPad Software, San Diego, CA, USA).

results show that there was progressive inactivation of both 1 mg/L EOW and 1 mg/L NaClO (Fig. 4c), as observed earlier for EOW (Fig. 4a,b) and as reported by other researchers[36,37]. The mechanism by which organic matter reduces the activity of EOW or NaClO is by quenching the activity of the free available chlorine, leading to lower concentrations of chlorine available to act on pathogens; if the concentration of free chlorine is reduced to below the effective concentration required to kill the pathogen, this might lead to reduced kill rates and/or induction of VBNC cells[38,39] (also see below). However, both EOW and NaClO were still strongly inhibitory in the presence of increasing organic matter when their starting concentration was set at 5 mg/L free chlorine (Fig. 4d). Together, these results strongly indicate that the efficacy of EOW to reduce the microbial load is (at least) comparable to that of the other chlorine-based sanitizers.

EOW is bactericidal and could potentially reduce induction of the VBNC state in bacterial populations. It has been widely reported in the literature that chlorine-based sanitizers have the propensity to induce the VBNC state in bacteria[25,27–29,40–42]. Despite the widespread use of chlorine-based sanitizers, testing for VBNC is not widely undertaken and effective concentrations for disinfection of irrigation or process wash water without induction of VBNC have not been established. The use of sanitizers at concentrations below the effective concentration could result in dissemination or transfer of VBNC cells, which can then resuscitate and lead to outbreaks of food-borne disease[25]. This is of particular concern in low-quality irrigation waters, where disinfection efficacy might be compromised by organic matter content or other factors. Therefore, it was of interest to investigate whether EOW also induces the VBNC state in the bacterial populations being tested. For this assessment, we initially examined the effects of different concentrations of EOW, NaClO or ClO_2 in the presence or absence of SRNOM or γ-sterilized cow manure on the viability and metabolic activity of bioluminescent *E. coli* Xen14. We found that, in the absence of organic matter, EOW and NaClO were bactericidal (and little to no metabolic activity was observed) in the range of concentrations (1–50 mg/L free chlorine) used over the 40 h incubation period (Fig. 5a,d). However,

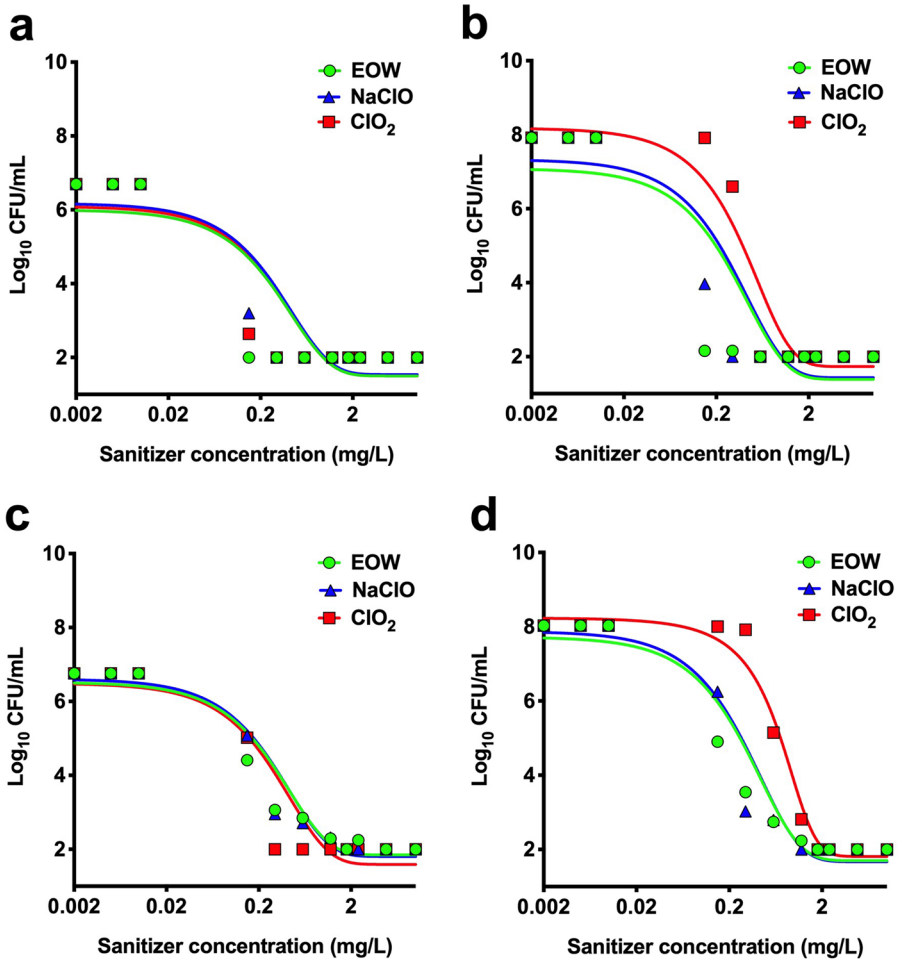

Figure 3. Comparison of electrolyzed oxidizing water (EOW) activity with equivalent concentrations of sodium hypochlorite (NaClO) and chlorine dioxide (ClO_2) expressed as mg/L of free chlorine. (**a**) *Escherichia coli*, (**b**) *Listeria innocua* 6a, (**c**) *Salmonella* Enteritidis 11RX, and (**d**) mixed bacterial culture. Figures were generated using Prism v8 (GraphPad Software, San Diego, CA, USA).

detectable metabolic activity was already observed for Xen14 treated with ClO_2 at as low as 1 mg/L free chlorine (Fig. 5g) despite the absence of visible growth on culture plates at this concentration, a strong indication of VBNC bacteria. Furthermore, we found that no metabolic activity was observed for Xen14 treated in the range of EOW and NaClO concentrations at which no growth was observed on agar plates used in the presence of SRNOM or cow manure over the 40 h incubation period (Fig. 5b,c,e). The efficacy of EOW was slightly superior to that of NaClO under these conditions, being bactericidal at 20 mg/L free chlorine, while NaClO was only bactericidal at 50 mg/L free chlorine in the presence of cow manure. The efficacy of ClO_2 was poor in the presence of organic matter, with complete kill only observed when it was used at the highest concentration (50 mg/L free chlorine) in the presence of SRNOM. Furthermore, ClO_2 exerted no measurable activity at this concentration in the presence of cow manure. Bacterial plate counts from all treatments were also determined for comparison (Table S1).

There are no equivalent studies for a comparison with the results of this study in terms of DOC as a measure of organic load. Han *et al.*[23] obtained an effective EOW concentration of 37 mg/L of free chlorine against suspensions of *E. coli*, *S.* Enteriditis and *Yersinia enterocolitica* in the absence of organic matter. A recent study by Afari *et al.*[39] showed the induction of VBNC *E. coli* and *L. monocytogenes* after treatment of inoculated lettuce wash water with acidic EO water; their results suggested the effective concentration of 9 mg/L acidic EO water in lettuce wash water. However, they used UV_{254} as a measure of the organic load, making it difficult to directly compare to the results of our study. To confirm the above findings using naturally-occurring DOC on sanitizer efficacy, another set of experiments using sterile RO water, tertiary treated wastewater effluent (containing 5.6 mg/L DOC), or secondary treated wastewater effluent (containing 19.2 mg/L DOC) was performed. Again, the efficacy of 1 mg/L EOW was slightly superior to the equivalent concentration of NaClO in RO water and tertiary-treated effluent water (Fig. 6a,b,d,e). The results also show the superior antibacterial efficacy of EOW and NaClO over ClO_2 under all the conditions tested, but particularly so in the secondary treated wastewater with high DOC content (Fig. 6c,f,i).

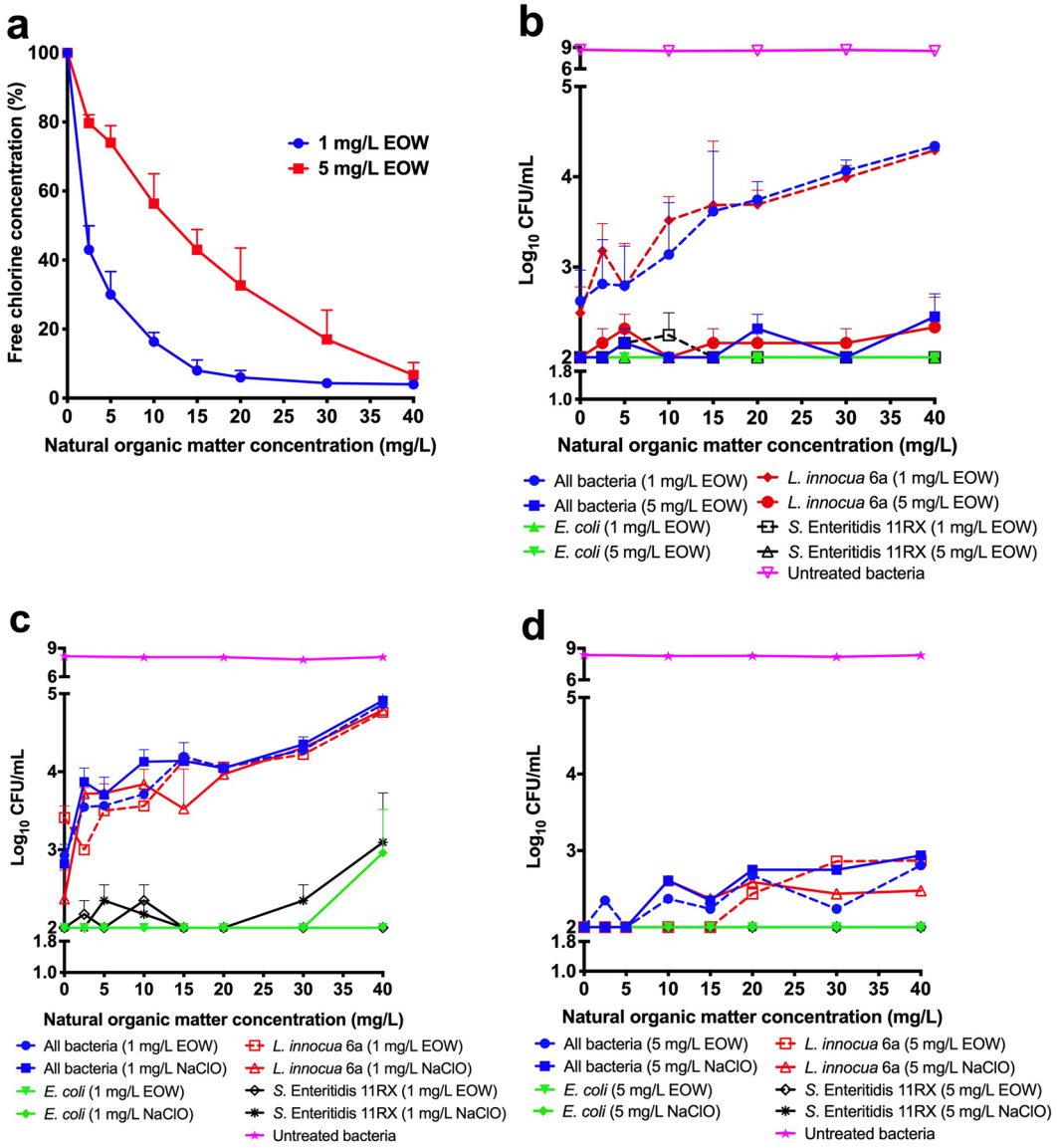

Figure 4. Effect of natural organic matter on activity of electrolyzed oxidizing water (EOW) and sodium hypochlorite (NaClO). (**a**) Quenching of free chlorine concentration by increasing organic matter content, (**b**) Inhibitory activity of EOW in the presence of increasing organic matter content, (**c**,**d**) Comparison of bactericidal activities of EOW and NaClO in the presence of increasing organic matter content. *E. coli*: *Escherichia coli*; *L. innocua*: *Listeria innocua*; *S.* Enteritidis: *Salmonella* Enteritidis. Figures were generated using Prism v8 (GraphPad Software, San Diego, CA, USA).

In a subsequent experiment where aliquots of samples analyzed in Fig. 6 were re-inoculated into fresh LB broth and re-incubated for an additional 40 h to examine the potential for regrowth, the results showed essentially similar trends to those of the initial 40 h incubation (Fig. 7). The results showed that at the effective concentration of the disinfectant, no metabolic activity was detected, indicating effectiveness of the disinfectant. VBNC cells were also undetectable under these conditions. Together with corresponding optical density (A_{600nm}) measurements (Fig. S1) these data corroborate our postulation that EOW does not appear to induce the VBNC state at its effective concentration.

To strengthen the results obtained in the metabolic activity assays, aliquots of the *E. coli* Xen14 cells treated with the various concentrations of EOW, NaClO or ClO_2 in the presence of sterile water, tertiary-treated effluent water or secondary-treated effluent water described above were treated with the photoreactive DNA binding dye PMAxx™, followed by real-time qPCR analysis. The results largely agree with the metabolic activity assay results, indicating overall superior antibacterial efficacy of EOW and NaClO over ClO_2 especially in RO water and tertiary-treated wastewater containing 5.6 mg/L DOC content (Fig. 8). Viable counts of bacteria from all treatments were also determined for comparison (Table S2).

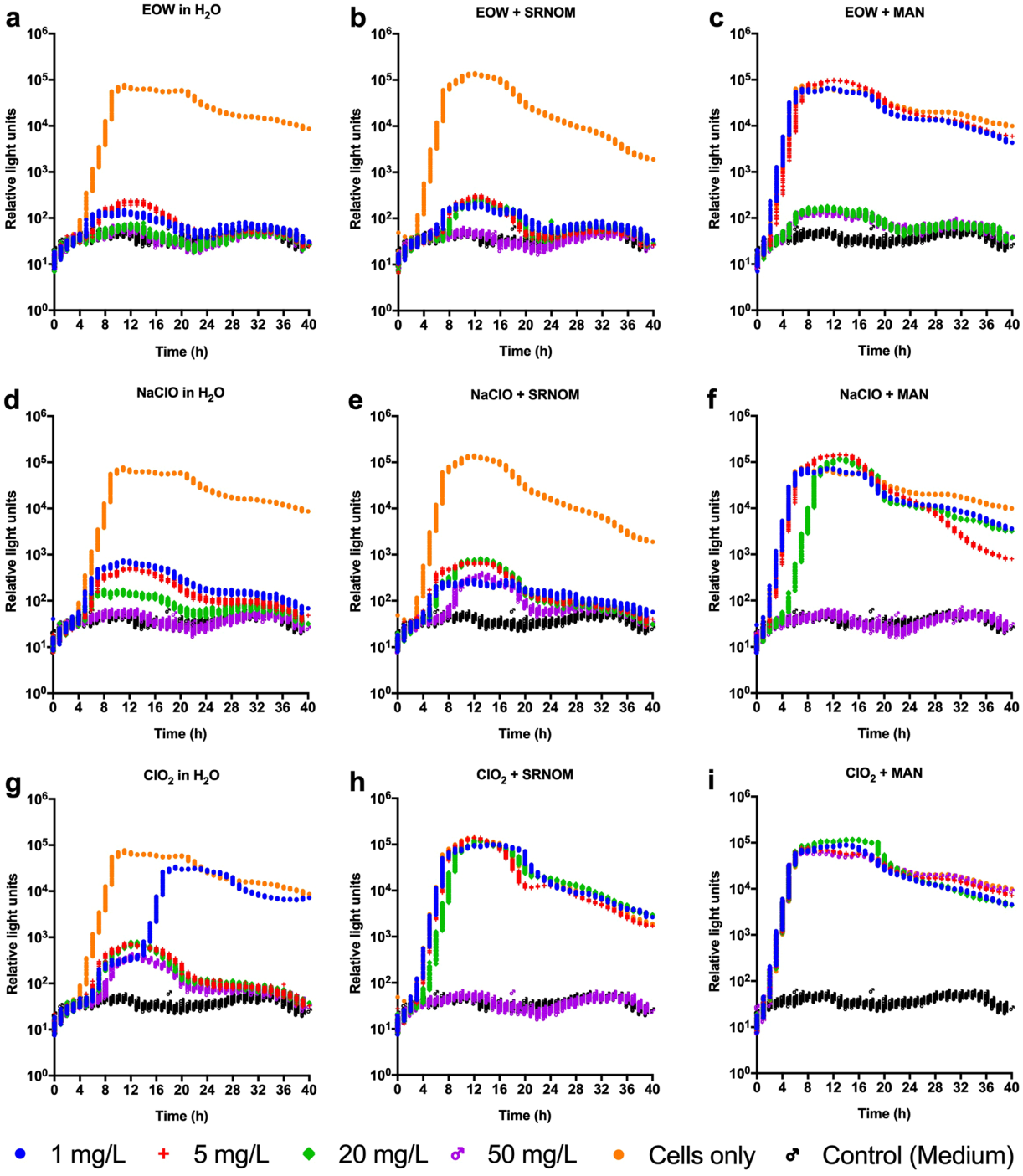

Figure 5. Metabolic activity measurements of bioluminescent *Escherichia coli* Xen14 treated with sanitizers added to artificially-contaminated water. Bacteria were treated with electrolyzed oxidizing water (EOW), sodium hypochlorite (NaClO) or chlorine dioxide (ClO_2) prepared at 0, 1, 5, 20 and 50 mg/L free chlorine in the presence of either 40 mg/L dissolved organic carbon (DOC) from Suwannee river natural organic matter (SRNOM) or 100 mg/L DOC from cow manure for 120 s. Untreated bacteria resuspended in sterile water, 40 mg/L DOC from SRNOM or 100 mg/L DOC from cow manure were used as controls. Aliquots of treated samples were added to 200 µL sterile Luria Bertani broth and then incubated at 37 °C in a Cytation 5 Cell Imaging Multi-Mode Reader. Total luminescent signals (relative light units) were collected over a 40 h incubation period. Figures were generated using Prism v8 (GraphPad Software, San Diego, CA, USA).

Conclusions

In this study, we have shown that EOW prepared using either Na or K salts significantly reduced the microbial load in artificially contaminated water and demonstrated that its efficacy was not affected under a range of pH conditions but rather by the organic matter content of the water. Furthermore, we showed that the efficacy of

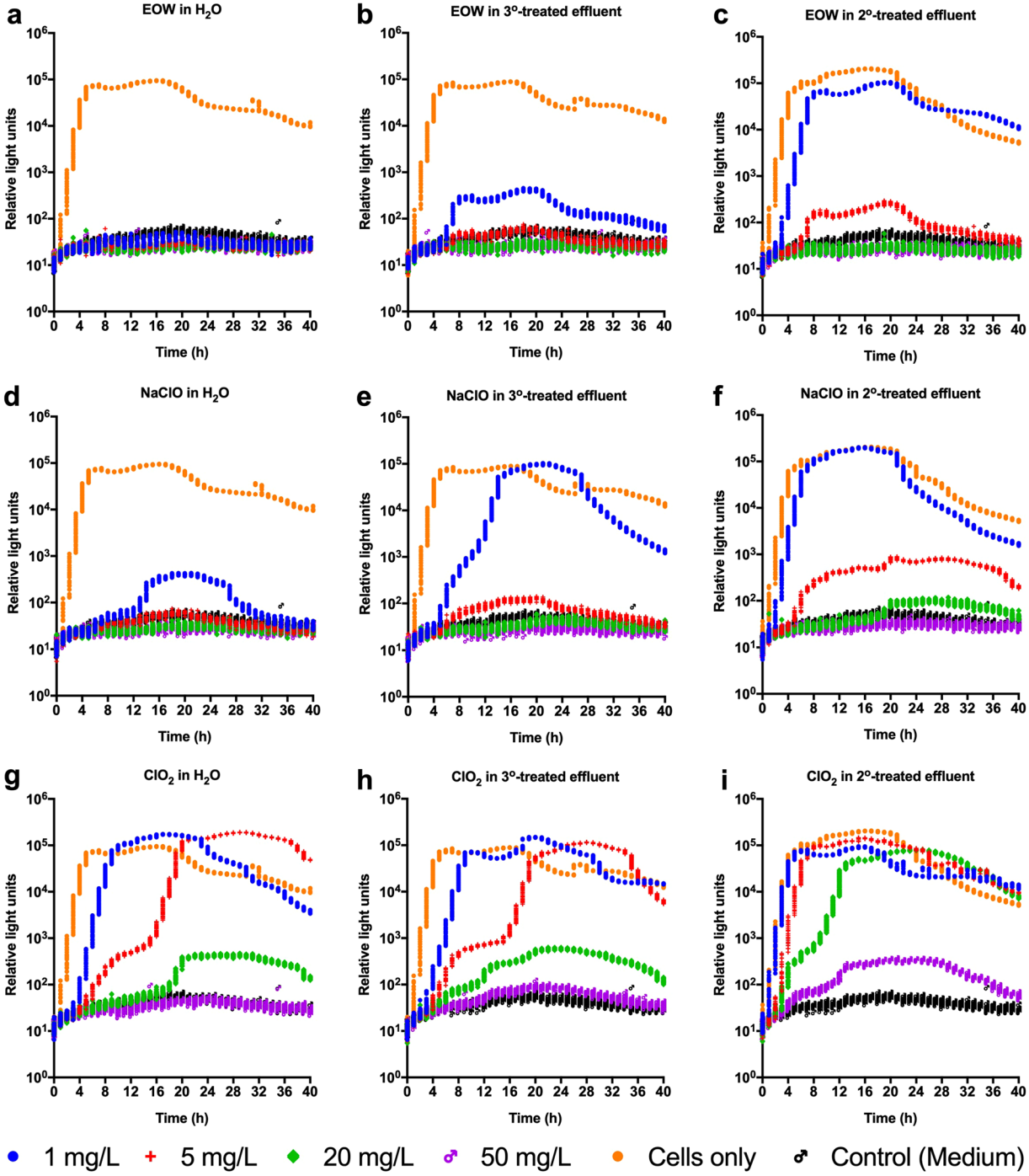

Figure 6. Metabolic activity measurements of bioluminescent *Escherichia coli* Xen14 treated with sanitizers added to wastewater effluent. Bacteria were treated with electrolyzed oxidizing water (EOW), sodium hypochlorite (NaClO) or chlorine dioxide (ClO_2) prepared at 0, 1, 5, 20 and 50 mg/L free chlorine in the presence of either tertiary (3°)-treated effluent water (containing 5.6 mg/L dissolved organic carbon [DOC]) or secondary (2°)-treated effluent water (containing 19.2 mg/L DOC) for 120 s. Untreated bacteria resuspended in sterile water, 3°-treated or 2°-treated water were used as controls. Aliquots of treated samples were added to 200 µL sterile Luria Bertani broth and then incubated at 37 °C in a Cytation 5 Cell Imaging Multi-Mode Reader. Total luminescent signals (relative light units) were collected over a 40 h incubation period. Figures were generated using Prism v8 (GraphPad Software, San Diego, CA, USA).

EOW to reduce the microbial load was comparable, and in some cases better than that of the other chlorine-based sanitizers (NaClO and ClO_2). Critically, we showed that at its effective concentration (20 mg/L), EOW did not induce VBNC cells of the surrogate bacterial pathogens tested. In comparison, the effective concentration of

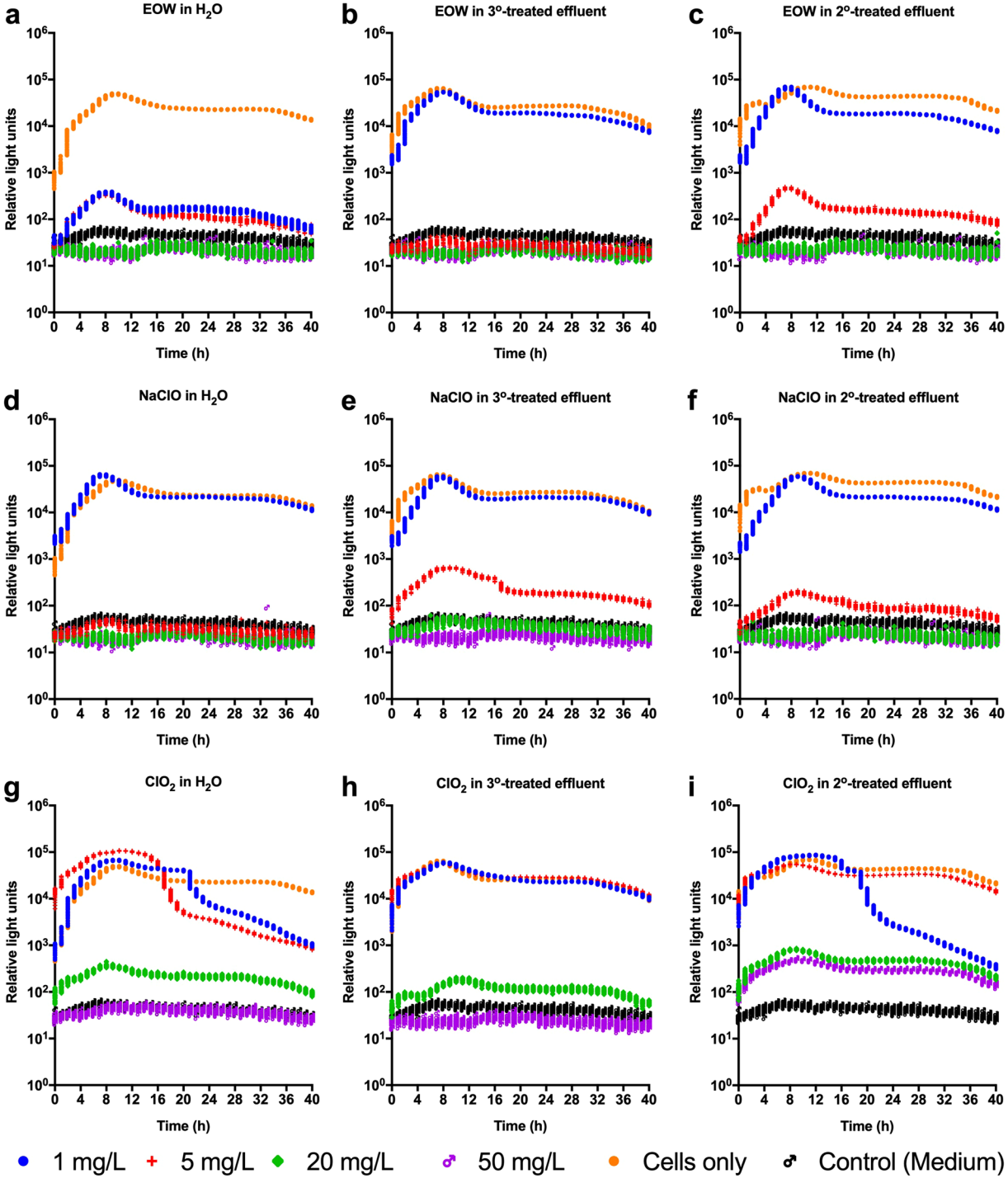

Figure 7. Regrowth of sanitizer-treated bioluminescent *Escherichia coli* Xen14. Aliquots of all samples treated with electrolyzed oxidizing water (EOW) sodium hypochlorite (NaClO) or chlorine dioxide (ClO_2) in Fig. 6 were added to fresh Luria Bertani broth and then incubated at 37 °C in a Cytation 5 Cell Imaging Multi-Mode Reader for another 40 h. Total luminescent signals (relative light units) were collected over a 40 h incubation period. 3°, tertiary treated effluent water; 2°, secondary treated effluent water. Figures were generated using Prism v8 (GraphPad Software, San Diego, CA, USA).

NaClO was 50 mg/L and ClO_2 was not effective at the highest concentration tested. The propensity for ClO_2 to induce the VBNC state in *E. coli* and other bacteria has been described[25,28,42–44]; the finding that EOW at its effective concentration in the presence of high organic matter did not induce the VBNC state is an additional feature that growers could find advantageous over the use of other chlorine-based sanitizers. To the best of our knowledge, this study is the first to systematically address the effect of organic matter content in terms of DOC on the efficacy of chlorine-based sanitizers, thus providing a benchmark for future studies and

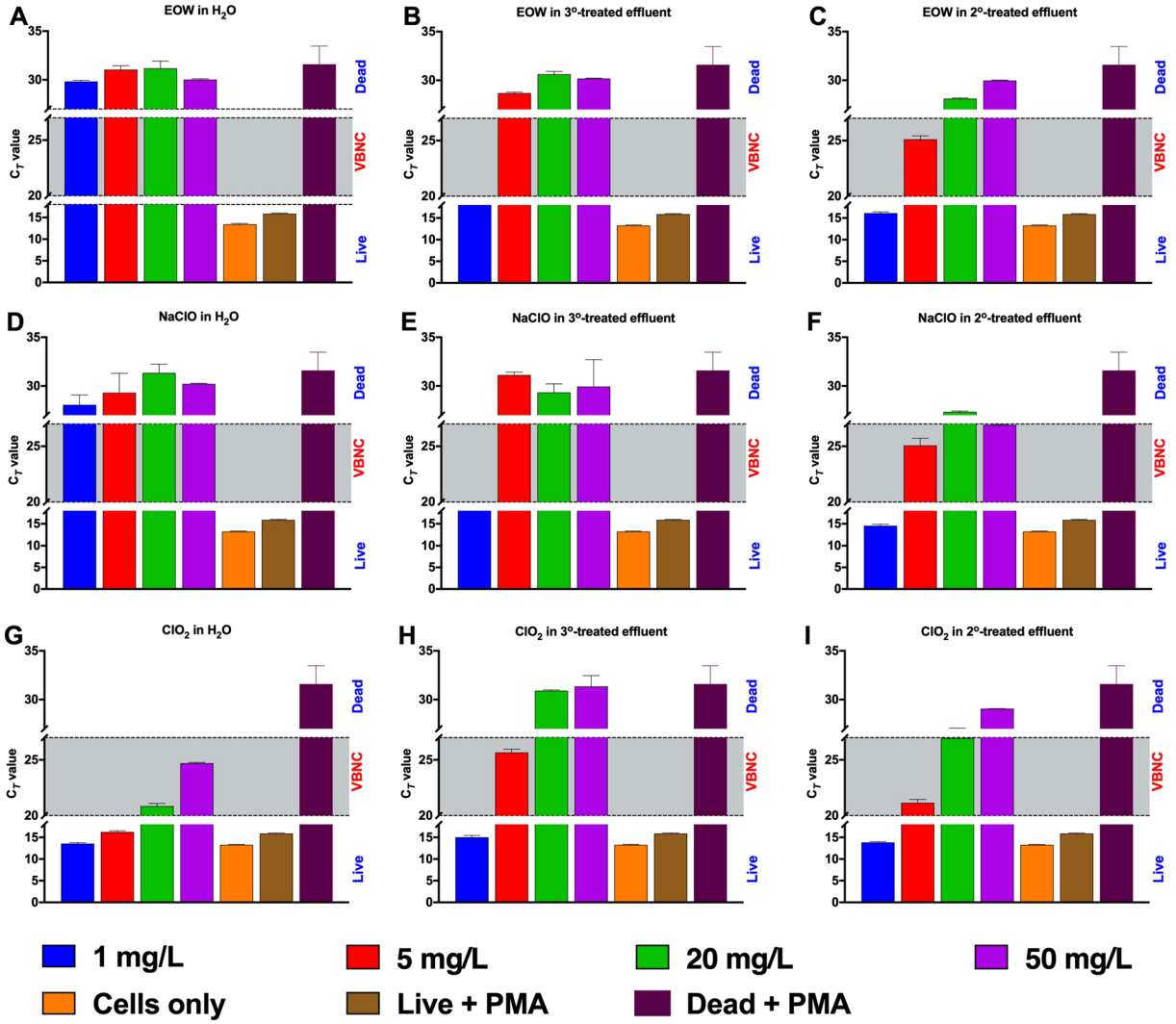

Figure 8. Generation of potential VBNC state in *Escherichia coli* Xen14. Bacteria were treated with various concentrations of sanitizers in the presence of different levels of dissolved organic matter content, after which propidium monoazide (PMAxx) was added. PMAxx is a membrane-impermeable dye that only penetrates and binds to DNA of damaged cells, preventing subsequent PCR amplification. As such, the cycle threshold (C_T) value of intact (live) cells are low (up to 19 C_T); values for potential viable but nonculturable (VBNC) cells range between 20 and 27 C_T, while values for dead cells are from 28 C_T upwards. For further explanation please see Tenzin *et al.*[32]. Figures were generated using Prism v8 (GraphPad Software, San Diego, CA, USA).

application in the field. With these results in mind, we suggest EOW has a strong potential for decontamination of microbiologically-impaired waters for irrigation of fruits and vegetables and/or for post-harvest sanitation of minimally processed fresh produce.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

A.D.O., C.E.D., B.D., E.D. and E.L. conceived and designed the study; A.D.O. C.E.D. and G.B. performed the experiments; A.D.O. and C.E.D. wrote the manuscript; A.D.O., C.D., H.V. and E.D. analyzed the data; S.F. provided the electrolyzed oxidizing water (EOW) used in this study; S.F., B.H., B.D., G.B., H.V., B.M., P.D., E.D. and E.L. assisted in experimental design and editing of the manuscript.

Competing interests

S.F. is Technical Manager for Ecas4 Australia Pty Ltd. Ecas4 Australia Pty Ltd had no role in data collection, analysis, decision to publish, or preparation of the manuscript. A.D.O., C.E.D., B.H., B.D., G.B., H.V., B.M., P.D., E.D. and E.L. declare no potential conflict of interest.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41598-019-56248-7.

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Materials and Methods

1. Bacterial inoculum

The bacterial strains used in this activity report were *Escherichia coli* (ATCC 25922), *Listeria innocua* 6a (ATCC 33090) and *Salmonella enterica* serovar Enteritidis 11RX (Ushiba *et al*., 1959; Ogunniyi *et al*., 1994). Glycerol stock cultures were maintained at –80°C and were streaked onto Luria Bertani (LB) agar (Oxoid) to obtain isolated colonies. Single colonies were streaked onto the following selective agar plates (Thermo Fisher Scientific) to confirm purity: Eosin Methylene Blue (EMB) agar (PP2169) for *E. coli*; Listeria Selective Oxford (OXF) agar (PP2141) for *L. innocua* 6a, and Xylose Lysine Deoxycholate (XLD) agar (PP2004) for *S.* Enteritidis 11RX.

For experiments, single colonies from selective agar plates were emulsified in LB broth and grown overnight at 37°C with aeration at 150 rpm on a digital platform mixer (Ratek Instruments). Thereafter, bacteria were subcultured at a 1:10 dilution into fresh LB broth and incubated further at 180 rpm for 2–3 h until A_{600}=1.0 (for *E. coli* and *S*. Enteritidis 11RX) or A_{600}=0.5 (for *L. innocua* 6a) was reached (equivalent to approx. 1×10^9 colony-forming units (CFU)/ml for *E. coli* and *S*. Enteritidis 11RX or approx. 5×10^8 CFU/ml for *L. innocua* 6a. Bacteria were then harvested and washed in autoclave-sterilised Milli-Q water (PURELAB Classic, ELGA) to remove residual culture medium and then resuspended in the filtered manure mix as described below.

2. Reagents, solutions and instruments

Electrolysed oxidising (EO) water was provided by ECAS4 Australia (Unit 8 / 1 London Road, Mile End South, SA 5031) at 300–350 mg/L free chlorine. Sodium hypochlorite (NaClO) was obtained as a 125 ml/L solution (UN No 1791) from Chemwell Pty Ltd (3 Clive St, Springvale, VIC 3171). The amount of free chlorine in EO water and NaClO was measured using the free chlorine and chlorine ultra-high range portable photometer (HI 96771C; Hanna Instruments) according to the manufacturer's recommendations. The pH and oxidation-reduction potential (ORP) of EO water, NaClO and water were measured on a EUTech PC700 meter with separate pH and ORP probes, respectively.

3. Manure mixture preparation

The manure used was a cow manure. The manure was gamma sterilised at Steritech (Melbourne, Australia). Sterilised manure was dried in an oven at 37^{o}C and ground to a fine powder using a coffee grinder (HOME COLLECTION). The manure suspension was prepared by weighing 10 g manure into 1 litre of sterile distilled water. To remove particles in the suspension that would clog the sprayer, the manure suspension was filtered through several layers of sterile paper towel prior to the addition of bacteria. The dissolved organic carbon (DOC) content in the manure suspension was measured on a Shimadzu TOC-L total organic carbon analyser. A mixed suspension of *E. coli*, *L. innocua* 6a and *S.* Enteritidis 11RX organisms pre-washed in sterile Milli-Q water were added to the manure suspension to a final concentration of approx. 1×10^8 CFU/ml of each strain).

4. Plant growth conditions

Cos lettuce, baby spinach and Italian [flat-leaf) parsley seedlings were purchased from commercial plant suppliers in the local area (Virginia Nursery, Virginia, SA; Bunnings Pty Ltd., Parafield, SA). Rockwool was purchased from Complete Hydroponics (Salisbury East, SA). Plants were grown in a hydroponic system of troughs, with 20 litres of nutrient solution supplied to each set of three troughs (containing 15 plants). The nutrient solution was a dilute Hoagland's solution (1/2 or 1/4 strength) prepared from stock solutions of concentrated nutrients (Hoagland and Arnon, 1950). Individual seedlings were re-potted into pre-wetted rockwool cubes in plastic mesh pots. The pots were then placed into the trough system and an aquarium pump (Aqua One Maxi 104) was used to circulate the nutrient solution through the troughs, wetting the rockwool and allowing the plants to access the nutrients for growth. Nutrient solution was continually circulated and replaced weekly. The plants were grown in a greenhouse with temperature settings of 22°C/15°C day/night 12 h:12 h for lettuce and 24°C/19°C day/night 12 h:12 h for spinach and parsley. No supplementary lighting was supplied.

5. Plant inoculation experiments and bacterial recovery from plant leaves

5.1 ***Plant inoculation*****:** Plant seedlings were pre-grown for 7 days (for cos lettuce and flat-leaf parsley) to 14 days (for baby spinach) prior to application of the bacterial inocula. The bacteria/manure mix was applied manually from a 500-ml spray bottle. Approximately 10 ml (measured) of the mixed bacterial suspension (equivalent to approx. 1×10^9 CFU total for each strain) was applied to each plant. Plants were allowed to dry after application of the bacterial inoculum for 2–3 h.

5.2 ***Plant washing*****:** Solutions for the washing procedure were prepared freshly at the time of application. EO water concentrate (~300 mg/l free available chlorine (FAC)) and NaClO (12,500 mg/l FAC) were diluted to the appropriate concentration and the FAC concentration of the diluted solutions were tested as described above; pH and ORP were also recorded for each trial. Sixty litre drums were filled with the diluted solutions, with tap water as the control. The sprinkler system was flushed with the test solutions prior to use on the plants. Plants were sprinkler irrigated from an overhead sprinkler system with 25 litres of solution over a period of approx. 10 mins. Plants were left to dry for 1–2 h after the washing procedure and prior to sampling.

5.3 ***Leaf harvesting and bacterial enumeration*****:** Leaves were harvested at Days 0, 3 and 7 after application of the inoculant and the washing procedure. Leaves were cut at the base of the stem and placed into sterile stomacher bags (Thermo Fisher Scientific, Scoresby, VIC Australia); the wet weight of plant material was recorded. Sterile peptone water (0.1%; Thermo Fisher Scientific) was added (50 or 100 ml) and the leaves were processed in a Seward BA6021 stomacher (Seward Limited, Worthing, UK) for 1 min. The bacterial suspension was then serially diluted in sterile peptone water and surviving bacterial colonies were enumerated on selective media as described above; total bacterial counts were also enumerated on plate count agar (PCA; PP2145, Thermo Fisher Scientific). Agar plates were

incubated at 37°C for 24–36 h. Bacterial counts were reported as CFU/g wet weight plant material.

5.4 ***Measurement of leaf chlorophyll concentration of plant leaves:*** The chlorophyll content of plant leaves for each treatment group was measured and recorded before bacterial inoculation (Day 0) and at Days 3 and 7 post-treatment using a lightweight handheld chlorophyll meter (SPAD 502-Plus, Konica Minolta).

6. Statistical analysis

All figures were drawn and statistical analyses performed using Prism v7 software package. A two-way analysis of variance (Tukey's multiple comparisons) was performed to evaluate statistical differences between mean (±SEM) bacterial counts or chlorophyll content between groups for each time point. A *p*-value of <0.05 was considered statistically significant.

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Results and discussion

Preliminary assessment of the efficacy of 5 mg/l available chlorine for either EO water or NaClO in reducing microbial load on contaminated cos lettuce leaves in the presence of 100 mg/l organic matter.

In an initial experiment, we investigated the ability of EO water at 5.7 mg/l FAC and NaClO at 5.9 mg/l FAC in the presence of 100 mg/l DOC to significantly reduce the microbial load on contaminated cos lettuce leaves relative to tap water-treated plants. Our analyses show that both treatments provided comparable efficacy with the tap water control treatment, with the exception of Day 3, where NaClO demonstrated significantly better efficacy over EO water and tap water in reducing total viable counts (Figure 1, Table 1).

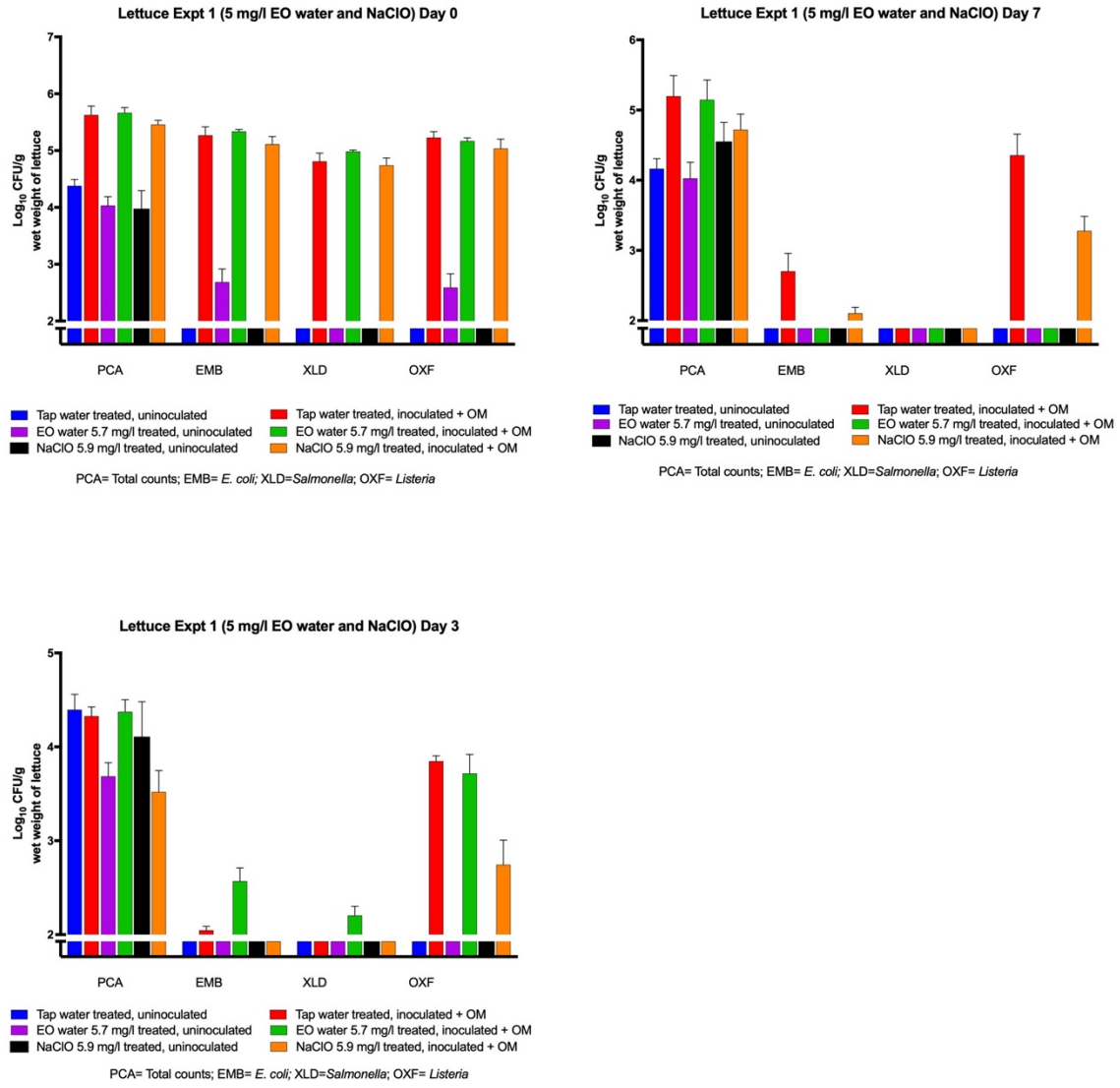


Figure 1. Preliminary assessment of the efficacy of 5 mg/l available chlorine for either EO water or NaClO in reducing microbial load on contaminated cos lettuce leaves in the presence of 100 mg/l organic matter.

A two-way analysis of variance (Tukey's multiple comparisons) was performed to evaluate statistical differences between mean (±SEM) bacterial counts between groups for each time point. A *p*-value of <0.05 was considered statistically significant.

Table 1. Statistical data for cos lettuce experiment 1 comparing efficacy of tap water, 5 mg/l EO water and 5 mg/l NaClO			
Days post-treatment	Growth medium	Comparison	*p*-value
Day 0	PCA	Tap water treated, uninoculated vs. Tap water treated, inoculated + OM	****
		Tap water treated, uninoculated vs. EO water 5.7 mg/l treated, inoculated + OM	****
		Tap water treated, uninoculated vs. NaClO 5.9 mg/l treated, inoculated + OM	**
		Tap water treated, inoculated + OM vs. EO water 5.7 mg/l treated, uninoculated	****
		Tap water treated, inoculated + OM vs. NaClO 5.9 mg/l treated, uninoculated	****
		EO water 5.7 mg/l treated, uninoculated vs. EO water 5.7 mg/l treated, inoculated + OM	****
		EO water 5.7 mg/l treated, uninoculated vs. NaClO 5.9 mg/l treated, inoculated + OM	**
		EO water 5.7 mg/l treated, inoculated + OM vs. NaClO 5.9 mg/l treated, uninoculated	****
		NaClO 5.9 mg/l treated, uninoculated vs. NaClO 5.9 mg/l treated, inoculated + OM	**
	EMB	Tap water treated, uninoculated vs. EO water 5.7 mg/l treated, inoculated + OM	*
		EO water 5.7 mg/l treated, uninoculated vs. EO water 5.7 mg/l treated, inoculated + OM	*
		EO water 5.7 mg/l treated, inoculated + OM vs. NaClO 5.9 mg/l treated, uninoculated	*
Day 3	PCA	Tap water treated, uninoculated vs. EO water 5.7 mg/l treated, uninoculated	**
		Tap water treated, uninoculated vs. NaClO 5.9 mg/l treated, inoculated + OM	**
		Tap water treated, inoculated + OM vs. EO water 5.7 mg/l treated, uninoculated	*
		Tap water treated, inoculated + OM vs. NaClO 5.9 mg/l treated, inoculated + OM	*
		EO water 5.7 mg/l treated, uninoculated vs. EO water 5.7 mg/l treated, inoculated + OM	*
		EO water 5.7 mg/l treated, inoculated + OM vs. NaClO 5.9 mg/l treated, inoculated + OM	**
Day 7	All	No significant difference in all comparisons	NS

PCA = Plate count agar for total viable counts

EMB = Eosin Methylene Blue (EMB) agar for selection of *E. coli*

OXF = *Listeria* Selective Oxford agar for selection of *L. innocua* 6a

XLD = Xylose Lysine Deoxycholate agar for selection of *S.* Enteritidis 11RX

*$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$; NS = not statistically significant

We attributed the overall lack of efficacy of EO water and NaClO to the consumption/quenching of the FAC by the DOC in the organic matter. Nonetheless, the leaf visual quality during the 7 days experimentation and shelf life post-harvest were not affected by the use of EO water or NaClO (Figure 2A, B).

Figure 2A. Lettuce plants Experiment 1 (Treatments: 5 mg/l EO water (A), 5 mg/l NaClO (B), Tap water (C))

EO water washed (Treatment A)

NaClO washed (Treatment B)

Tap water washed (Treatment C)

Note: no plants showed any negative effects of any washing treatment.

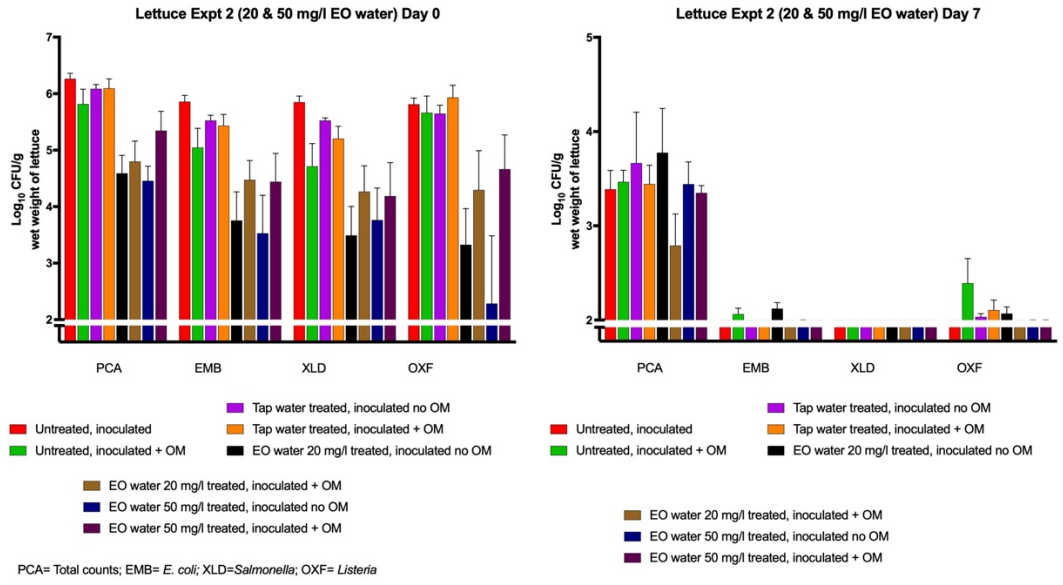
Figure 2B. Experiment 1 post-harvest 11-09-18 and 20-09-18 – Lettuce (5 mg/l EO water (A), 5 mg/l NaClO (B), Tap water (C))

A inoculated 11-09-18 	A inoculated 20-09-18 
B inoculated 11-09-18 	B inoculated 20-09-18 
C inoculated 11-09-18 	C inoculated 20-09-18 

Note: no leaves showed any negative effects of any washing treatment after 9 days storage at 4°C.

Further assessment of the efficacy of 20 mg/l and 50 mg/l available chlorine for EO water in reducing microbial load on contaminated cos lettuce leaves in the presence of 100 mg/l organic matter.

In order to overcome the limited efficacy of EO water and NaClO imposed by the presence of high DOC content, we examined whether the efficacy of EO water in reducing the microbial load on lettuce plants can be significantly improved in the presence of high DOC content by testing the anolyte at higher concentrations (20 mg/l and 50 mg/l FAC) as described in Materials and Methods. Our results show that at these concentrations, EO water was able to substantially reduce the microbial contamination of the lettuce leaves, with the 50 mg/l anolyte showing statistically significant difference in reducing *L. innocua* populations from the lettuce leaves by Day 3 post-inoculation (Figure 3, Table 2).



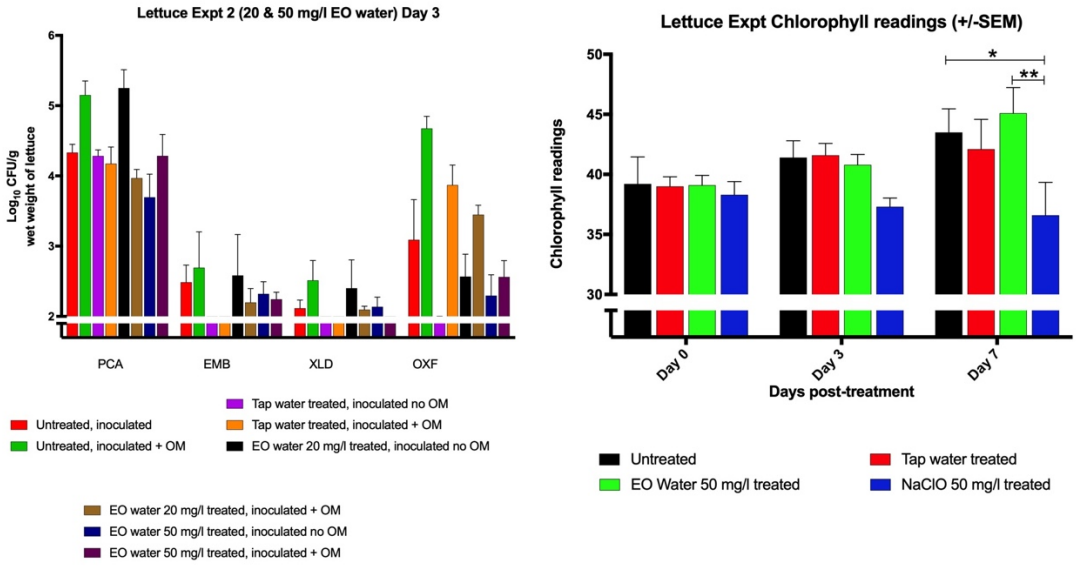


Figure 3. Assessment of the efficacy of 20 mg/l and 50 mg/l available chlorine for EO water in reducing microbial load on contaminated cos lettuce leaves in the presence of 100 mg/l organic matter.

Table 2. Statistical data for cos lettuce experiment 2 comparing efficacy of tap water, 20 mg/l and 50 mg/l EO water

Days post-treatment	Growth medium	Comparison	*p*-value
Day 0	EMB	Untreated, inoculated vs. EO water 20 mg/l treated, inoculated no OM	*
		Untreated, inoculated vs. EO water 50 mg/l treated, inoculated no OM	**
		Tap water treated, inoculated no OM vs. EO water 50 mg/l treated, inoculated no OM	*
	XLD	Untreated, inoculated vs. EO water 20 mg/l treated, inoculated no OM	**
		Untreated, inoculated vs. EO water 50 mg/l treated, inoculated no OM	*
		Tap water treated, inoculated no OM vs. EO water 20 mg/l treated, inoculated no OM	*
	OXF	Untreated, inoculated vs. EO water 20 mg/l treated, inoculated no OM	**
		Untreated, inoculated vs. EO water 50 mg/l treated, inoculated no OM	****
		Untreated, inoculated + OM vs. EO water 20 mg/l treated, inoculated no OM	**
		Untreated, inoculated + OM vs. EO water 50 mg/l treated, inoculated no OM	****
		Tap water treated, inoculated no OM vs. EO water 20 mg/l treated, inoculated no OM	**
		Tap water treated, inoculated no OM vs. EO water 50 mg/l treated, inoculated no OM	****
		Tap water treated, inoculated + OM vs. EO water 50 mg/l treated, inoculated no OM	****
		EO water 20 mg/l treated, inoculated + OM vs. EO water 50 mg/l treated, inoculated no OM	*
		EO water 50 mg/l treated, inoculated no OM vs. EO water 50 mg/l treated, inoculated + OM	**
Day 3	PCA	Untreated, inoculated + OM vs. EO water 20 mg/l treated, inoculated + OM	*
		Untreated, inoculated + OM vs. EO water 50 mg/l treated, inoculated no OM	**
		EO water 20 mg/l treated, inoculated no OM vs. EO water 20 mg/l treated, inoculated + OM	*
		EO water 20 mg/l treated, inoculated no OM vs. EO water 50 mg/l treated, inoculated no OM	**
	OXF	Untreated, inoculated vs. Untreated, inoculated + OM	**
		Untreated, inoculated + OM vs. Tap water treated, inoculated no OM	****
		Untreated, inoculated + OM vs. EO water 20 mg/l treated, inoculated no OM	****
		Untreated, inoculated + OM vs. EO water 20 mg/l treated, inoculated + OM	*
		Untreated, inoculated + OM vs. EO water 50 mg/l treated, inoculated no OM	****

		Untreated, inoculated + OM vs. EO water 50 mg/l treated, inoculated + OM	****
		Tap water treated, inoculated no OM vs. Tap water treated, inoculated + OM	****
		Tap water treated, inoculated no OM vs. EO water 20 mg/l treated, inoculated + OM	**
		Tap water treated, inoculated + OM vs. EO water 20 mg/l treated, inoculated no OM	*
		Tap water treated, inoculated + OM vs. EO water 50 mg/l treated, inoculated no OM	**
		Tap water treated, inoculated + OM vs. EO water 50 mg/l treated, inoculated + OM	*
		EO water 20 mg/l treated, inoculated + OM vs. EO water 50 mg/l treated, inoculated no OM	*
Day 7	PCA	Tap water treated, inoculated no OM vs. EO water 20 mg/l treated, inoculated + OM	**
		EO water 20 mg/l treated, inoculated no OM vs. EO water 20 mg/l treated, inoculated + OM	**

PCA = Plate count agar for total viable counts

EMB = Eosin Methylene Blue (EMB) agar for selection of *E. coli*

OXF = *Listeria* Selective Oxford agar for selection of *L. innocua* 6a

XLD = Xylose Lysine Deoxycholate agar for selection of *S.* Enteritidis 11RX

*$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$

Again, the overall leaf quality during the 7 days evaluation and the shelf life of the leaves post-harvest were not affected by the use of EO water at either 20 mg/l or 50 mg/l (Figure 4A, B), leading to the choice of the 50 mg/l anolyte for further experimentation.

Figure 4A. Lettuce plants experiment 2. Treatments: A – unwashed, uninoculated; B – unwashed, inoculated (inoc.); C – unwashed, inoc. + organic matter (OM); D – tap water (TW) washed, inoc.; E – TW washed, inoc. + OM; F – EO water washed (20 mg/l), inoc.; G – EO water washed (20 mg/l), inoc. + OM; H – EO water washed (50 mg/l), inoc.; I – EO water washed (50 mg/l), inoc. + OM.

A – uninoculated, unwashed.	B – unwashed, inoc.	C – unwashed, inoc. + OM
		

D – TW washed, inoc. 	E – TW washed, inoc. + OM 	F – EO 20 washed, inoc. 
G – EO 20 washed, inoc + OM 	H – EO 50 washed, inoc. 	I – EO 50 washed, inoc + OM. 

Figure 4B. Lettuce leaves experiment 2 post-harvest 25-09-18 and 10-10-18. For treatments, please see Figure 4A legend.

A – unwashed, uninoculated 25-09-18 	A – unwashed, uninoc. 10-10-18 
B – unwashed, inoc. 25-09-18 	B – unwashed, inoc. 10-10-18 
C – unwashed, inoc. + OM 25-09-19 	C – unwashed, inoc. + OM 10-10-18 

D – TW washed, inoc. 25-09-18 	D – TW washed, inoc. 10-10-18 
E – TW washed, inoc. + OM 25-09-18 	E – TW washed, inoc. + OM 10-10-18 
F – EO 20 washed, inoc. 25-09-18 	F – EO 20 washed, inoc. 10-10-18 

G – EO 20 washed, inoc + OM 25-09-18 	G – EO 20 washed, inoc + OM 10-10-18 
H – EO 50 washed, inoc. 25-09-18 	H – EO 50 washed, inoc. 10-10-18 
I – EO 50 washed, inoc + OM 25-09-18 	I – EO 50 washed, inoc + OM 10-10-18 

Note: there was no deterioration in leaf quality indicators for any treatment within this 10-day period post-harvest.

In depth evaluation of the comparative efficacy of 50 mg/l available chlorine for either EO water or NaClO in reducing microbial load on contaminated cos lettuce leaves.

The finding that 50 mg/l available chlorine for EO water was the best overall of all tested EO water concentrations at reducing the microbial contamination of lettuce leaves without compromising leaf quality or shelf life was encouraging. Therefore, we carried out a detailed comparative efficacy assessment of 50 mg/l available chlorine for either EO water or NaClO in reducing microbial load on contaminated cos lettuce leaves. In this experiment, EO water treatment showed statistically significant reduction of *E. coli* and *L. innocua* compared to tap water treatment Day 0, while NaClO showed statistically reduced *L. innocua* populations at the same time point. Furthermore, total bacteria counts was significantly reduced by EO water treatment at Day 7 post-infection (Figure 5, Table 3).

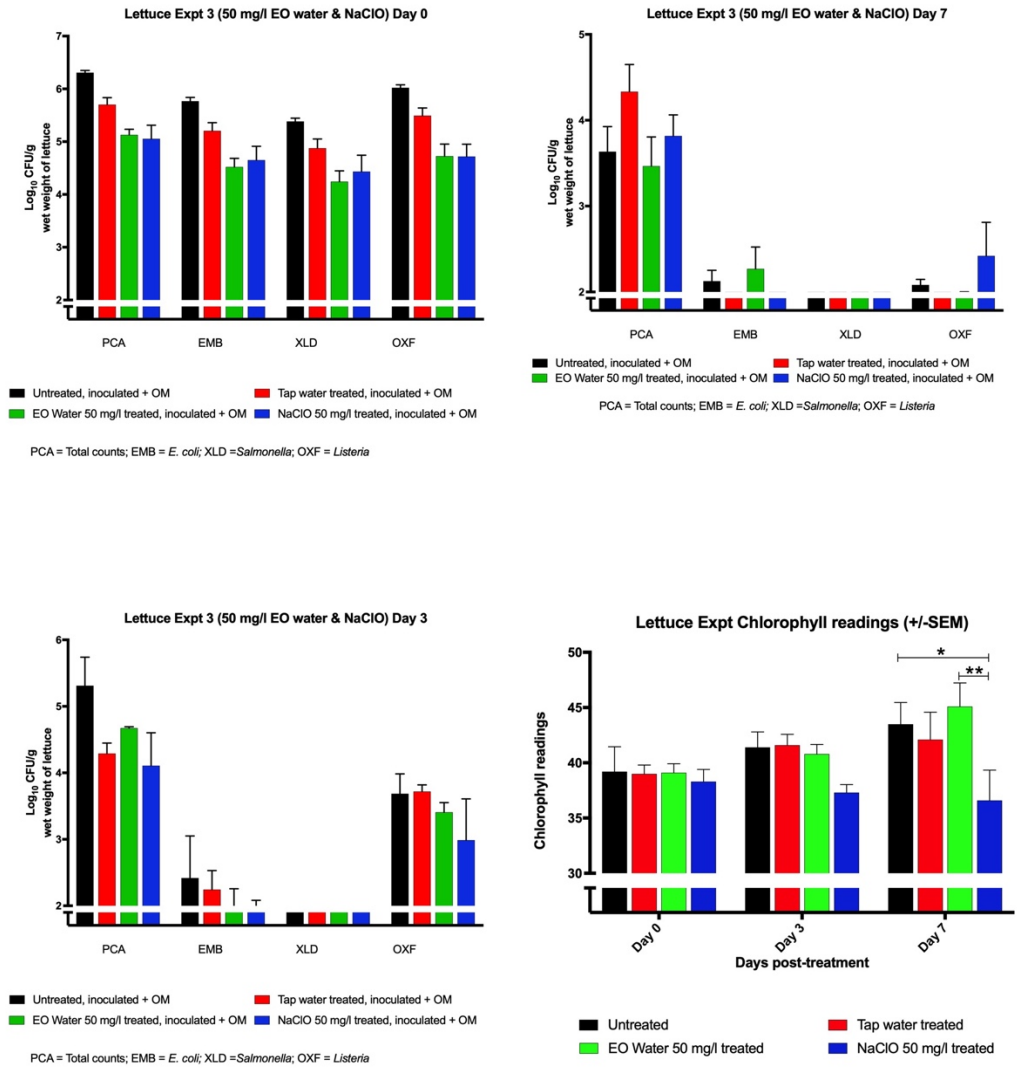


Figure 5. Detailed evaluation of the comparative efficacy of 50 mg/l available chlorine for either EO water or NaClO in reducing microbial load on contaminated cos lettuce leaves.

Table 3. Statistical data for cos lettuce experiment 3 comparing efficacy of tap water, 50 mg/l EO water and 50 mg/l NaClO

Days post-treatment	Growth medium	Comparison	*p*-value
Day 0	PCA	Untreated, inoculated + OM vs. EO Water 50 mg/l treated, inoculated + OM	***
		Untreated, inoculated + OM vs. NaClO 50 mg/l treated, inoculated + OM	****
	EMB	Untreated, inoculated + OM vs. EO Water 50 mg/l treated, inoculated + OM	****
		Untreated, inoculated + OM vs. NaClO 50 mg/l treated, inoculated + OM	***
		Tap water treated, inoculated + OM vs. EO Water 50 mg/l treated, inoculated + OM	*
	XLD	Untreated, inoculated + OM vs. EO Water 50 mg/l treated, inoculated + OM	***
		Untreated, inoculated + OM vs. NaClO 50 mg/l treated, inoculated + OM	**
	OXF	Untreated, inoculated + OM vs. EO Water 50 mg/l treated, inoculated + OM	****
		Untreated, inoculated + OM vs. NaClO 50 mg/l treated, inoculated + OM	****
		Tap water treated, inoculated + OM vs. EO Water 50 mg/l treated, inoculated + OM	*
		Tap water treated, inoculated + OM vs. NaClO 50 mg/l treated, inoculated + OM	*
Day 3	All	No significant difference in all comparisons	NS
Day 7	PCA	Tap water treated, inoculated + OM vs. EO Water 50 mg/l treated, inoculated + OM	*

PCA = Plate count agar for total viable counts

EMB = Eosin Methylene Blue (EMB) agar for selection of *E. coli*

OXF = *Listeria* Selective Oxford agar for selection of *L. innocua* 6a

XLD = Xylose Lysine Deoxycholate agar for selection of *S.* Enteritidis 11RX

*$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$; NS = not statistically significant

EO water treatment did not affect the quality of the leaves whereas the overall quality of the leaves treated with NaClO deteriorated from Day 3 onwards (Figure 6A, B).

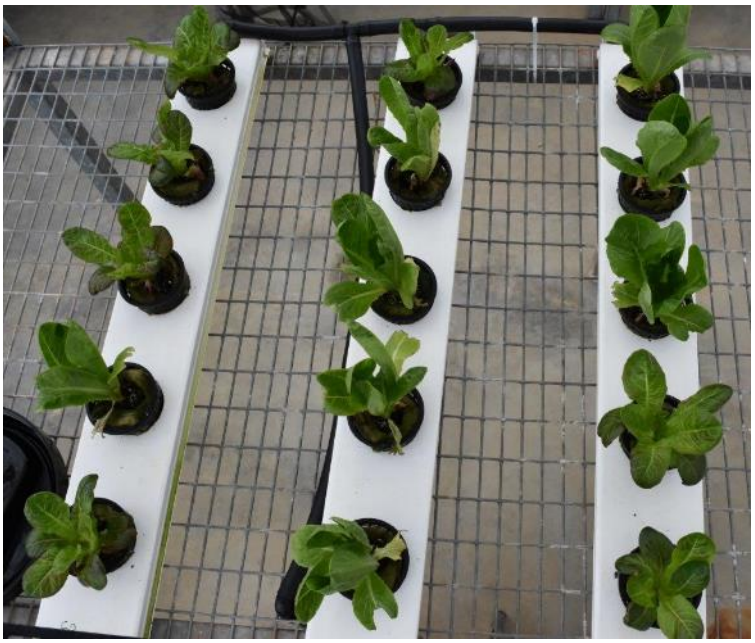
Figure 6A. Lettuce plants experiment 3. Treatments (all inoc. + OM): A: unwashed; B; TW washed; C: EO water (50 mg/l); D: NaClO (50 mg/l).

A: unwashed

B and E: TW washed. Left two rows, single wash (B), right row, double wash (E)

C and F: EO (50) washed. Left two rows, single wash (C), right row, double wash (F).

D and G: NaClO (50) washed. Left two rows, single wash (D), right row, double wash (G).



Note: Severe wilting and brown/yellow spots observed on NaClO treated plants. No negative impacts observed on other treatments.

Figure 6B. Lettuce experiment 3 post-harvest 16-10-18 (A–D) and 19-10-18 (E–G) and 24-10-18 (all). For treatments, please see Figure 6A legend.

A: unwashed 16-10-18 	A: unwashed 24-10-18 
B: TW washed (single wash) 16-10-18 	B: TW washed (single wash) 24-10-18 
C: EO 50 water washed (single wash) 16-10-18 	C: EO 50 water washed (single wash) 24-10-18 

D: NaClO 50 washed (single wash) 16-10-18 	D: NaClO 50 washed (single wash) 24-10-18 
E: TW washed (double wash) 19-10-18 	E: TW washed (double wash) 24-10-18 
F: EO 50 water washed (double wash) 19-10-18 	F: EO 50 water washed (double wash) 24-  10-18

G: NaClO 50 washed (double wash) 19-10-18

Close-up example of damage from NaClO

G: NaClO 50 washed (double wash) 24-10-18

Note: extensive brown/yellow spots on NaClO treated leaves, especially with double wash.

Comparative efficacy assessment of 50 mg/l available chlorine for either EO water or NaClO in reducing microbial load on contaminated baby spinach leaves.

The experiment performed on lettuce leaves using 50 mg/l available chlorine for either EO water or NaClO was recapitulated for baby spinach treatment. In this experiment, EO water treatment showed statistically significant reduction of *S.* Enteritidis 11RX and *L. innocua* compared to tap water treatment Day 0. While the *L. innocua* numbers were higher for EO water treatment compared to NaClO treatment at Day 3, there was no statistically significant difference in numbers by Day 7 between the treatment groups (Figure 7, Table 4).

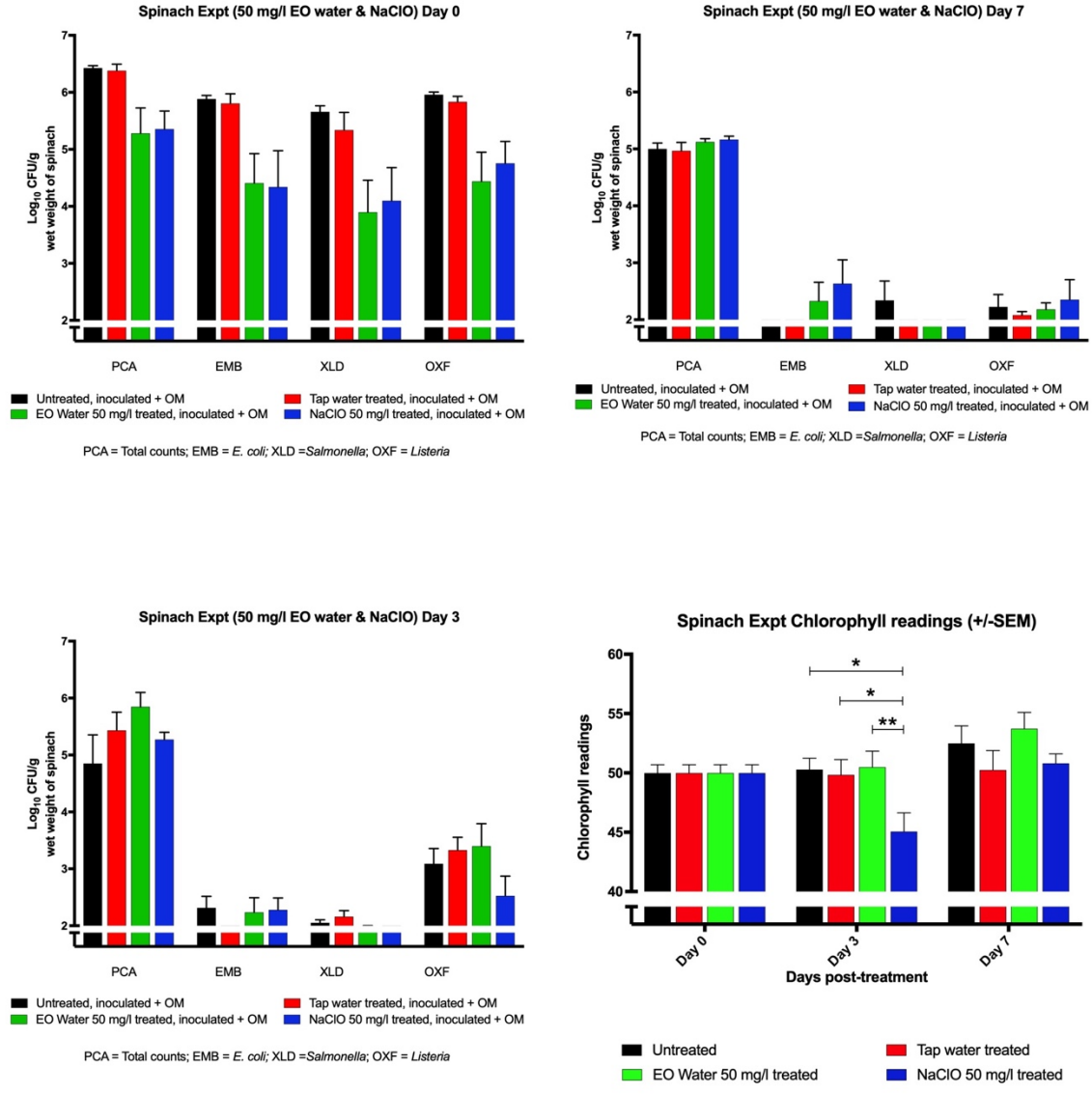


Figure 7. Comparative efficacy assessment of 50 mg/l available chlorine for either EO water or NaClO in reducing microbial load on contaminated baby spinach leaves.

Table 4. Statistical data for baby spinach experiment comparing efficacy of tap water, 50 mg/l EO water and 50 mg/l NaClO			
Days post-treatment	Growth medium	Comparison	*p*-value
Day 0	EMB	Untreated, inoculated + OM vs. EO Water 50 mg/l treated, inoculated + OM	*
		Untreated, inoculated + OM vs. NaClO 50 mg/l treated, inoculated + OM	*
	XLD	Untreated, inoculated + OM vs. EO Water 50 mg/l treated, inoculated + OM	**
		Untreated, inoculated + OM vs. NaClO 50 mg/l treated, inoculated + OM	*
		Tap water treated, inoculated + OM vs. EO Water 50 mg/l treated, inoculated + OM	*
	OXF	Untreated, inoculated + OM vs. EO Water 50 mg/l treated, inoculated + OM	*
		Tap water treated, inoculated + OM vs. EO Water 50 mg/l treated, inoculated + OM	*
Day 3	PCA	Untreated, inoculated + OM vs. EO Water 50 mg/l treated, inoculated + OM	*
	OXF	EO Water 50 mg/l treated, inoculated + OM vs. NaClO 50 mg/l treated, inoculated + OM	*
Day 7	All	No significant difference in all comparisons	NS

PCA = Plate count agar for total viable counts

EMB = Eosin Methylene Blue (EMB) agar for selection of *E. coli*

OXF = *Listeria* Selective Oxford agar for selection of *L. innocua* 6a

XLD = Xylose Lysine Deoxycholate agar for selection of *S.* Enteritidis 11RX

*$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$; NS = not statistically significant

As seen with lettuce leaves, EO water treatment did not affect the quality of the baby spinach leaves whereas the overall quality of the leaves treated with NaClO deteriorated from Day 3 onwards (Figure 8A, B).

Figure 8A. Spinach plants experiment. All plants inoculated + organic matter; Treatments - A: unwashed; B: Tap water (TW) washed; C: EO water washed (50 mg/l); D: NaClO washed (50 mg/l))

Spinach plants post-inoculation:

Note: Plants were spray inoculated with bacteria in suspension with manure/water. The spray covered all leaves and was left on the leaves to dry prior to the washing treatments.

Spinach plants post-treatment:

B: Tap water washed

C: EO water washed

D: NaClO washed

Note: The washing step used a rotary sprinkler. Twenty-five litres of water/EO/NaClO was applied to the plants over an approximate 10 min period. The sprinkler system gave good coverage of all plants on the growing tables.

Spinach plants at harvest.

A: unwashed

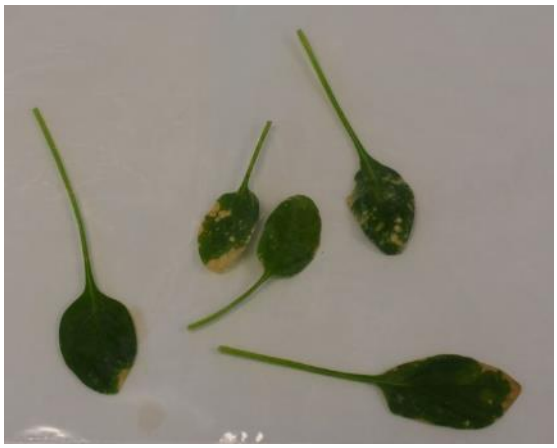
B: TW washed

C: EO 50 water washed

D: NaClO 50 washed

Figure 8B: Spinach plants post-harvest

A 20-11-18 	A 28-11-18 
B 20-11-18 	B 28-11-18 
C 20-11-18 	C 28-11-18 

D 20-11-18	D 28-11-18
	

Note: no deterioration of leaves was seen for any treatment in the post-harvest storage period.

Comparative efficacy assessment of 50 mg/l available chlorine for either EO water or NaClO in reducing microbial load on contaminated flat-leaf parsley leaves.

We also investigated the efficacy of 50 mg/l available chlorine for either EO water or NaClO in reducing microbial contamination of flat-leaf parsley leaves. The effect of EO water and NaClO on inoculum survival on parsley was difficult to assess; there were no significant differences in inoculum survival with any treatment on Day 0 or Day 3, however the inoculum survival was lower than that for lettuce and spinach on Day 0, which suggests that there might be other factors affecting the inoculum survival on parsley leaves that were not able to be accurately assessed in the scope of this study. Figure 9, Table 5).

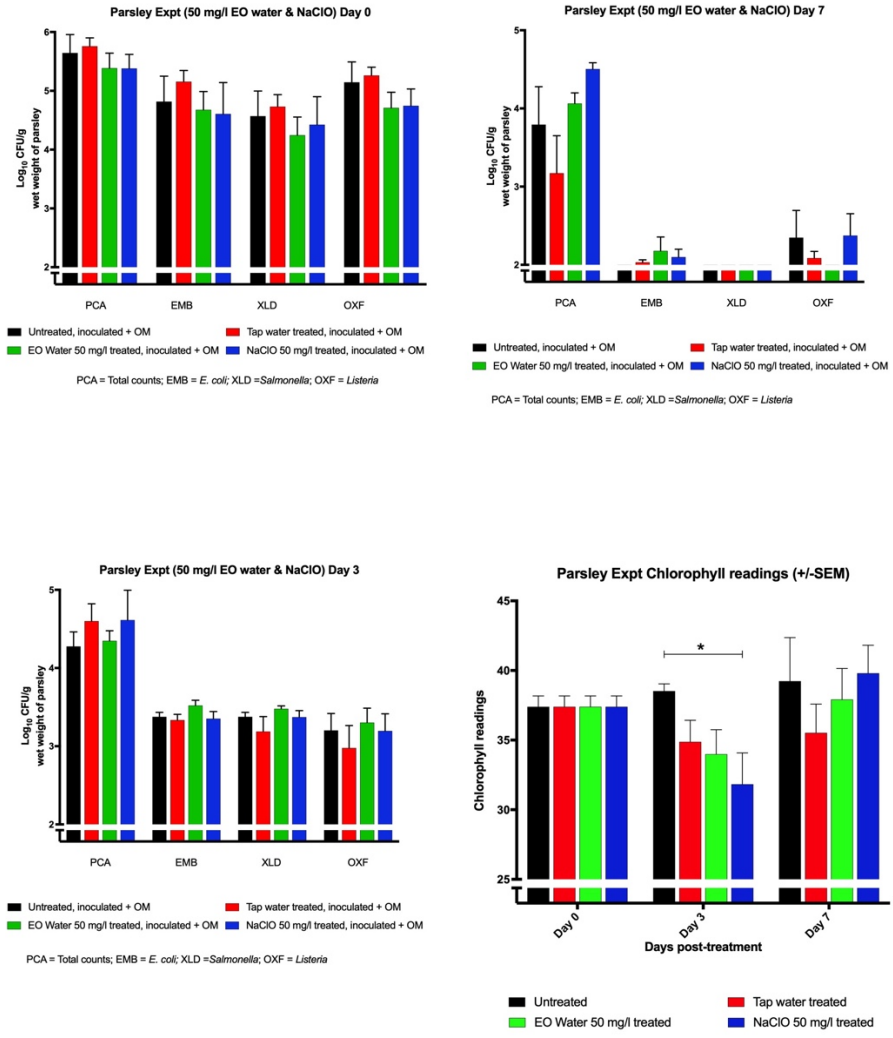


Figure 9. Comparative efficacy assessment of 50 mg/l available chlorine for either EO water or NaClO in reducing microbial load on contaminated flat-leaf parsley leaves.

Table 5. Statistical data for Italian flat-leaf parsley experiment comparing efficacy of tap water, 50 mg/l EO water and 50 mg/l NaClO			
Days 0 & 3	All	No significant difference in all comparisons	NS

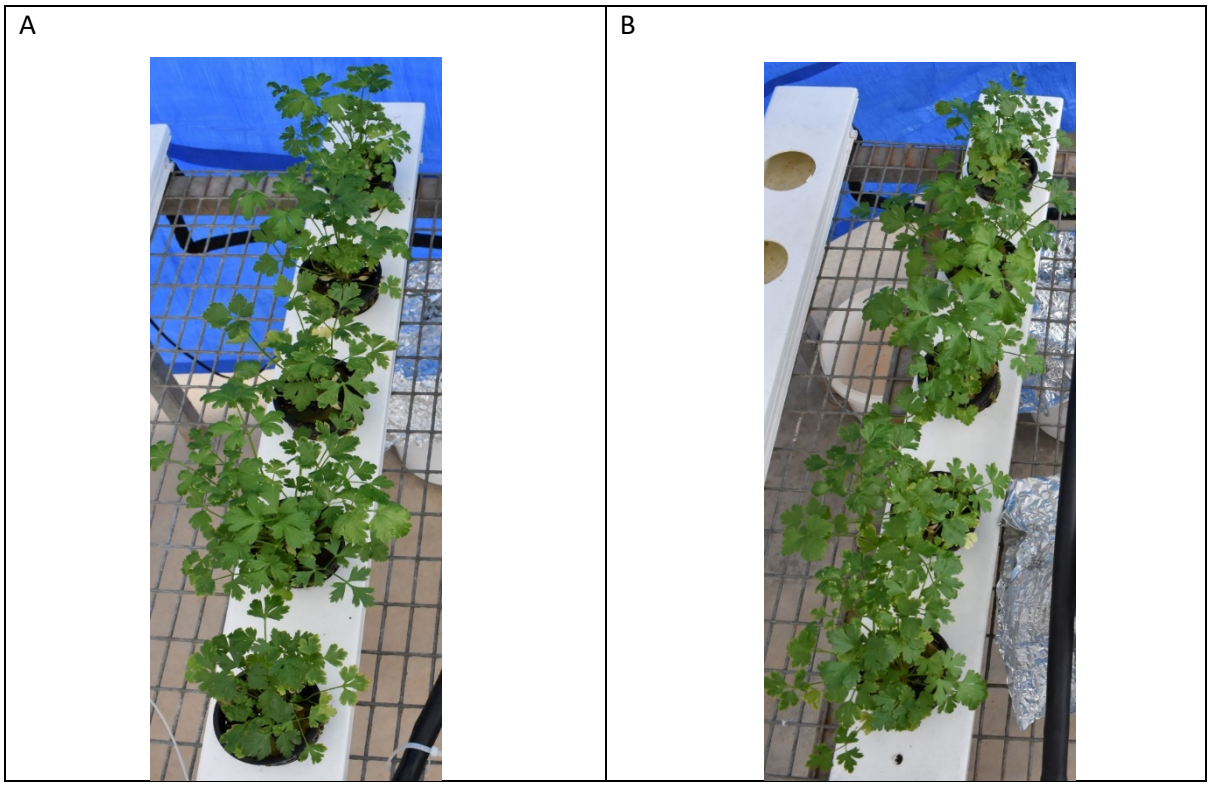
Day 7	PCA	Tap water treated, inoculated + OM vs. EO Water 50 mg/l treated, inoculated + OM	*
		Tap water treated, inoculated + OM vs. NaClO 50 mg/l treated, inoculated + OM	***

PCA = Plate count agar for total viable counts

*$p < 0.05$; ***$p < 0.001$; NS = not statistically significant

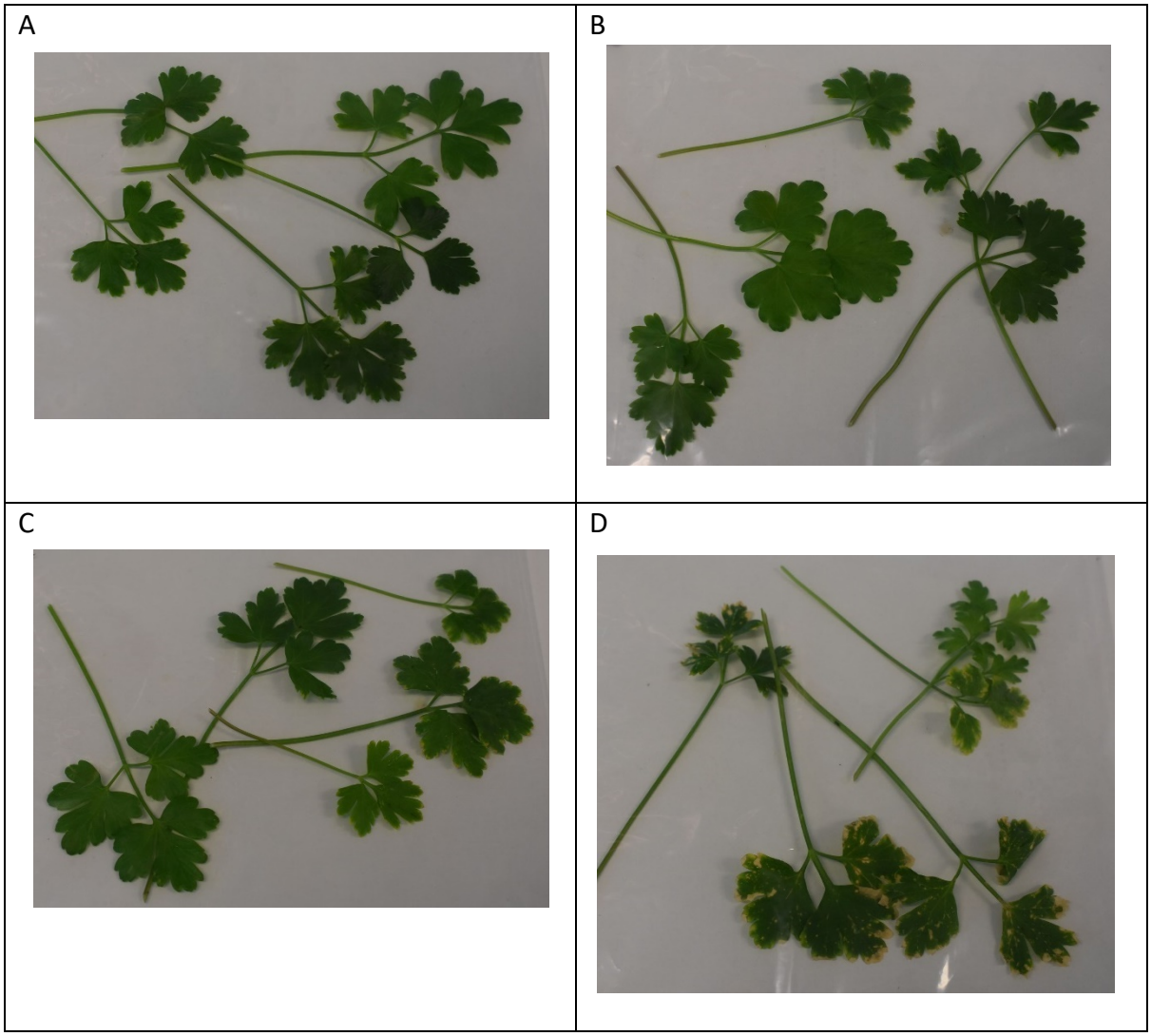
Again, EO water treatment did not affect the quality of the parsley leaves whereas the overall quality of the leaves treated with NaClO deteriorated from Day 3 onwards (Figure 10A, B).

Figure 10A. Parsley plants experiment 1 (Treatments: A – unwashed; B – tap water (TW) washed; C – EO water washed (50 mg/l); D – NaClO washed (50 mg/l))



C 	D 

Figure 10B. Parsley leaves post-harvest 20-11-18



Note: Treatment D showed leaf damage (yellow spots) as a result of NaClO treatment.

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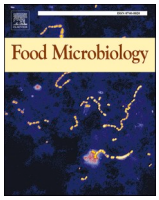

Neutral electrolyzed oxidizing water is effective for pre-harvest decontamination of fresh produce

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ABSTRACT

Pre-harvest sanitization of irrigation water has potential for reducing pathogen contamination of fresh produce. We compared the sanitizing effects of irrigation water containing neutral electrolyzed oxidizing water (EOW) or sodium hypochlorite (NaClO) on pre-harvest lettuce and baby spinach leaves artificially contaminated with a mixture of *Escherichia coli*, *Salmonella* Enteritidis and *Listeria innocua* (~1×10^8 colony-forming units/mL each resuspended in water containing 100 mg/L dissolved organic carbon, simulating a splash-back scenario from contaminated soil/manure). The microbial load and leaf quality were assessed over 7 days, and post-harvest shelf life evaluated for 10 days. Irrigation with water containing EOW or NaClO at 50 mg/L free chlorine significantly reduced the inoculated bacterial load by $\geq$ 1.5 $\log_{10}$, whereas tap water irrigation reduced the inoculated bacterial load by an average of 0.5 $\log_{10}$, when compared with untreated leaves. There were no visual effects of EOW or tap water irrigation on baby spinach or lettuce leaf surfaces pre- or post-harvest, whereas there were obvious negative effects of NaClO irrigation on leaf appearance for both plants, including severe necrotic zones and yellowing/browning of leaves. Therefore, EOW could serve as a viable alternative to chemical-based sanitizers for pre-harvest disinfection of minimally processed vegetables.

1. Introduction

Microbial contamination of fresh, minimally processed foods such as lettuce, spinach, parsley and other leafy greens by opportunistic and human pathogens is of serious health and economic concern worldwide (Food and Agriculture Organization of the United Nations, 2018; World Health Organization, 2018). Microbiologically impacted irrigation water or splash-back from contaminated soil during irrigation can function as a conduit for pathogen transfer to fresh produce (Jongman and Korsten, 2018; Lee et al., 2019; Markland et al., 2017). Leafy greens are particularly vulnerable to irrigation-mediated contamination with opportunistic human pathogens because they have large surface areas, are often grown in close proximity to soil, are irrigated intensively, and are mostly consumed raw (De Keuckelaere et al., 2015). In their investigation of splash transfer of *Salmonella* to a range of field-grown produce, Lee and colleagues (Lee et al., 2019) demonstrated the potential for splash transfer as a route of pre-harvest contamination. For fresh produce, pre-harvest (i.e. irrigation water) and post-harvest (i.e. washing water) water sources have been identified as the main sources of contamination associated with illnesses (FSANZ, 2011). Indeed, investigations of recent outbreaks have focused on the quality of water used in produce processing, for instance in outbreaks associated with *Salmonella* spp. and *Listeria monocytogenes* in cantaloupes, pre-packed lettuce and baby spinach leaves (FSANZ, 2016; Zhu et al., 2017).

Abbreviations: FAC, free available chlorine; EOW, electrolyzed oxidizing water; SEM, standard error of the mean; DOC, dissolved organic carbon; OM, organic matter.

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Contaminated irrigation water has been implicated in outbreaks of verotoxigenic *E. coli* in lettuce in Sweden and Denmark (Ethelberg et al., 2010; Söderström et al., 2008) and in outbreaks of *Salmonella* in tomatoes and serrano pepper in the US (Greene et al., 2008; Hanning et al., 2009). Thus, the quality of irrigation water is paramount in ensuring the safety of edible produce and it is important to select an appropriate water source and/or disinfection method to reduce the potential for fresh produce contamination (De Keuckelaere et al., 2015; Mogren et al., 2018; Uyttendaele et al., 2015).

Disinfection processes for on-site treatment of microbiologically-impaired irrigation water commonly involve application of chemicals, such as chlorine, ozone, peroxyacetic acid or hydrogen peroxide (Dandie et al., 2019; Premier, 2013). While there is a substantial industry around post-harvest washing/processing of fresh produce (Premier, 2013), there are few studies on the application of such processes for pre-harvest sanitization of fresh produce. However, it is becoming increasingly evident that the best strategy to reduce fresh produce contamination is to prevent contamination occurring in the first instance, for various reasons. This is a significant part of the hurdle approach to reducing food safety risks (Mogren et al., 2018; Sigge et al., 2016), which emphasizes pre-harvest treatments and using clean irrigation water. This approach is further supported by the fact that removing/eliminating bacteria from leaf surfaces through post-harvest washing is not always possible once the bacteria are irreversibly attached (Yaron and Romling, 2014). For example, it has been demonstrated that even with several washes of a chlorine-based sanitizer, pathogens such as *E. coli* and *S.* Typhimurium were difficult to remove from produce surfaces once firmly attached (Banach et al., 2017). There is further evidence that bacteria can be internalized through various routes and thus are not generally susceptible to removal by post-harvest washing procedures (Alegbeleye et al., 2018).

Given the paucity of data on the pre-harvest sanitization of fresh produce, there is a great need to evaluate the efficacy of on-farm irrigation of fresh produce using a common chlorine based sanitizer, sodium hypochlorite (NaClO), and examine its effects on overall leaf quality and post-harvest shelf life. However, currently used chemical-based sanitizers such as NaClO have a number of drawbacks including efficacy, limited range of application and safety concerns (Dandie et al., 2019). It has recently been highlighted that growers need more alternatives for the treatment of irrigation water (Allende et al., 2018), therefore global efforts are focused on the development and testing of alternative sanitization methods that address these shortcomings, without compromising efficacy. Electrolyzed oxidizing water (EOW) is an alternative sanitization technology (Rahman et al., 2016; Veasey and Muriana, 2016) mainly used in the healthcare industry to control *Legionella* in water supplies (Ferro, 2015; Migliarina and Ferro, 2014). EOW has also gained attention in the food industry (Hricova et al., 2008; Khazandi et al., 2017; Rahman et al., 2016; Veasey and Muriana, 2016) and has been used successfully for sanitizing household utensils such as plastic and wooden kitchen cutting boards (Deza et al., 2007). Of the various types of EOW, the pH-neutral EOW is considered the most promising as it contains predominantly HOCl, which is more effective than ClO^- in NaClO for microbial cell wall penetration and oxidative attack while not presenting the corrosiveness of the acidic or slightly acidic forms (Rahman et al., 2016; Veasey and Muriana, 2016). In a recent study, we demonstrated that EOW was as effective or better than two other chemical based sanitizers (NaClO and ClO_2) for pathogen reduction in contaminated water, and that its efficacy was not affected under a range of pH or buffer conditions (Ogunniyi et al., 2019). In that study, the efficacy of EOW was superior to that of NaClO and ClO_2, being bactericidal at 20 mg/L of free available chlorine (FAC) in the presence of high organic matter content (100 mg/L), while NaClO was only bactericidal at 50 mg/L of FAC, and ClO_2 showed no effectiveness at the highest concentration used (equivalent to 50 mg/L of FAC).

In this study, we compared the sanitizing effects of irrigation water containing either EOW or NaClO on pre-harvest lettuce and baby spinach leaves artificially contaminated with a mixture of *E. coli*, *S.* Enteritidis and *Listeria innocua*. *L. innocua* is routinely used as a proxy of *L. monocytogenes*, a pathogen of fresh produce, since it displays similar behavior; the main advantage is that it does not require Biosafety level 2 containment (Rasch, 2004). We created a worst-case scenario of splash-back of contaminated soil/manure by preparing a high-concentration bacterial inoculant in a manure suspension and then manually spraying this onto the plants. We hypothesized that the EOW would be at least as effective, if not more effective, than NaClO in reducing concentrations of the target microorganisms, whilst also being less harmful to the crop at the equivalent concentration of free chlorine. Changes in the abundance of inoculated bacteria, total bacterial and fungal populations were monitored for 7 days after irrigation treatment. The overall quality and post-harvest shelf life of the vegetables were evaluated.

2. Materials and methods

2.1. Bacterial inocula

The bacterial strains used in this study were *Escherichia coli* (ATCC 25922), *Listeria innocua* 6a (ATCC 33090) and *Salmonella enterica* serovar Enteritidis 11RX (Ogunniyi et al., 1994; Ushiba et al., 1959). Glycerol stock cultures were maintained at −80 °C until use and were streaked onto Luria Bertani (LB) agar (Oxoid; Thermo Fisher Scientific Australia Pty Ltd., Scoresby, Australia) to obtain isolated colonies. Single colonies were streaked onto the following selective agar plates (Thermo Fisher Scientific) to confirm purity: eosin methylene blue (EMB) agar (PP2169) for *E. coli*; *Listeria* selective Oxford (OXF) agar (PP2141) for *L. innocua* 6a, and xylose lysine deoxycholate (XLD) agar (PP2004) for *S.* Enteritidis 11RX.

For experiments, single colonies from selective agar plates were suspended in LB broth and grown overnight at 37 °C with aeration at 150 rpm on a digital platform mixer (Ratek Instruments Pty Ltd., Boronia, Australia). Thereafter, bacteria were subcultured at a 1:10 dilution into fresh LB broth and incubated further at 180 rpm for 2–3 h until optical densities of $A_{600} = 1.0$ (for *E. coli* and *S.* Enteritidis 11RX) and $A_{600} = 0.5$ (for *L. innocua* 6a) were reached, equivalent to approx. 1×10^9 colony-forming units (CFU)/mL for *E. coli* and *S.* Enteritidis 11RX and to approx. 5×10^8 CFU/mL for *L. innocua* 6a. Bacteria were then harvested and washed in autoclave-sterilized Milli-Q water (PURELAB Classic, ELGA; Thermo Fisher Scientific) to remove residual culture medium and resuspended in the filtered manure mix as described below.

2.2. Manure mixture preparation

Blended cow manure (Fine Farm Organics, Charlton, Australia) was obtained from a local distributor and was γ-sterilized at Steritech (Melbourne, Australia). The sterilized manure was subsequently dried in an oven at 37 °C and ground to a fine powder using an analytical mill (IKA, Selangor, Malaysia), resuspended to the equivalent of 10 g/L in sterile Milli-Q water (pH 7.0) and then passed through a 0.45 μm filter to remove particulate matter (Bolan et al., 2011). The dissolved organic carbon (DOC) content in the manure suspension was measured on a Shimadzu TOC-L total organic carbon analyzer (Shimadzu Australasia, Rydalmere, Australia). A mixed suspension of *E. coli*, *L. innocua* 6a and *S.* Enteritidis 11RX organisms pre-washed in sterile Milli-Q water was added to the manure suspension to a final concentration of approx. 1×10^8 CFU/mL of each strain.

2.3. Plant growth conditions

Cos lettuce (*Lactuca sativa* L. var. *longifolia*) and baby spinach (*Spinacia oleracea* L.) seedlings were purchased from commercial plant suppliers in the local area (Virginia Nursery, Virginia, SA, Australia; Bunnings Pty Ltd., Parafield, SA, Australia). Rockwool was purchased

from Complete Hydroponics (Salisbury East, SA, Australia). Plants were grown in a hydroponic system of troughs, with 20 L of nutrient solution supplied to each set of three troughs (containing 15 plants). The nutrient solution was a dilute Hoagland's solution (1/2 or 1/4 strength for lettuce and spinach, respectively) prepared from stock solutions of concentrated nutrients (Hoagland and Arnon, 1950). Individual seedlings were re-potted into pre-wetted rockwool cubes in plastic mesh pots. The pots were then placed into the trough system and a pump (Aqua One Maxi 104, Kong's Australia Pty Ltd., Ingleburn, Australia) submerged in a storage tank was used to circulate the nutrient solution through the troughs, wetting the rockwool to allow access to the nutrients for growth. The nutrient solution was continually recirculated and replaced weekly. The plants were grown in a greenhouse with temperature settings of 22 °C/15 °C day/night 12 h:12 h for lettuce and 24 °C/19 °C day/night 12 h:12 h for spinach. No supplementary lighting was supplied.

2.4. Reagents, solutions and instruments

Electrolyzed oxidizing water (EOW) was kindly provided by Ecas4 Australia at 300–350 mg/L of FAC. Sodium hypochlorite (NaClO) was obtained as a 12.5% solution (CAS no. 7681-52-9) from Chemwell Pty. Ltd., Melbourne, Australia. The amount of FAC in EOW and NaClO was measured using a free chlorine and chlorine ultra-high range portable photometer (HI 96771C; Hanna Instruments Australia, Keysborough, Australia) according to the manufacturer's instructions. The pH and oxidation-reduction potential (ORP) of EOW, NaClO and tap water were measured using a Eutech PC700 m (John Morris Scientific, Wayville, Australia) with separate pH and ORP probes, respectively.

2.5. Plant inoculation experiments and bacterial recovery from plant leaves

2.5.1. Plant inoculation

Plant seedlings were grown for 7 days (for cos lettuce) or 14 days (for baby spinach) prior to application of the bacterial inocula. The bacteria/manure mix was applied manually using a 500-mL spray bottle. Approximately 10 mL (measured) of the mixed bacterial suspension (equivalent to approx. 1×10^9 CFU total for each strain) was applied to each plant. Plants were allowed to air-dry for 2–3 h after application of the bacterial inocula in a manner similar to that described by others (Jacob and Melotto, 2020; Van der Linden et al., 2014).

2.5.2. Plant washing

Solutions for the washing procedure were freshly prepared at the time of application. EOW (~350 mg/L FAC) and NaClO (12,500 mg/L FAC) were diluted to the appropriate concentration with tap water (0.16 mg/L FAC) and the FAC content of the diluted solutions was tested as described above; pH and ORP were also recorded for each trial. The pH and ORP of the test solutions were as follows: EOW pH 6.8, ORP 855 mV; NaClO pH 10.3, ORP 616 mV. Sixty-liter drums were filled with the diluted solutions and tap water was used as the control. A sprinkler system was flushed with the test solutions prior to use on the plants. Plants were sprinkler irrigated from an overhead sprinkler system with approx. 25 L of solution over a period of 10 min (equivalent to a 6 mm irrigation event). Plants were left to dry for 1–2 h after the washing procedure and prior to sampling.

2.5.3. Leaf harvesting and bacterial enumeration

Leaves were harvested from growing plants at days 0, 3 and 7 after application of the inocula and the washing procedure ($n = 5$ per treatment per time point). Leaves were cut at the base of the stem and placed into sterile stomacher bags (Thermo Fisher Scientific); the wet weight of plant material was recorded. Sterile peptone water (0.1%; Thermo Fisher Scientific) was added (50 or 100 mL) and the leaves were processed in a Seward BA6021 Stomacher (Seward Limited, Worthing, UK) for 1 min to extract and wash intact microbes into solution. The bacterial suspension was then serially diluted in sterile peptone water and surviving bacterial colonies were enumerated on selective media as described above; total bacterial counts were also enumerated on plate count agar (PCA; PP2145, Thermo Fisher Scientific). Agar plates were incubated at 37 °C for 24–36 h. Bacterial counts were reported as CFU/g wet weight plant material.

2.5.4. Sensory evaluation of lettuce and spinach leaves

A post-harvest quality rating scheme for cos lettuce and baby spinach leaves was used for post-harvest quality assessment. For each treatment, five individual leaves were packed in separate sealable plastic bags. All bags with leaves were stored in a container with ice or ice packs at 4 °C for 10 days. Photographs of the leaves were taken on days 0 and 10 post sampling and the samples were independently assessed for post-harvest quality by five trained sensory panelists. For sensory evaluation, a previously optimized shelf-life quality rating scheme was used (Table 1).

2.5.5. DNA extraction, quantitative PCR and microbial ecology analysis

In order to determine the overall microbial composition of leaves, 15 mL aliquots from the processed (homogenized) samples from Section 2.5.3 ($n = 5$) of each treatment at days 0 and 3 were centrifuged at 4,000×*g* for 7 min, the supernatant was decanted and DNA was extracted from the pellet using the DNeasy PowerSoil® kit (QIAGEN Cat No 12888-100). DNA was eluted in 100 μL of RNAse and DNAse free water and the amount of DNA extracted from each sample was determined on a DS-11 Series spectrophotometer (DeNovix Inc, Wilmington, DE, USA). Quantitative PCR (qPCR) was performed in a LightCycler®480 II instrument (Roche Diagnostics GmbH) using gene-specific primers and associated cycling parameters (*cdsA* for *L. innocua*, 16S rRNA gene for total bacteria and internal transcribed spacer (ITS) region for fungi). The numbers of copies of the qPCR standards were calculated by assuming average molecular masses of 340 Da for 1 nucleotide of single-stranded DNA according to the following equation: copies per nanogram = (amount × NL)/(n × 10^9 x *mw*), where amount is the concentration of template in ng, NL is the Avogadro constant (6.022 × 10^{23} molecules per mol), n is the length of the strain in base pairs or nucleotides and *mw* is the average molecular weight per bp or nucleotide. The sample copy numbers were determined from the standard curve and subsequently standardized to copy numbers per gram of material. In all runs, standard curves and the amplification efficiency were calculated using the software manufactured by Roche. The efficiency of the different real-time PCRs ranged from 95 to 100%. The threshold of each single run was placed above any baseline activity and within the exponential increase phase. The cycle thresholds (C_T) were determined by a mathematical analysis of the resulting curve using the software manufactured by Roche. The C_T values of the no-template controls were always around 40, indicating no amplification and

Table 1
Quality rating scheme for cos lettuce and baby spinach leaves used in this study.

Criteria	Rating				
Yellowing	No yellowing	slight yellowing	just acceptable	unacceptable yellowing	very severe yellowing
Bruising	No bruising	slight bruising	just acceptable	unacceptable bruising	very severe bruising
Wilting	No wilting	slight wilting	just acceptable	unacceptable wilting	very severe wilting
Sliming	No Sliming	No rating	sliming evident	bad sliming	very severe sliming
Browning	No browning,	slight browning	just acceptable	unacceptable browning	very severe browning

internal positive control strains were around 25. Melting curves were determined for qPCR products to confirm product integrity and assess the presence of inhibitors, including the presence of primer-dimers. Among the different qPCR coefficients, attention was given to the R^2 coefficient which was used to analyze the standard curves obtained by linear regression analysis. For each run, the R^2 was $\geq$0.99 (values between zero and −1 a negative correlation and between zero and +1 for a positive correlation). Most of the samples, and all standards, were assessed in at least two different runs to confirm the reproducibility of the quantification and all the samples were free of PCR inhibitors. The primers used are listed in Table 2.

2.6. Statistical analyses

All figures were drawn and statistical analyses performed using Prism v8 (GraphPad Software, San Diego, CA, USA). A two-way analysis of variance (Tukey's multiple comparisons) was performed to evaluate statistical differences between mean bacterial counts, chlorophyll content or gene copy numbers between groups for each time point. A *p*-value of <0.05 was considered statistically significant.

3. Results

3.1. Determination of the effective concentrations of EOW and NaClO for contaminated lettuce leaves

In a preliminary experiment, we investigated the ability of EOW and NaClO solutions at an average value of 5.8 mg/L of FAC in the presence of 100 mg/L of DOC to significantly reduce the microbial load on contaminated cos lettuce leaves relative to tap water-treated plants. There were no statistically significant differences among the treatments at this concentration of FAC (Fig. 1). We attributed the overall lack of efficacy of EOW and NaClO at this concentration to the consumption/quenching of the FAC by the DOC in the organic matter (Ogunniyi et al., 2019). The leaf visual quality during the 7-days experimentation and shelf life post-harvest were not affected by the use of EOW or NaClO at the above FAC concentration (Figure S1A, B).

3.2. EOW at 20 mg/L and 50 mg/L FAC is effective in reducing microbial load on contaminated cos lettuce leaves in the presence of 100 mg/L DOC

Because of the quenching of EOW and NaClO at high DOC content, we examined EOW efficacy in reducing bacterial contamination on plant leaves at increased FAC concentrations of 20 and 50 mg/L. At both of these concentrations, EOW substantially reduced the microbial contamination of the lettuce leaves, with EOW at 50 mg/L of FAC showing the most pronounced and statistically significant difference in reducing *L. innocua* populations from the lettuce leaves by day 3 post-inoculation (>2 $\log_{10}$ reduction; Fig. 2).

Again, the overall leaf appearance during the 7-days evaluation and the shelf life of the leaves post-harvest were not affected by the use of EOW at either 20 mg/L or 50 mg/L of FAC (Figure S2A, B), leading to the choice of EOW at 50 mg/L of FAC for further experimentation.

Table 2
Primers used for quantification of surrogate bacterial pathogens.

Primer name	Primer sequence (5' → 3')	Product length	Source/reference
cdsA F	GTGGTTAGTTGTCGTGCCAGATAG	163	This work
cdsA R	AGCAGCAACCATACAAATTCCAAC		
16S F	TCCTACGGGAGGCAGCAGT	195	Modified from (
16S R	ATTACCGCGGCTGCTGG		Muyzer et al., 1993)
ITS F	AGAGCACTGTGCACTTAAG	208	Chiang et al. (2011)
ITS R	CATTATCACGGTAATTAGTG		

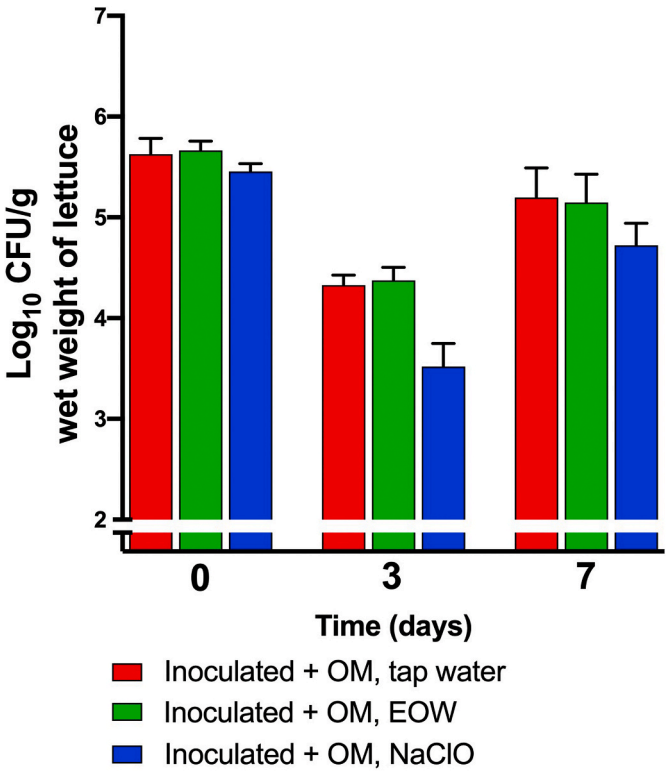

Fig. 1. Preliminary assessment of the efficacy of ~5.8 mg/L of free available chlorine for either electrolyzed oxidizing water (EOW) or sodium hypochlorite (NaClO) in comparison with tap water (control) in reducing total microbial load (on plate count agar) on contaminated cos lettuce leaves in the presence of organic matter (OM: 100 mg/L of dissolved organic carbon). CFU: colony forming units. Values presented are mean ± SEM (n = 3); horizontal segment shows the limit of detection (100 CFU).

3.3. EOW is more effective than NaClO at 50 mg/L FAC in reducing microbial load on contaminated cos lettuce leaves

We carried out a further assessment of 50 mg/L of FAC for either EOW or NaClO in reducing microbial load on contaminated cos lettuce leaves. In this experiment, both EOW and NaClO treatment led to statistically significant reductions in abundance of all microbial populations tested when compared with untreated plants (mean reductions of 1.2, 1.2, 1.0 and 1.3 $\log_{10}$ for total bacteria, *E. coli*, *S.* Enteritidis 11RX and *L. innocua*, respectively; $p < 0.001$). In addition, EOW treatment resulted in statistically significant reductions in *E. coli* (0.7 $\log_{10}$ reduction; $p < 0.05$) and *L. innocua* (0.8 $\log_{10}$ reduction; $p < 0.05$) abundance while NaClO treatment resulted in a statistically significant reduction in *L. innocua* (0.8 $\log_{10}$ reduction; $p < 0.05$) abundance when compared with tap water treatment at day 0 (Fig. 3). EOW treatment did not affect leaf quality during the lettuce growth period whereas the overall leaf quality after NaClO treatment deteriorated from day 3 onwards with severe necrotic zones, yellowing and browning of leaves (Figure S3A, B).

3.4. EOW and NaClO at 50 mg/L of FAC are both effective in reducing microbial load on contaminated baby spinach leaves

The experiment performed on lettuce leaves using 50 mg/L of FAC for either EOW or NaClO was repeated for baby spinach. In this experiment, EOW treatment resulted in statistically significant reductions in *E. coli* (1.5 $\log_{10}$ reduction; $p < 0.05$), *S.* Enteritidis 11RX (1.8 $\log_{10}$ reduction; $p < 0.01$) and *L. innocua* (1.5 $\log_{10}$ reduction; $p < 0.05$) abundance, while NaClO treatment resulted in statistically significant reductions in *E. coli* (1.5 $\log_{10}$ reduction; $p < 0.05$) and *S.* Enteritidis

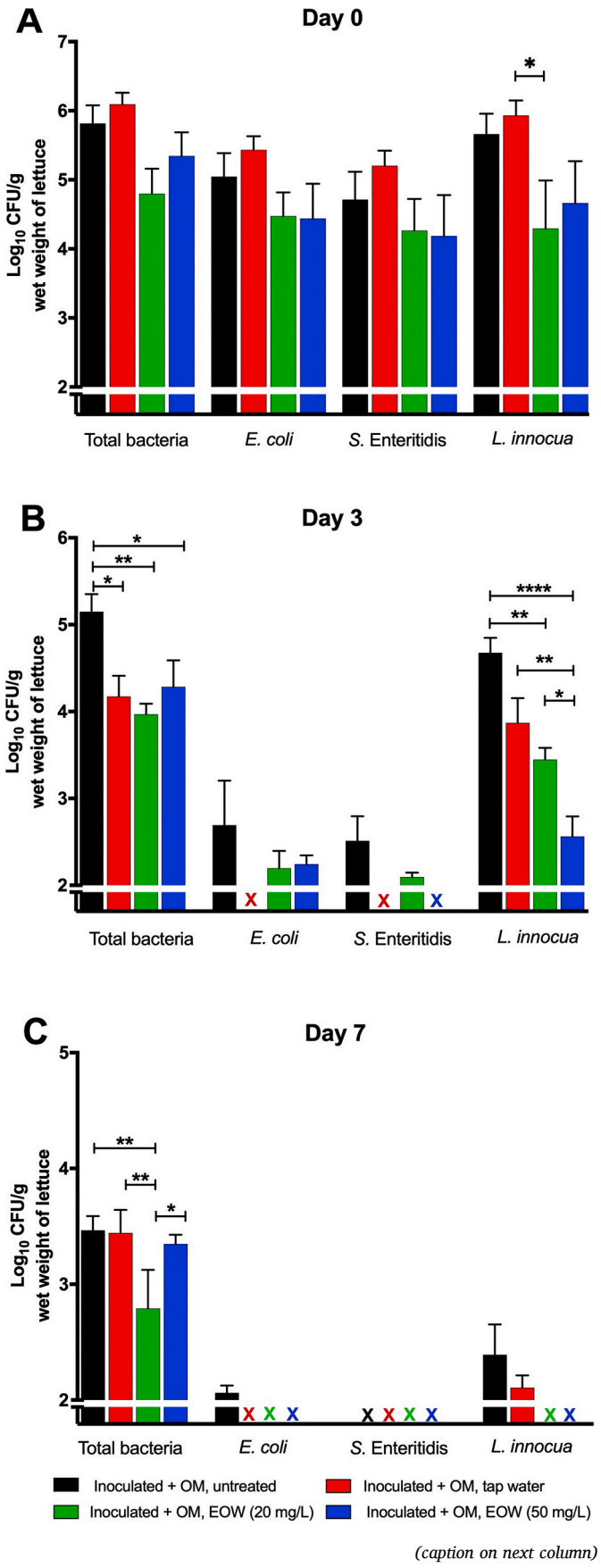

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Fig. 2. Assessment of the efficacy of 20 and 50 mg/L of free available chlorine for electrolyzed oxidizing water (EOW) in reducing microbial load on contaminated cos lettuce leaves in the presence of 100 mg/L of dissolved organic carbon. A: day 0; B: day 3; C: day 7 post-irrigation treatment. *E. coli*: *Escherichia coli*; *S.* Enteritidis: *Salmonella* Enteritidis 11RX; *L. innocua*: *Listeria innocua* 6a. CFU: colony-forming units; OM: 100 mg/L of dissolved organic carbon. Values presented are mean ± SEM ($n = 3$); horizontal segment shows the limit of detection (100 CFU); X in colour denotes no colonies detected. *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$; two-way analysis of variance (Tukey's multiple comparisons). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

11RX (1.5 $\log_{10}$ reduction; $p < 0.05$) when compared with untreated plants at Day 0 (Fig. 4). EOW treatment also showed statistically significant reductions in *S.* Enteritidis 11RX (1.4 $\log_{10}$ reduction; $p < 0.05$) and *L. innocua* (1.4 $\log_{10}$ reduction; $p < 0.05$) abundance when compared with tap water treatment at day 0. The *L. innocua* numbers were higher for EOW treatment compared to NaClO treatment at day 3, but there was no statistically significant difference in numbers by day 7 between the treatment groups (Fig. 4). As observed for lettuce leaves, EOW treatment did not affect the quality of baby spinach leaves whereas the overall quality of the leaves treated with NaClO deteriorated from day 3 onwards (Figure S4A, B).

3.5. EOW treatment does not affect the post-harvest quality of lettuce and spinach leaves

To assess any post-harvest effects of irrigating lettuce and spinach plants with EOW or NaClO, photographs of five individual leaves packed in separate sealable bags were taken at days 0 and 10 post-harvest, and were independently assessed for post-harvest quality by five trained sensory panelists using a previously optimized shelf-life quality rating scheme. We found that there were no visual effects of EOW or tap water irrigation on baby spinach or lettuce leaf surfaces pre- or post-harvest, whereas there were obvious negative effects of NaClO irrigation on leaf appearance for both plants, including severe necrotic zones and yellowing/browning of leaves (Fig. 5).

3.6. EOW and NaClO significantly reduce bacterial contamination, but not the overall microbial load of leaves

In order to complement the results obtained on the effectiveness of EOW or NaClO at decontaminating or reducing the microbial contamination of lettuce and spinach leaves, the *cdsA* gene was used to determine the abundance of *L. innocua* populations, 16S rRNA was used for total bacterial load and ITS was used for total fungal load by quantitative PCR. There was a statistically significant reduction in gene copy numbers for the *L. innocua cdsA* gene from lettuce leaves treated with EOW (1.1 $\log_{10}$ reduction; $p < 0.05$) or NaClO (1.4 $\log_{10}$ reduction; $p < 0.05$) when compared with untreated leaves at day 0 (Fig. 6). There were statistically significant reductions in *cdsA* gene copy number for baby spinach leaves treated with EOW or NaClO when compared with tap water (0.7 $\log_{10}$ reduction, $p < 0.01$ and 1.2 $\log_{10}$ reduction, $p < 0.001$, respectively) or untreated leaves (0.7 $\log_{10}$ reduction, $p < 0.001$ and 1.2 $\log_{10}$ reduction, $p < 0.0001$, respectively) at this time point; no statistically significant differences were seen at day 3 for both leaves. (Fig. 6). Quantitative PCR was not carried out for day 7 as viable counts were very low across all the samples at this time point. Together, these results corroborate the reduction in the *L. innocua* viable counts obtained from both types of fresh produce. However, apart from a statistically significant reduction in the ITS gene abundance in the EOW-treated spinach leaves at day 0 (0.9 $\log_{10}$ reduction, $p < 0.05$), there were no statistically significant differences in the copy numbers for 16S rRNA and ITS genes. The lack of decrease in total abundance of bacteria and fungi on the leaf surface indicated that the EOW treatment did not result in substantial disruption of the resident leaf microbiota, but was effective at reducing

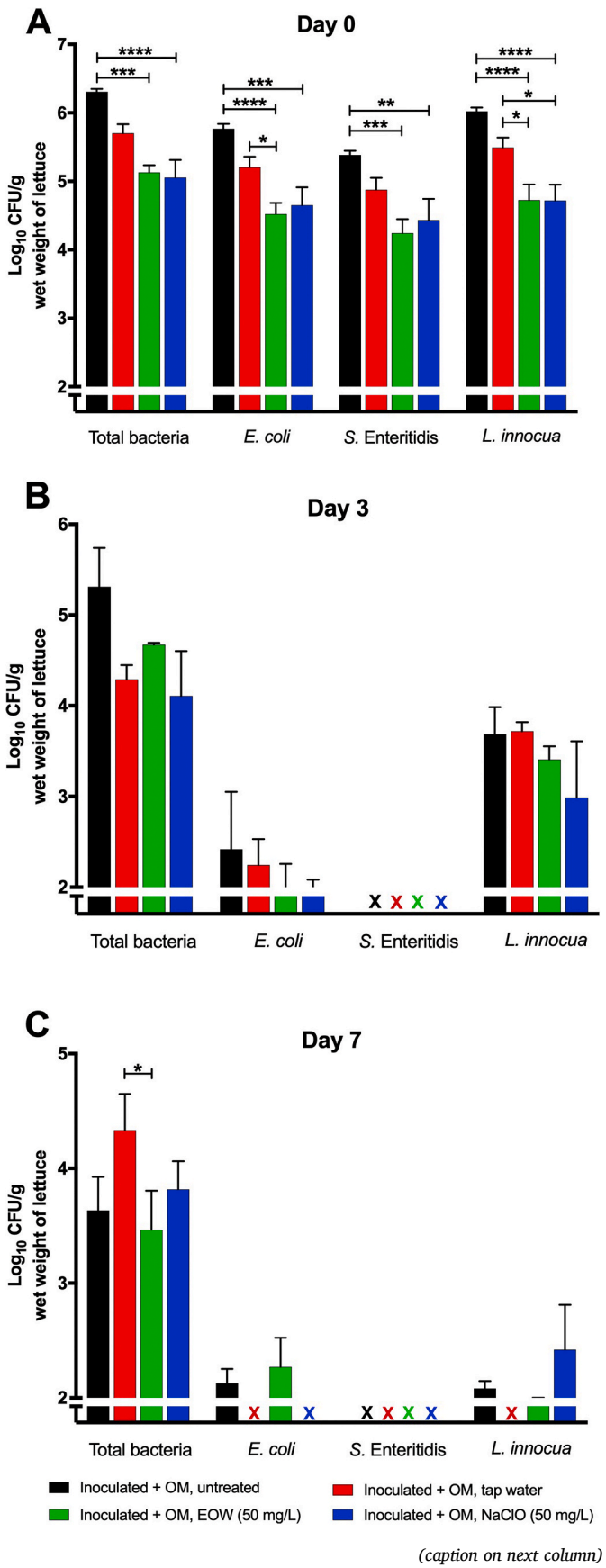

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Fig. 3. Comparative assessment of 50 mg/L of free available chlorine for either electrolyzed oxidizing water (EOW) or sodium hypochlorite (NaClO) in reducing microbial load on contaminated cos lettuce leaves. A: day 0; B: day 3; C: day 7 post-irrigation treatment. *E. coli*: *Escherichia coli*; *S.* Enteritidis: *Salmonella* Enteritidis 11RX; *L. innocua*: *Listeria innocua* 6a. CFU: colony-forming units; OM: 100 mg/L of dissolved organic carbon. Values presented are mean ± SEM ($n = 5$); horizontal segment shows the limit of detection (100 CFU); X in colour denotes no colonies detected. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$; two-way analysis of variance (Tukey's multiple comparisons). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the abundance of newly-inoculated bacteria in the scenario tested here.

4. Discussion

Safe, effective and environmentally-friendly strategies for sanitization of fresh produce are being evaluated and promoted worldwide. Sanitization strategies should ideally target spoilage and foodborne pathogens without affecting the indigenous microbiome present on fresh produce, which acts as a "natural biological barrier" against colonization by spoilage organisms and pathogens (Andrews and Harris, 2000; Barth et al., 2009; Janisiewicz and Korsten, 2002). As such, it is crucial to avoid contamination of leaf surfaces in the first instance as post-harvest washing to remove bacteria from leaf surfaces is not always effective once the bacteria are firmly attached (Sigge et al., 2016; Yaron and Romling, 2014). Therefore, to maximize the quality of fresh produce, it is critical that good agricultural practices are implemented before, during and after harvest to maintain good soil and water quality and promote a balanced and functioning microbial ecosystem, as defined in the Codex General Principles of Food Hygiene (Codex Alimentarius Commission, 2003).

In this work, we compared the efficacy of EOW and NaClO in reducing artificial microbial contamination of pre-harvest cos lettuce and baby spinach plants grown under hydroponic conditions in a greenhouse. Lettuce and spinach are minimally processed ready-to-eat vegetables known to be susceptible to colonization by foodborne pathogens (Hackl et al., 2013). The plants were spray-inoculated in a manure suspension with a mixture of three model organisms (*E. coli*, *S.* Enteritidis and *L. innocua*) that are representatives or surrogates of important pathogenic bacteria of fresh produce. This simulated a worst-case scenario of soil/manure splash-back onto plant leaves with high contamination levels. The plants were then irrigated with tap water, EOW at 5.8, 20 and 50 mg/L FAC or NaClO at 5.8 and 50 mg/L FAC. The controlled greenhouse environment allowed us to assess the effect of the irrigation treatment without introducing any further confounding factors. The concentrations of these sanitizers were chosen based on our recent work, which showed that concentrations of EOW and NaClO at 20–50 mg/L FAC were effective in eliminating microbial contamination from contaminated water in the presence of very high DOC (Ogunniyi et al., 2019). Post-irrigation evaluation of the sanitizer efficacy at the highest examined rate of 50 mg/L FAC showed that treatment with EOW and NaClO resulted in significant reductions in inoculum survival at day 0 for both lettuce and spinach leaves. Furthermore, there were no visual effects of irrigation with EOW or tap water on the lettuce and spinach leaf surfaces; however, there were obvious negative effects of NaClO at that concentration on leaf appearance, with severe necrotic zones, yellowing and browning of the leaves appearing from day 3 post-irrigation. These findings clearly indicate the potential for EOW pre-harvest sanitization of fresh produce, even at free chlorine concentrations that would otherwise be detrimental to produce quality.

Although significant reductions in viable counts were observed for the irrigation water treatments applied, these did not reduce the concentrations of applied bacteria to below acceptable guideline levels for consumption [set at less than 3 cfu for *E. coli* per 25 g and no cfu for *Listeria* spp or *Salmonella* spp per 25 g of ready-to eat foods] (FSANZ,

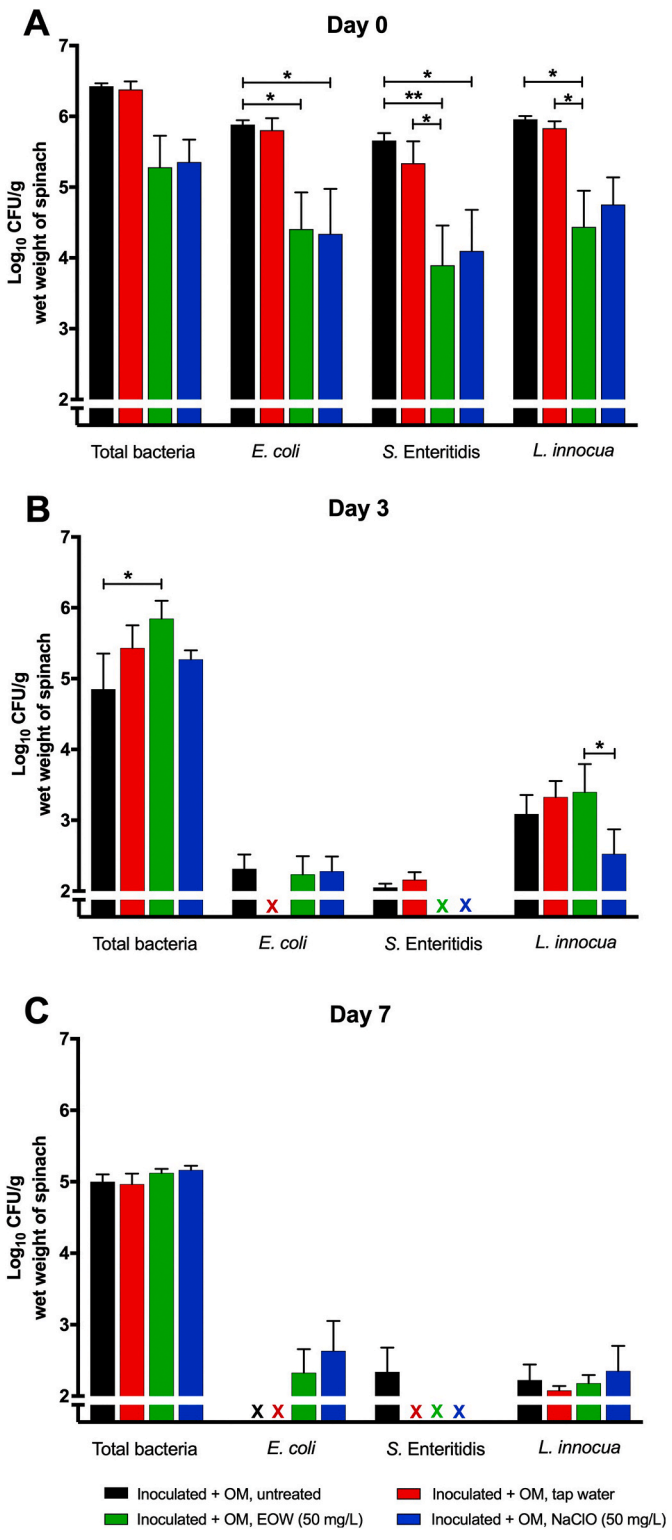

Fig. 4. Comparative assessment of 50 mg/L of free available chlorine for either electrolyzed oxidizing water (EOW) or sodium hypochlorite (NaClO) in reducing microbial load on contaminated baby spinach leaves. A: day 0; B: day 3; C: day 7 post-irrigation treatment. *E. coli*: *Escherichia coli*; *S.* Enteritidis: *Salmonella* Enteritidis 11RX; *L. innocua*: *Listeria innocua* 6a. CFU: colony-forming units; OM: 100 mg/L of dissolved organic carbon. Values presented are mean ± SEM ($n = 5$); horizontal segment shows the limit of detection (100 CFU); X in colour denotes no colonies detected. *$p < 0.05$; **$p < 0.01$; two-way analysis of variance (Tukey's multiple comparisons). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2018). However, the very high concentrations of bacteria applied in this scenario (10^6 cfu/g wet weight leaf material) means that it would be extremely unlikely for any treatment to reduce counts by 4–6 $\log_{10}$ to comply with the food guideline requirements. This experiment was a worst case scenario to demonstrate the potential efficacy of the irrigation water treatment in reducing microbial counts on contaminated leaves, as part of a multiple hurdle approach to reducing the risk of pathogen contamination of leafy greens. Other components of this hurdle approach might include the use of withholding periods prior to harvest, post-harvest washing and other treatments, each of which might not on their own be sufficient to control foodborne pathogens, but which together significantly reduce the risk (Mogren et al., 2018).

The microbiome on fresh produce can act as a natural biological barrier against spoilage organisms and invading pathogens (Andrews and Harris, 2000; Barth et al., 2009; Janisiewicz and Korsten, 2002), and treatments should preferably not affect this barrier. The main contributing factors to changes in the microbial ecology in soil, vegetables and fruits after treatment include irrigation water quality, soil type, harvest season, harvest techniques, pre- and post-harvest sanitization practices, nature and relative abundance of resident microbiota in the rhizosphere, and treatment processes (Allende and Monaghan, 2015; Berg and Smalla, 2009; Cluff et al., 2014; Frenk et al., 2014). There are few published articles on changes to the microbial ecology of soil and foliar tissues after irrigation with treated irrigation water. Yin et al. (2019) showed that the transfer of indicator organisms from irrigation waters to spinach leaves was dependent on the type of water and growing season.

Chlorine-based sanitizers have been shown to reduce beneficial inhibitory microbes in lettuce and spinach (Johnston et al., 2009). However, a study using low residual ClO_2 concentrations (approx. 0.25 mg/L) to treat irrigation water throughout the growing period of baby spinach concluded that the phyllosphere bacterial community of the leaves was mainly influenced by the soil bacterial community (Truchado et al., 2018). The study also demonstrated that the ClO_2 treatment only caused changes in two bacterial families of the baby spinach and soil microbiota, without affecting the major phyla and classes, and our preliminary investigations support this finding. While treatment of lettuce and baby spinach leaves with EOW and NaClO significantly reduced the abundance of the bacteria inoculated onto the leaves, neither EOW nor NaClO significantly changed the overall abundance of microbial (bacterial and fungal) communities present on the leaves, except for a significant reduction in the ITS gene in the EOW-treated spinach leaves. Although the abundance did not generally change, it is possible that irrigation with treated water could change the microbial community composition, with potential effects on disease resistance, growth rate, post-harvest losses or other properties. The effect of EOW on the microbial ecology of ready-to-eat leafy vegetables requires further evaluation.

There are several other considerations in the application of EOW in the field, including potential effects on soil, cost and feasibility of large-scale application. The use of NaCl-based EOW has potential negative effects because of the addition of Na ions to soil, which could result in problems with sodicity and dispersive soils. The use of KCl could overcome some of these issues, given that K-based EOW is just as effective as Na-based EOW (Ogunniyi et al., 2019) and that K can be used as a plant nutrient. The cost and feasibility of application are issues that would have to be addressed at a local site scale.

5. Conclusions

We tested the efficacy of EOW and NaClO at 50 mg/L FAC as pre-harvest sanitizers of artificially-contaminated lettuce and spinach grown under hydroponic greenhouse conditions. While both sanitizers were effective in reducing microbial counts on contaminated leaves, NaClO had severe negative effects on leaf quality both pre- and post-harvest, whereas EOW showed no discernible negative effects on leaf quality throughout the experiment. This study provides essential

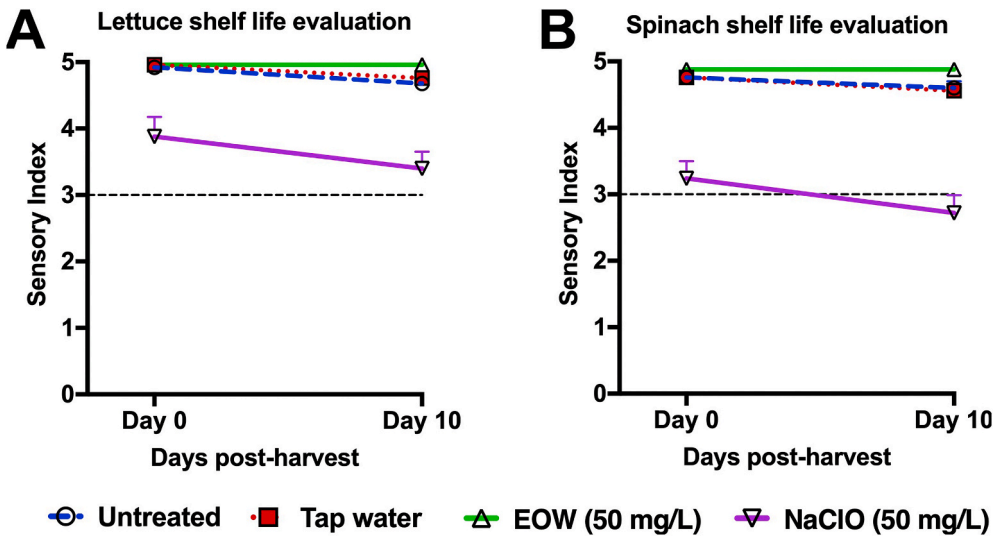

Fig. 5. Shelf life assessment of lettuce (A) and spinach (B) leaf quality after treatment with electrolyzed oxidizing water (EOW) or sodium hypochlorite (NaClO) at 50 mg/L of free available chlorine, compared with tap water-treated or untreated leaves. The results presented are mean (±SEM) scores of 5 sensory panelists based on the following sensory attributes: yellowing, bruising, wilting, sliming and browning. The sensory cut-off score was fixed at a SI of 3.

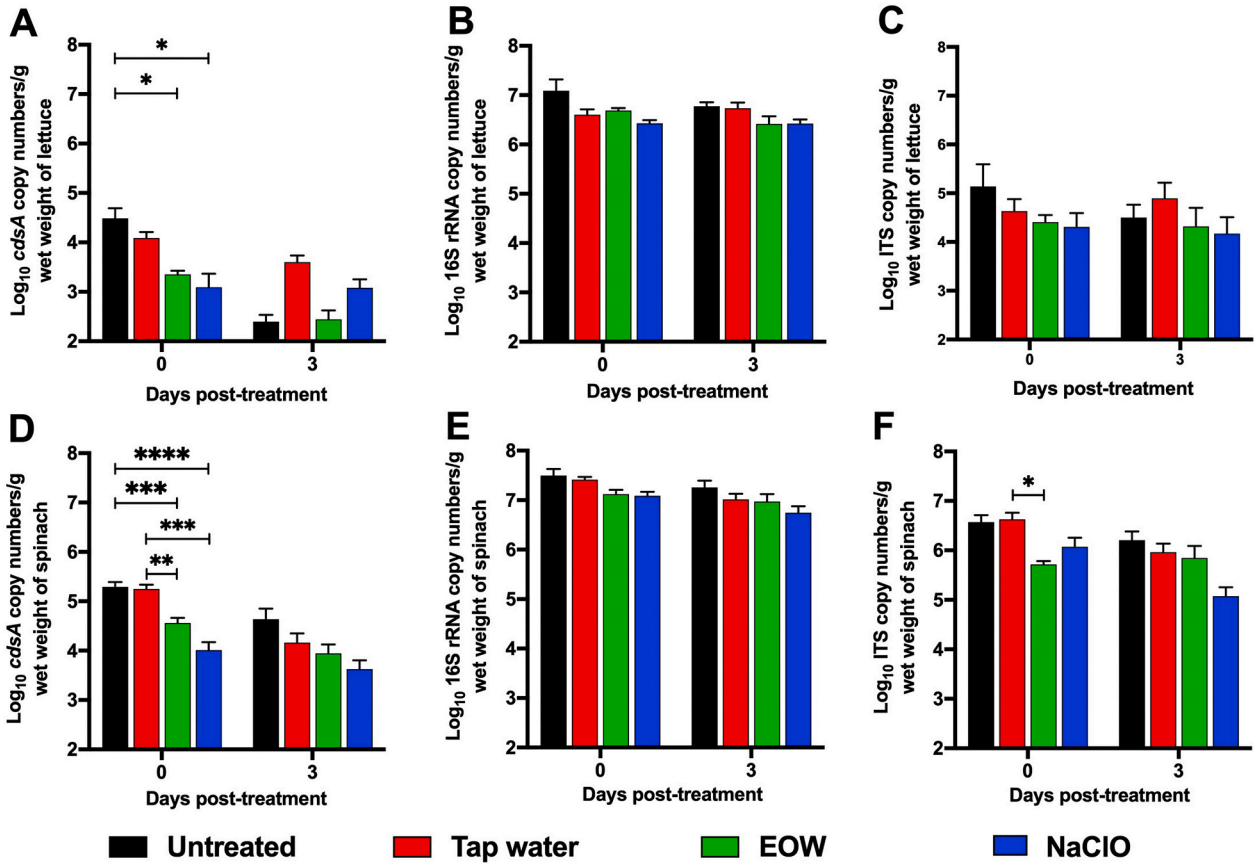

Fig. 6. Quantitative PCR analysis of the *Listeria innocua cdsA* (A, D), 16S rRNA (B, E) and internal transcribed spacer (ITS; C, F) genes from lettuce (A–C) and baby spinach (D–F) leaves treated with electrolyzed oxidizing water (EOW) or sodium hypochlorite (NaClO) at 50 mg/L of free available chlorine, compared with tap water-treated or untreated leaves. Values presented are mean ± SEM ($n = 5$) gene copy numbers at days 0 and 3 post treatment; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ two-way analysis of variance (Tukey's multiple comparisons).

information on the conditions required for efficient use of EOW for pre-harvest sanitization of fresh produce such as lettuce and spinach. However, the preliminary results obtained here on hydroponically-grown immature plants should be verified with large-scale field trials in agricultural soils. For a single application, FAC concentrations in EOW of up to 50 mg/L were not harmful to lettuce or spinach leaves under the conditions tested.

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Declaration of competing interest

Sergio Ferro is the technical manager of Ecas4 Australia.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2020.103610.

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Materials and Methods

1. Soil samples and collection

Soil samples were obtained from several vegetable growing regions around Australia (Fig. 1 and Table 1). Intact soil columns (12) were sampled from a single vegetable field at each site and transported back to the University of South Australia, Mawson Lakes campus, for further work.

Table 1. Soil samples location and characteristics

Site, State (Code):	Collected by:	Site information/ characteristics:
Parilla, Mallee region, SA (SAM)	Barbara Hall/Michael Rettke, SARDI	Sandy soil, site currently producing carrots, previously potato and cereals for many years
Virginia, SA (SAVC)	Cathy Dandie/David Ogunniyi	Currently under cauliflower production
Virginia, SA (SAVL)	Cathy Dandie/David Ogunniyi	Currently under lettuce production
Clyde, VIC (VIC)	Clinton Muller/Ian Douglas, RMCG	Britten sand soil type, producing celery, leek and peas over the last three years
Wesley Vale, TAS (TAS)	Doris Blaesing/Theresa Chapman, RMCG	Currently producing broccoli, previously peas, under vegetable rotation for many years
Gatton, QLD (QLD)	John Duff, Horticulture and Plant Science, QLD Dept of Forestry and Fisheries	Horticultural trial site with a range of crops and activities, dark cracking clay.

Fig. 1 Air-dried soils in weighing trays

2. Basic soil characterization

Soils were characterized in terms of their basic characteristics with standard methods (Table 2). Soil pH was determined on a 1:5 soil:solution suspension using water (pH_w) or 0.01 M $CaCl_2$ (pH_{Ca}) (Rayment and Higginson, 1992). Electrical conductivity (EC) was determined on a 1:5 soil:water suspension (Rayment and Higginson, 1992). Total organic carbon was determined on 0.25 g ground dried soil treated with sulphurous acid and analysed by LECO CNS (LECO Australia Pty Ltd, Castle Hill, NSW). Soil texture was determined according to standard methods (Miller and Miller, 2008) by APAL Laboratories (Hindmarsh, SA).

Table 2. Basic soil characteristics

Soil	pH_w	pH_{Ca}	EC	TOC (%)	Clay (%)	Sand (%)	Silt (%)	Soil texture
SAM	7.1	7.0	417	0.3	10	87	3	Sandy loam
SAVC	7.6	7.4	405	1.2	16	73	11	Sandy loam
SAVL	7.3	7.3	914	1.0	28	51	21	Clay loam
VIC	6.2	6.3	400	2.2	9	79	12	Loamy sand
TAS	6.2	6.3	112	2.2	7	67	26	Silty loam
QLD	7.5	7.3	685	1.2	24	44	32	Silty loam

EC: electrical conductivity, µS cm^{-1}; TOC: total organic carbon.

3. Experimental treatments

The aim of this activity was to test the repeated application of sanitised water on the soil microbial community, key soil functions driving nutrient cycles and soil physical-chemical parameters. To achieve this aim, soils were incubated in a greenhouse with repeated application of 1) water, 2) electrolysed oxidising (EO) water at 5 mg/l or 3) sodium hypochlorite (NaOCl) at 5 mg/l. The choice of 5 mg/l free chlorine concentration was driven by the acceptable chlorine residual for recycled water (up to 5 mg/l; NRMMC, EPHC and AHMC, 2006), noting that there are no specific limits for free chlorine residual in irrigation water (ANZECC and ARMCANZ, 2000). Concentrated solutions of EO water and NaOCl were diluted in the same Milli-Q water that was used for the control irrigation water treatment. A summary of the irrigation water characteristics is shown in Table 3. Soils were watered over a period of 14 weeks, with 250 ml of the watering treatment applied to each core each week. Soils were rotated weekly within the greenhouse. The greenhouse temperature was adjusted to 24°C:19°C for 12 h:12 h.

Table 3. Irrigation water characteristics

Treatment	Available chlorine (mg l^{-1})	ORP (mV)	pH
Milli-Q water Electrolysed	0 (0)	NA	NA
oxidising water Sodium	5.26 (0.18)	762 (26)	6.95 (0.13)
hypochlorite	5.10 (0.27)	425 (63)	10.34 (0.11)

Values are means (standard deviation), *n* = 14. ORP: oxidising reduction potential.

4. Soil analyses

At the time of soil core sampling, soil cores were destructively sampled and sectioned at different depths (0–2, 2–5, 5–12, 12–20 cm). Fresh soil samples were used for analysis of extracellular enzyme activities (section 4.1) and for determination of moisture content. Aliqouts of samples were stored at −80°C for DNA and RNA analyses and the rest of the soil was air dried at room temperature for physico-chemical analyses.

4.1 Extracellular enzyme activities

Extracellular enzyme activities were determined on fresh soil samples at the time of soil core sampling. Enzyme activities were assayed as described in Bell et al. (2013) and Vasileiadis et al. (2018). Details of the enzymes tested and their substrates are listed in Table 4.

Table 4. Extracellular enzymes and their substrates

Enzyme	Enzyme consort. (EC) no.	Code	Dye-substrate conjugate	Nutrient cycle
α-1,4-glucosidase	3.2.1.20	AG	4-methylumbelliferyl *α*-D-	C

			glucopyranoside	
β-1,4-glucosidase	3.2.1.21	BG	4-methylumbelliferyl *β*-D-glucopyranoside	C
β-D-cellobiohydrolase	3.2.1.91	CB	4-methylumbelliferyl *β*-D-cellobioside	C
β-xylosidase	3.2.1.37	XYL	4-methylumbelliferyl *β*-D-xylopyranoside	C
L-leucine aminopeptidase	3.4.11.1	LAP	L-leucine-7-amido-4-methylcoumarin hydrochloride	N
β-1,4-N-acetylglucosaminidase	3.2.1.96	NAG	4-methylumbelliferyl N-acetyl-*β*-*D*-glucosaminide	N
Phosphatase	3.1.3.2/3.1.3.1	PHOS	4-methylumbelliferyl phosphate	P
Arylsulfatase	3.1.6.1	SUL	4-methylumbelliferyl sulphate potassium salt	S

Enzymes were assayed in modified universal buffer with the pH adjusted to that of the original soil pH. The buffer was prepared as follows: A stock solution was prepared by dissolving 12.1 g of tris(hydroxymethyl)aminomethane (THAM/Trizma), 11.6 g of maleic acid, 14.0 g of citric acid, and 6.3 g of boric acid (H3BO3) in 488 m of 1 M sodium hydroxide (NaOH); the solution was adjusted to 1 litre with Milli-Q water. The stock solution was stored at 4ºC. The working solution was prepared by placing 200 ml of the stock solution in a beaker, adjusting the pH to the required pH with 0.1 M HCl or 0.1 M NaOH, and then adjusting the volume to 1 litre with Milli-Q water.

Each soil sample was blended in a buffer solution to dissolve the enzymes, and the resulting soil slurry was incubated together with the fluorescently labelled substrate. For each soil sample, 2.75 g of soil was mixed in a blender with 91 ml of buffer. The resulting slurry was transferred into a container with continuous stirring and used to measure all enzyme activities for that soil sample. Standard curves for the methylumbelliferone (MUB)- and 7-amino-methylcoumarin (AMC)-based dyes were set up in two control plates for each soil slurry. For each test, 800 µl of slurry was incubated with either 200 µl of 0.2 mM of fluorescently labelled substrates in the test plate or 200 µl of 0, 2.5, 5, 10, 25, 50, and 100 µM dye solutions (MUB or AMC) in the corresponding control plates (for establishing standard curves and controlling for background soil fluorescence/ quenching). The test and control plates were incubated at 37 °C in the dark for 1.5 h. Post incubation, the plates were centrifuged for 3 min at 2900 × *g*, and 250 µl of each supernatant was transferred into black plates and analysed with the FLUOstar Optima (BMG Labtech, Cary, NC) microplate absorbance/fluorescence reader using the 355/460 nm excitation/emission filter-set. The substrate degradation rates were calculated according to the control plate standard curve intensity values.

4.2 Soil physicochemical analyses

Soil moisture content was determined by drying at 105°C to constant weight. Soil pH_w and EC were determined as described above in section 2.

5. Statistical analyses

All figures were drawn and statistical analyses performed using Prism v8 software package. A two-way analysis of variance (Tukey's multiple comparisons) was performed to evaluate statistical

differences between mean (±SEM) values among treatment groups and depths. A *p*-value of <0.05 was considered statistically significant.

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Soil physico-chemical characteristics

Soil pH and electrical conductivity (EC) were analysed for each soil at each depth for the three irrigation water treatments (Milli-Q, electrolysed oxidising (EO) water, sodium hypochlorite (NaOCl)).

Results summary

Soil pH (Table 1):

SA Mallee soil: There were significant differences in soil pH for NaOCl irrigation in comparison with Milli-Q water irrigation at depths of 0–2, 2–5 and 12–20 cm, but not for 5–12 cm. No significant differences in pH were obtained for EO water irrigation in comparison with Milli-Q water irrigation at any depth.

SA Virginia Lettuce soil: There were no significant differences in soil pH for any treatment in comparison with the Milli-Q water irrigation at any depth.

SA Virginia Cauliflower soil: There were significant differences in soil pH for NaOCl irrigation in comparison with Milli-Q water irrigation at depths of 0–2 and 5–12 cm. No significant differences in pH were obtained for EO water irrigation in comparison with Milli-Q water irrigation at any depth.

VIC soil: There were no significant differences in soil pH for any treatment in comparison with the Milli-Q water irrigation at any depth.

TAS soil: There were significant differences in soil pH for NaOCl irrigation in comparison with Milli-Q water irrigation at depths of 0–2 and 5–12 cm. No significant differences in pH were obtained for EO water irrigation in comparison with Milli-Q water irrigation at any depth.

QLD soil: There were significant differences in soil pH for EO water irrigation in comparison with Milli-Q water irrigation at depths of 0–2 and 5–12 cm. Significant differences in soil pH were obtained for NaOCl irrigation in comparison with Milli-Q water irrigation at depths of 0–2 and 2–5 cm. There were no significant differences among treatments at the depth of 12–20 cm.

In summary, the main effect on soil pH was derived from NaOCl irrigation treatment, with only QLD soil showing a pH response to EO water irrigation in comparison with Milli-Q water irrigation treatment. The pH generally increased in response to the NaOCl irrigation treatment, because of the high pH (average value of 10.34) of the NaOCl irrigation solution.

Soil pH

Table 1. Soil pH values by depth

Soil	Depth (cm)	Milli-Q	EO water	NaOCl
SAM	0–2	7.39 (0.08) a	7.63 (0.14) a	8.37 (0.10) b
	2–5	7.06 (0.10) a	7.29 (0.10) ab	7.57 (0.08) b
	5–12	7.10 (0.01) a	7.23 (0.10) a	7.31 (0.07) a
	12–20	7.81 (0.12) a	7.99 (0.10) a	7.45 (0.04) b
SAVL	0–2	8.24 (0.13) a	8.20 (0.21) a	8.02 (0.01) a
	2–5	8.10 (0.09) a	8.05 (0.19) a	7.74 (0.02) a
	5–12	7.94 (0.11) a	7.92 (0.20) a	7.71 (0.02) a
	12–20	7.85 (0.05) a	7.74 (0.11) a	7.73 (0.01) a
SAVC	0–2	7.98 (0.16) a	8.04 (0.05) a	8.64 (0.23) b
	2–5	7.85 (0.14) a	7.85 (0.04) a	8.31 (0.23) a
	5–12	7.87 (0.09) a	7.89 (0.03) a	8.48 (0.23) b
	12–20	8.17 (0.11) a	8.04 (0.10) a	8.44 (0.20) a
VIC	0–2	6.16 (0.13) a	6.38 (0.04) a	6.43 (0.07) a
	2–5	6.15 (0.13) a	6.31 (0.06) a	6.43 (0.07) a
	5–12	6.13 (0.10) a	6.29 (0.06) a	6.41 (0.07) a
	12–20	6.21 (0.08) a	6.28 (0.10) a	6.36 (0.07) a
TAS	0–2	6.45 (0.11) a	6.87 (0.14) ab	7.26 (0.11) b
	2–5	6.47 (0.07) a	6.73 (0.20) a	6.94 (0.18) a
	5–12	6.17 (0.05) a	6.39 (0.17) ab	6.70 (0.18) b
	12–20	6.10 (0.05) a	6.28 (0.22) a	6.56 (0.17) a
QLD	0–2	7.70 (0.07) a	8.03 0(.04) b	8.17 (0.09) b
	2–5	7.51 (0.12) a	7.48 (0.06) a	7.84 (0.08) b
	5–12	7.41 (0.08) b	7.12 (0.10) a	7.42 (0.01) b
	12–20	7.26 (0.08) a	7.06 (0.12) a	7.02 (0.02) a

Values are means (SEM) for $n = 4$. Mean values within each row with different lowercase letters (a or b) are significantly different ($p < 0.05$).

Soil EC (Table 2):

SA Mallee soil: There were no significant differences in soil EC among treatments at depths of 0–2, 2–5 and 5–12 cm. Soil EC was significantly different between EO water and Milli-Q irrigation and between EO water and NaOCl at the depth of 12–20 cm.

SA Virginia Lettuce soil: There were significant differences in soil EC between NaOCl and Milli-Q water irrigation at depths of 2–5, 5–12 and 12–20 cm. There were no significant differences among treatments at the depth of 0–2 cm.

SA Virginia Cauliflower soil: There were no significant differences in soil EC among treatments at depths of 0–2, 2–5 and 5–12 cm. Soil EC was significantly different between NaOCl and Milli-Q irrigation at the depth of 12–20 cm.

VIC soil: There were significant differences between NaOCl and Milli-Q water irrigation only at the depth of 0–2 cm. There were no significant differences among treatments at the other depths.

TAS soil: There were no significant differences in soil EC among treatments at depths of 0–2, 2–5 and 5–12 cm. Soil EC was significantly different between EO water and Milli-Q irrigation and between EO water and NaOCl at the depth of 12–20 cm.

QLD soil: There were no significant differences in soil EC among treatments at depths of 0–2, 2–5 and 5–12 cm. There were significant differences among all treatments at the depth of 12–20 cm.

In general, the EC results demonstrated leaching of soluble salts down the soil columns, and accumulation of salts in the deepest section of the soil columns (12–20 cm), therefore the results of the 12–20 cm sections might be confounded by this accumulation of salts and will not be further considered.

There were no significant effects of EO water irrigation on soil EC for any of the soils tested. There were significant effects of NaOCl irrigation only for the 2–12 cm layer of SA Virginia Lettuce soil and for the 0–2 cm layer of VIC soil, where there were significant increases in the soil EC for these treatments.

Soil EC

Table 2. Soil EC values by depth

Soil	Depth (cm)	Milli-Q	EO water	NaOCl
SAM	0–2	61 (2) a	102 (14) a	141 (14) a
	2–5	51 (3) a	62 (3) a	71 (2) a
	5–12	71 (4) a	80 (5) a	71 (2) a
	12–20	492 (59) b	310 (33) a	453 (99) b
SAVL	0–2	362 (114) a	361 (137) a	810 (37) a
	2–5	252 (69) a	417 (233) a	1109 (17) b
	5–12	503 (152) a	592 (300) a	1306 (43) b
	12–20	1109 (269) a	971 (235) a	2157 (69) b
SAVC	0–2	69 (7) a	96 (2) a	148 (14) a
	2–5	68 (6) a	79 (1) a	119 (5) a
	5–12	76 (6) a	91 (4) a	145 (23) a
	12–20	285 (43) a	199 (27) a	486 (68) b
VIC	0–2	110 (53) a	329 (146) a	768 (229) b
	2–5	56 (9) a	99 (20) a	109 (12) a
	5–12	60 (11) a	83 (13) a	102 (7) a
	12–20	124 (25) a	184 (18) a	397 (117) a
TAS	0–2	61 (6) a	65 (9) a	66 (3) a
	2–5	28 (1) a	41 (3) a	38 (2) a
	5–12	29 (1) a	30 (2) a	39 (2) a
	12–20	171 (8) b	116 (29) a	203 (28) b
QLD	0–2	46 (5) a	63 (3) a	91 (15) a
	2–5	43 (3) a	50 (2) a	67 (4) a
	5–12	44 (2) a	48 (3) a	69 (2) a
	12–20	635 (105) b	419 (84) a	1036 (157) c

Values are means (SEM) for $n = 4$. Mean values within each row with different lowercase letters (a, b or c) are significantly different ($p < 0.05$).

Soil extracellular enzyme activities

Although eight soil extracellular enzymes were assayed, there was limited activity for the majority of enzymes assayed in these soils. Therefore, results are only presented for those enzymes for which activity was measured across all depths and treatments for each soil type.

SA Mallee soil

In the SA Mallee soil, three enzymes (*β*-1,4-glucosidase (BG), L-leucine aminopeptidase (LAP) and phosphatase (PHOS)) showed activity across all samples (Fig. 1).

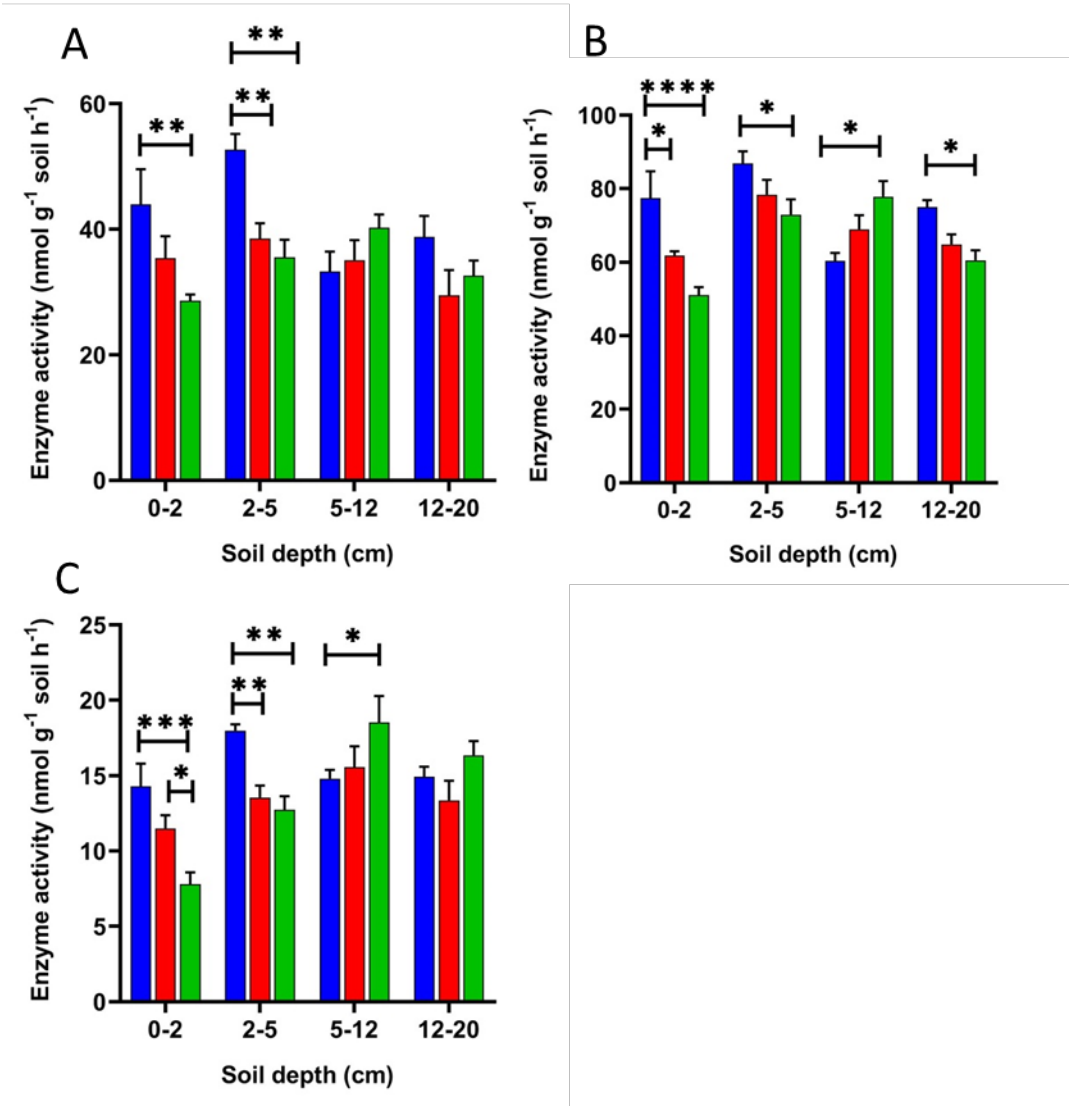


Fig. 1 Extracellular enzyme activity for SA Mallee soil. A) BG: *β*-1,4-glucosidase; B) LAP: L-leucine aminopeptidase; C) PHOS: phosphatase. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. **Blue:** Milli-Q water irrigation, **Red:** Electrolysed oxidising water irrigation, **Green:** sodium hypochlorite irrigation. Values are means ± SEM ($n = 4$).

BG activity: There was a significant decrease in BG activity in the 0–2 cm depth layer for NaOCl irrigation when compared with Milli-Q irrigation. There were significant decreases in the 2–5 cm layer for both EO water and NaOCl when compared with Milli-Q irrigation, while there were no significant differences between EO water and NaOCl treatments at this depth. There were no significant differences in enzyme activity for the soil at 5–20 cm depth between the 3 treatments.

LAP activity: There were significant differences in LAP activity between Milli-Q and NaOCl irrigation at all depths. There were significant decreases in LAP activity for NaOCl at depths of 0–2 (p<0.0001), 25 and 12–20 cm, while there was a significant increase in LAP activity for NaOCl at 5–12 cm. There was a significant decrease in LAP activity for EO water irrigation in comparison with Milli-Q irrigation only at the depth of 0–2 cm.

PHOS activity: There were significant differences in PHOS activity between Milli-Q and NaOCl irrigation at the depths of 0–2, 2–5 and 5–12 cm, but not at 12–20 cm. Similar to the LAP activity above, there was a significant decrease in PHOS activity at the depths of 0–2 and 2–5 cm, but there was a significant increase at 5–12 cm for NaOCl irrigation in comparison with Milli-Q irrigation. There was a significant difference in PHOS activity between EO water and NaOCl at the depth of 0–2 cm, and a significant different between Milli-Q and EO water at 2–5 cm.

SA Virginia Lettuce soil

In the SA Virginia Lettuce soil, three enzymes (*β*-1,4-glucosidase (BG), L-leucine aminopeptidase (LAP) and phosphatase (PHOS)) showed activity across all samples (Fig. 2).

BG activity: There were no significant differences in BG activity for any treatment in comparison with the Milli-Q water irrigation at any depth.

LAP activity: There was a significant increase in LAP activity for the NaOCl treatment in comparison with both Milli-Q water and EO water irrigation at the depth of 12–20 cm. There were no significant differences in LAP activity for any treatment at the other depths.

PHOS activity: There were no significant differences in PHOS activity for any treatment in comparison with the Milli-Q water irrigation at any depth.

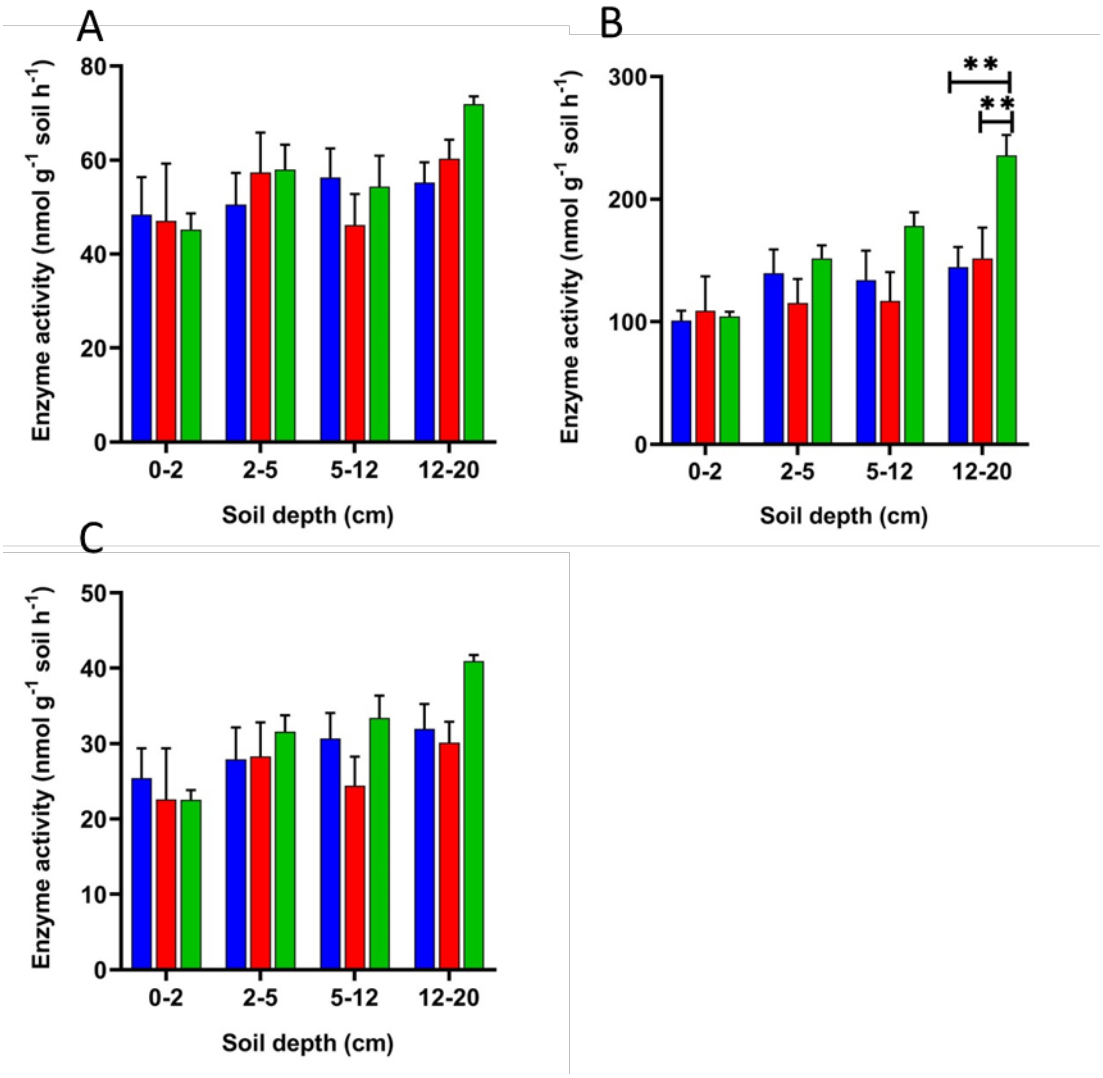


Fig. 2 Extracellular enzyme activity for SA Virginia Lettuce soil. A) BG: *β*-1,4-glucosidase; B) LAP: L-leucine aminopeptidase; C) PHOS: phosphatase. ** $p<0.01$. **Blue:** Milli-Q water irrigation, **Red:** Electrolysed oxidising water irrigation, **Green:** sodium hypochlorite irrigation. Values are means ± SEM ($n = 4$).

SA Virginia Cauliflower soil

In the SA Virginia Cauliflower soil, three enzymes (*β*-1,4-glucosidase (BG), L-leucine aminopeptidase (LAP) and phosphatase (PHOS)) showed activity across all samples (Fig. 3).

BG activity: There were no significant differences in BG activity for any treatment in comparison with the Milli-Q water irrigation at any depth.

LAP activity: There were no significant differences in LAP activity for any treatment in comparison with the Milli-Q water irrigation at any depth

PHOS activity: There were no significant differences in PHOS activity for any treatment in comparison with the Milli-Q water irrigation at any depth.

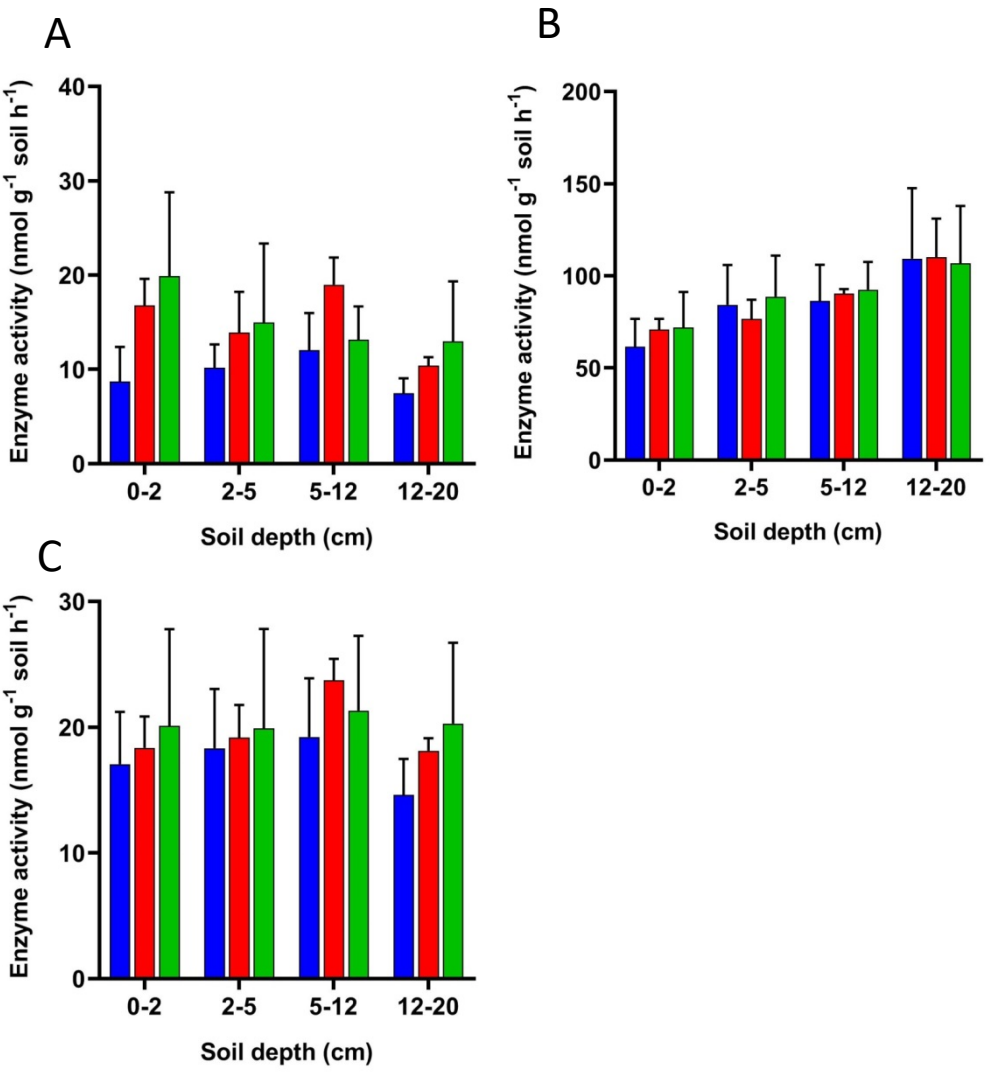


Fig. 3 Extracellular enzyme activity for SA Virginia Cauliflower soil. A) BG: *β*-1,4-glucosidase; B) LAP: L-leucine aminopeptidase; C) PHOS: phosphatase. No statistically-significant differences in enzyme activities between irrigation treatments. **Blue:** Milli-Q water irrigation, **Red:** Electrolysed oxidising water irrigation, **Green:** sodium hypochlorite irrigation. Values are means ± SEM (*n* = 4).

VIC soil

In the VIC soil, two enzymes (L-leucine aminopeptidase (LAP) and phosphatase (PHOS)) showed activity across all samples (Fig. 4).

LAP activity: There were significant increases in LAP activity for EO water irrigation in comparison with Milli-Q at depths of 2–5 and 5–12 cm. There were significant increases in LAP activity with NaOCl irrigation in comparison with Milli-Q at depths of 2–5, 5–12 and 12–20 cm. There were no significant differences in LAP activity at the depth of 0–2 cm.

PHOS activity: Although there were increases in activity with EO water and NaOCl irrigation in comparison with Milli-Q irrigation at all depths, these increases were not significant.

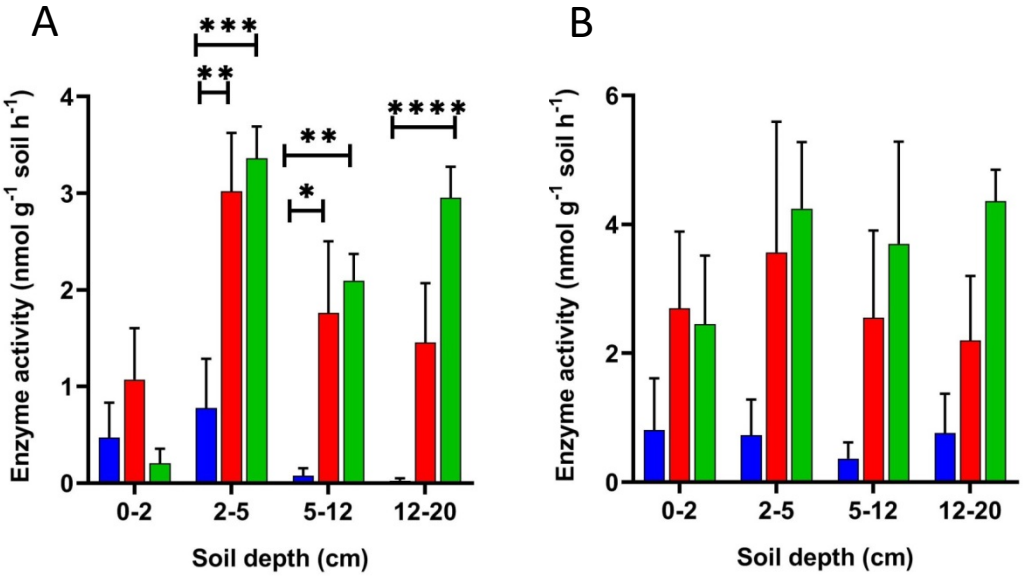


Fig. 4 Extracellular enzyme activity for VIC soil. A) LAP: L-leucine aminopeptidase; B) PHOS: phosphatase. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. **Blue:** Milli-Q water irrigation, **Red:** Electrolysed oxidising water irrigation, **Green:** sodium hypochlorite irrigation. Values are means ± SEM ($n = 4$).

TAS soil

In the TAS soil, one enzyme, phosphatase (PHOS) showed activity across all samples (Fig. 5).

PHOS activity: There were significant decreases in PHOS activity for both EO water and NaOCl irrigation in comparison with Milli-Q irrigation for the depths of 2–5 and 5–12 cm. There were no significant differences in PHOS activity at the other soil depths.

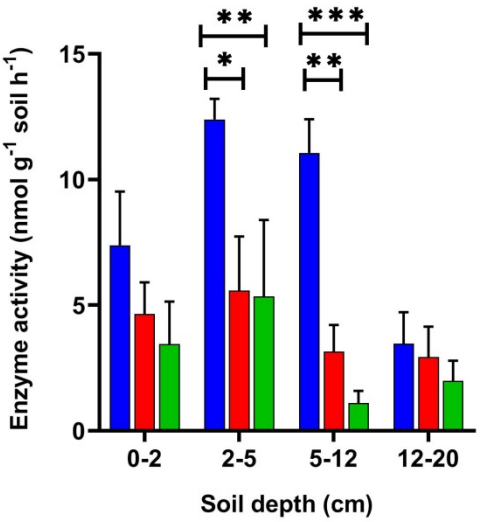


Fig. 5 Phosphatase activity for TAS soil. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. **Blue:** Milli-Q water irrigation, **Red:** Electrolysed oxidising water irrigation, **Green:** sodium hypochlorite irrigation. Values are means ± SEM ($n = 4$).

QLD soil

In the QLD soil, one enzyme, L-leucine aminopeptidase (LAP) showed activity across all samples (Fig. 6).

LAP activity: There were no significant differences in LAP activity for any treatment in comparison with the Milli-Q water irrigation at any depth.

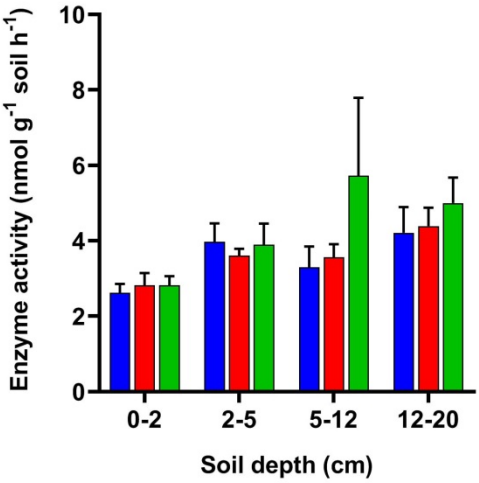


Fig. 6 L-leucine aminopeptidase activity for QLD soil. No statistically-significant differences in enzyme activity between irrigation treatments. **Blue:** Milli-Q water irrigation, **Red:** Electrolysed oxidising water irrigation, **Green:** sodium hypochlorite irrigation. Values are means ± SEM ($n = 4$).

Appendix 8: VG15068 Milestone report 106 appendices

Materials and methods

1. Preliminary Booster reactor testing

1.1 Description of the 'Booster' reactor

Because of practical considerations, the technology for on-farm disinfection was changed from the original technology (water disinfection system, used for dosing water with electrolysed oxidising (EO) water anolyte/ disinfectant) to an in-line 'Booster' reactor electrolysis treatment that generates free chlorine from the natural salts contained in the irrigation water.

The Booster is an electrochemical reactor developed by the project's subcontracted service providers Ecas4 Australia (http://www.ecas4.com.au/wp/wp-content/uploads/2019/01/Ecas4_Booster_WDS- FLP-001_1.0.pdf). The Booster reactor works by electrochemical conversion of the chloride salts present in the water into active chlorine, the main disinfecting agent. Irrigation water would generally have plenty of salts present, thus requiring no addition of any additional salt to facilitate this procedure, however if water does not have the required salt concentration, additional salts can be added (sodium chloride or potassium chloride).

Laboratory testing was conducted to investigate the efficacy of the Booster reactor to reduce microbial contamination on test microorganisms in a range of water samples and in real farm (recycled) water samples.

The Booster reactor generated free chlorine from a range of water samples, including tap water and farm water. Variation in the current resulted in variation in the free chlorine generated. Recirculation of water through the Booster reactor resulted in a time-dependent increase in free chlorine concentration.

Different size/capacity Booster reactors were used during different sections of the testing and experimentation described below.

For all tests, free chlorine was measured in a Hanna Instruments Ultra High Range Chlorine Portable Photometer (H196771), using either free chlorine reagents (H193701) for concentrations up to 5 mg/L or ultra-high-range reagents (H195771) for concentrations up to 500 mg/L. The pH and oxidation- reduction potential (ORP) of water were measured on a EUTech PC700 meter with separate pH and ORP probes, respectively.

1.2 Booster reactor testing in tap water or distilled water containing sodium chloride (NaCl)

Tap water was passed through a model "130" Booster reactor (with 130 cm^2 of anode surface) for 2 min at 600 mL/h at varying amperage/voltage to determine the relationship between these parameters and the free chlorine generated.

Pure distilled water was amended with 0.01M NaCl and compared with unamended water for the generation of free active chlorine over time. Samples were withdrawn for free chlorine analysis at 0, 2, 5 and 10 min of recirculation of water through the Booster reactor. The water was inoculated with a pure culture of *Pseudomonas aeruginosa* at a concentration of ~1×10^6 colony-forming units per mL. Colony counts were conducted on Luria-Bertani agar incubated at 37°C for 24 h to determine viable counts of bacteria pre- and post-Booster reactor treatment.

1.3 Booster reactor testing in farm dam water

Water was obtained from two vegetable farm dams filled using recycled water in Virginia, SA, used for growing lettuce and cauliflower. The water was passed through a model "130" Booster reactor for periods of up to 10 min at two different current settings (1.0 A and 4.0 A). Water samples were withdrawn and the free chlorine content was quenched with 0.05% (v/v) sodium thiosulfate at the time of sampling. Bacterial counts were monitored using Colilert and Enterolert tests for coliforms/*E. coli* and enterococci, respectively (Idexx Australia, Rydalmere, NSW, Australia).

2. Greenhouse trial

2.1 Soil and plant growth conditions

Soil was obtained from the Virginia farm site as described above in December 2019. The soil was Virginia lettuce soil, a clay loam, with pH 7.3 and total organic carbon content of 1.0%.

Plant growth experiments were undertaken at Mawson Lakes campus of UniSA in a greenhouse with temperature settings of 24°C/19°C day/night 12 h:12 h. No supplementary lighting was supplied. Soil was well-mixed and placed into round plastic pots of height 187 mm and diameter 205 mm. Lettuce (*Lactuca sativa*) and baby spinach (*Spinacia oleracea* L.) seedlings were sourced from Virginia Nursery (Virginia, SA, Australia) and planted at a density of three (lettuce) or five

(spinach) seedlings per pot. Seedlings were established in the soil by overhead irrigation with tap water for 15 min every 3–4 days.

2.2 Booster reactor irrigation treatments

2.2.1 Plant irrigation

Farm dam water was obtained in 1000 L food-grade intermediate bulk containers (IBCs) from the property in Virginia, SA, and transported to the greenhouse at Mawson Lakes. During the experiment, the lids were loosened, and water was recirculated in the IBCs using aquarium pumps and risers, to ensure that it did not stagnate or become anaerobic during the period of the experiment.

For each irrigation event during the plant growth period, three treatments were prepared: (1) no treatment (control); (2) low Booster treatment (single pass through a model "2k" Booster reactor, with 2000 cm^2 of anode surface) at 35 A to give ~5 mg/L free chlorine concentration); and (3) high Booster treatment (recirculation of water through a model 2k Booster reactor to give ~20 mg/L free chlorine concentration). Free chlorine concentration, ORP and pH of the water were tested for every irrigation event. Colilert testing and total aerobic heterotroph counts (3M Petrifilm Aerobic Plate Count; Thermo Fisher Scientific, Scoresby, VIC, Australia) were used to monitor microbial load in each of the irrigation treatments.

Plants were irrigated using an overhead sprinkler system for 15 min every 3–4 days during the plant growth period. Additional water from each treatment was supplied directly to the soil to ensure that sufficient water was available to plants to support growth.

2.2.2 Additional plant treatment/fertilisation

Because of the presence of aphids and caterpillars on the plants, pyrethrum spray was used for insect control throughout the growth period. Pyrethrum insect spray concentrate containing pyrethrins (4 g/L) and piperonyl butoxide (16 g/L; Amgrow Pty Ltd, Stapylton, Qld, Australia) was diluted and used according to the manufacturer's instructions.

Fertilizer was applied twice during the growth period. Seasol fertiliser (nitrogen 7%, phosphorus 0.8% and potassium 5.4% (w/v)) was diluted and applied according to the manufacturer's instructions.

2.3 Plant sampling

Plant leaves were sampled fortnightly over 8 weeks to assess microbial load on the leaves. Plant leaves were sampled from randomly selected pots for each treatment, to provide ~15 g (lettuce) or ~10 g (spinach) fresh leaf weight. Six samples of each leaf type were taken for each sample time point. Leaves were cut at the base of the stem (using scissors wiped with 70% (v/v) ethanol between each sampling) and placed into sterile stomacher bags (Thermo Fisher Scientific); the wet weight of plant material was recorded. Sterile peptone water (0.1%; Thermo Fisher Scientific) was added (100 ml) and the leaves were processed in a Seward BA6021 stomacher (Seward Limited, Worthing, UK) for 1 min. Bacterial suspensions were enumerated on *E. coli*/coliform and total aerobic count petrifilms (3M *E. coli*/Coliform and Aerobic Plate Count Petrifilms, respectively; Thermo Fisher Scientific). Bacterial counts were reported as cfu/g wet weight plant material.

3. Field trial

3.1 Field trial setup

A field trial of the Booster reactor technology was undertaken at a Virginia farm site used for lettuce production. Booster-treated water was used to irrigate lettuce plants through a complete growth cycle and compared to adjacent plants irrigated solely with untreated water. The setup of the field trial was as follows:

Booster treatment planting date: 21 April 2020

Control area planting date: 31 March 2020

Booster planted area: 48 m × 11 m, 25 m from the Control planted area

2 lines of 7 sprinklers each

27 rows of lettuce plants (9 sets of 3 rows each)

20 cm plant spacing

Irrigation water properties were measured at the site: pH 7.8, conductivity 1400 µS/cm, temperature 20.7°C, total dissolved solids 996 mg/L. Two model "10k" Booster reactors (10,000 cm^2 of anode surface each) were installed in parallel and fed with a current of 150 A, in order to produce a free chlorine concentration of ~5 mg/L at a sampling point just after the Booster system. Free chlorine was measured at the first and last sprinklers along the irrigation line, giving values of 4.8–4.9 mg/L on 22/4/20.

Note: initiation of the field trial occurred at the time of COVID-19 restrictions, which caused considerable problems with setting up and monitoring of the field site. As a result, we were not able to travel to the field site during the early period of the growth period, thus limiting our ability to sample and control all aspects of the field experiment. Sampling and monitoring of the trial were performed by personnel from Ecas4 Australia and UniSA staff once travel restrictions were lifted.

3.2 Water sampling

Irrigation water was sampled on several occasions during the growing period to determine the free chlorine content being generated by the Booster system and the efficacy in reducing microbial counts in the treated water. Samples were taken from a sampling port before the Booster system, a sampling port post-Booster system, and from the first and last sprinklers along the irrigation line. Free chlorine was determined on site as above from all samples. Water samples for microbial counts were neutralised with sodium thiosulfate (0.05%) and transported back to the laboratory for analysis by Colilert and total heterotroph counts, as described above.

3.3 Leaf sampling

Leaf samples were taken from plants irrigated with Booster-treated and Untreated water at several time points throughout the growth period. Leaf samples were processed as described above (resuspension of microbes in peptone water and stomacher homogenisation). Bacterial suspensions were enumerated on *E. coli*/coliform and total aerobic count petrifilms (3M *E. coli*/Coliform and Aerobic Plate Count Petrifilms, respectively; Thermo Fisher Scientific).

4. Results

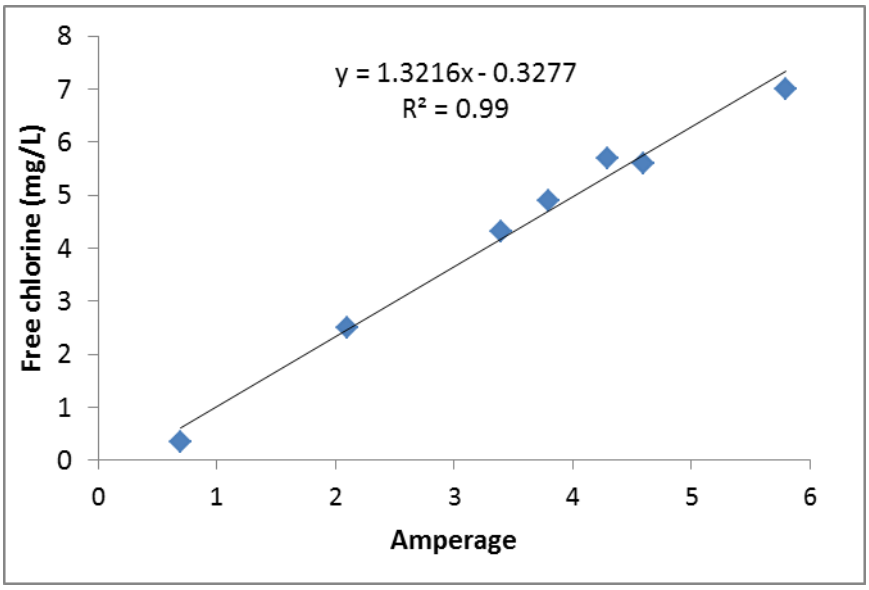
4.1 Booster reactor testing results

4.1.1 Booster reactor testing

4.1.1.1 Booster reactor testing in tap water or distilled water containing sodium chloride (NaCl)

Preliminary Booster reactor testing with tap water (no additional salts) showed a linear increase in free chlorine with increased amperage and/or voltage of the Booster reactor (Fig. 1A and B).

A



B

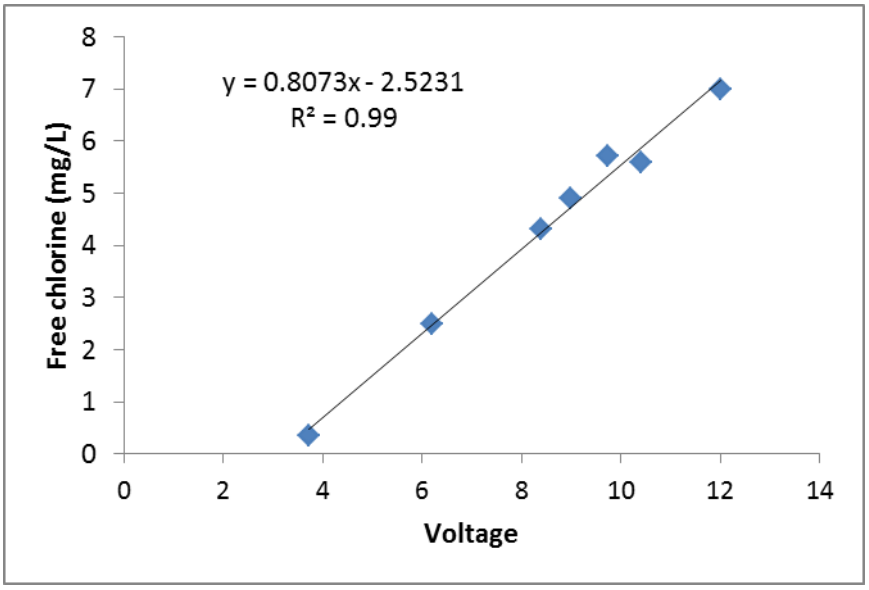


Fig. 1 Booster reactor testing in tap water with varying current; effects of varying amperage (A) and voltage (B) on free chlorine generated.

Booster reactor testing in distilled water containing NaCl showed a linear increase in free chlorine concentration with time of recirculation through the Booster and an increase in ORP from initial levels concurrently with the increase in free chlorine at 2 min onwards (Fig. 2). Viable counts showed that from an inoculant level of ~1×10^6 cfu, **no viable bacteria** were obtained after 2 min of recirculation (free chlorine concentration >5 mg/L).

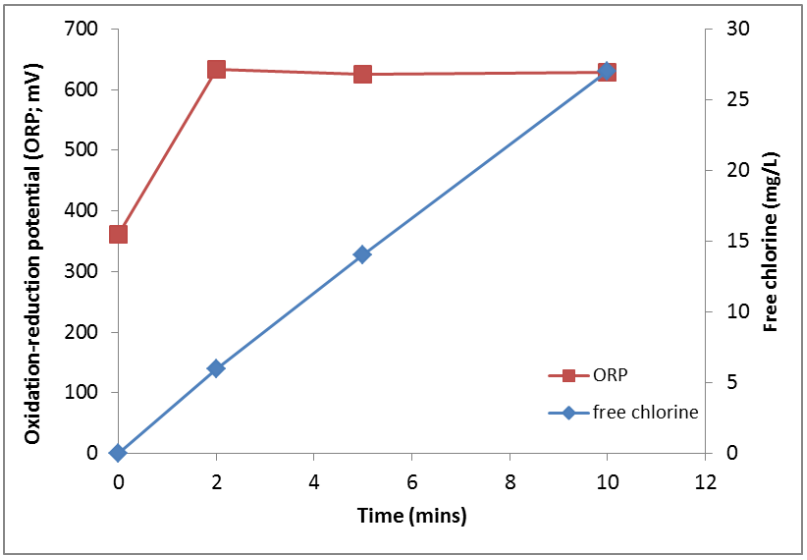


Fig. 2 Booster reactor testing in distilled water with sodium chloride over time of recirculation; effects on free chlorine and oxidation-reduction potential.

4.1.1.2 Booster reactor testing in farm dam water

Water from two farm dams was collected and run through a Booster reactor at two amperage settings (1.0 or 4.0 A) for up to 10 min (Fig. 3).

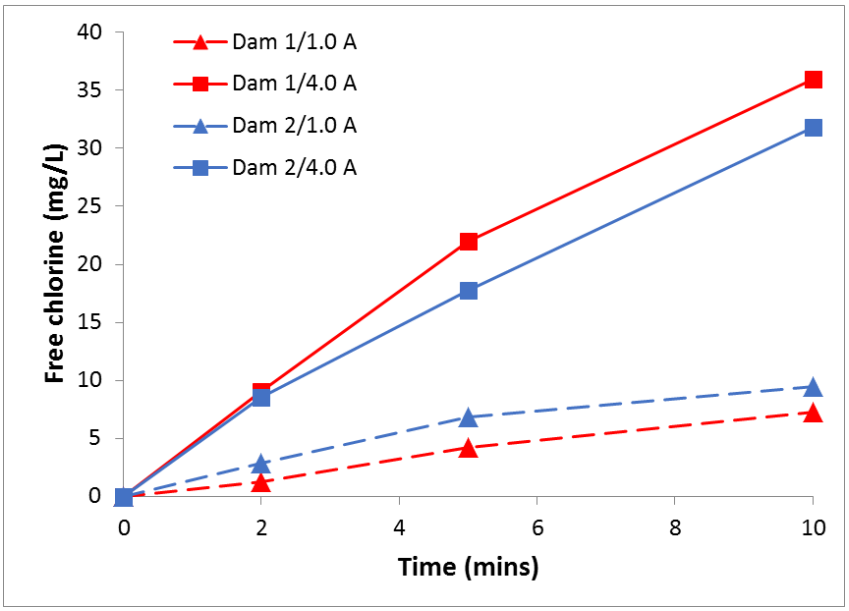
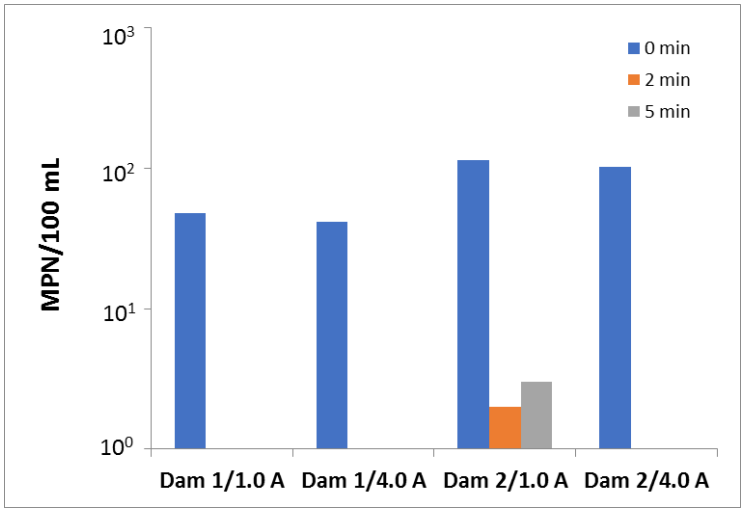


Fig. 3 Booster reactor testing on farm dam water at two different amperage settings and effect on free chlorine generated over time.

B



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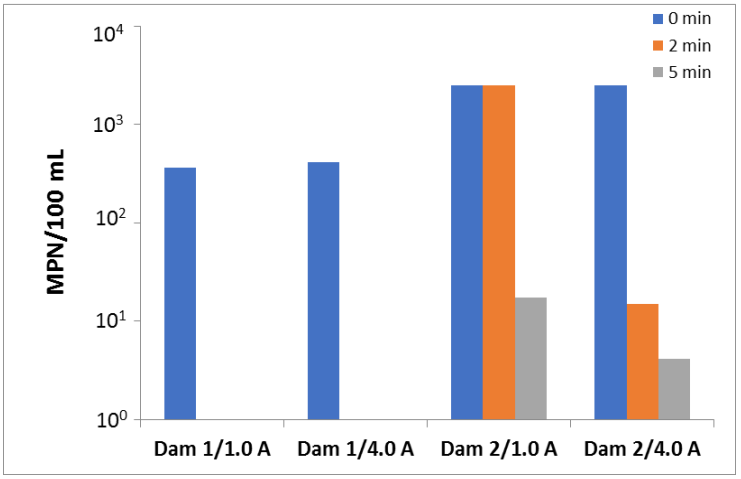


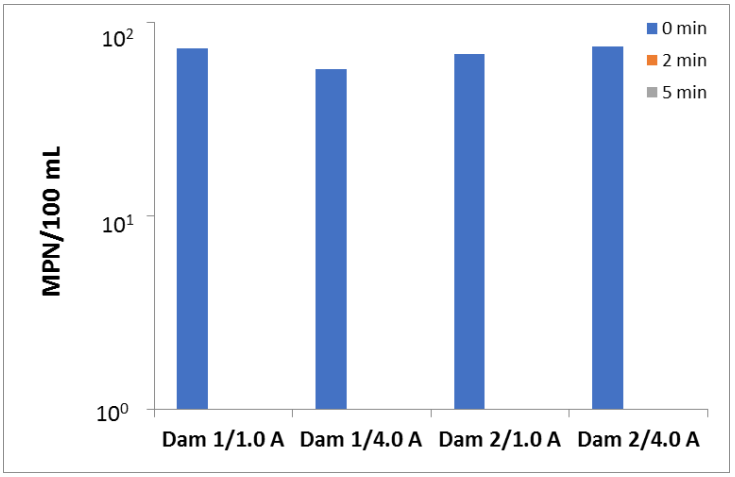


Fig. 4 Booster reactor testing on farm dam water. Most probable number (MPN) microbial counts per 100 mL of water were monitored for (A) enterococci; (B) coliforms; and (C) *E. coli*.

Microbial counts were monitored in the farm dam water during Booster reactor treatment up to 5 min (Fig. 4). Enterococci counts were reduced to below the detection limit (<1 most-probable number (MPN)/100 mL) for all except Dam 2 at 1.0 A (Fig. 4A). Coliforms were reduced to below the detection limit in Dam 1 at the 2 current settings tested (Fig. 4B) but for time periods of up to 5 min, coliform counts were reduced but not completely removed for Dam 2. *E. coli* counts were reduced to below the detection limit for all treatments (Fig. 4C).

The effect of Booster reactor treatment on water pH was assessed over time for all treatments (Fig. 5). The pH increased gradually over time in Dam 1 water with either 1.0 or 4.0 A current, but there was minimal pH increase in Dam 2 water, perhaps because of its higher starting pH when compared with that of Dam 1. The electrochemical treatment can result in an increase in water pH because of the generation of OH^- ions at the cathode.

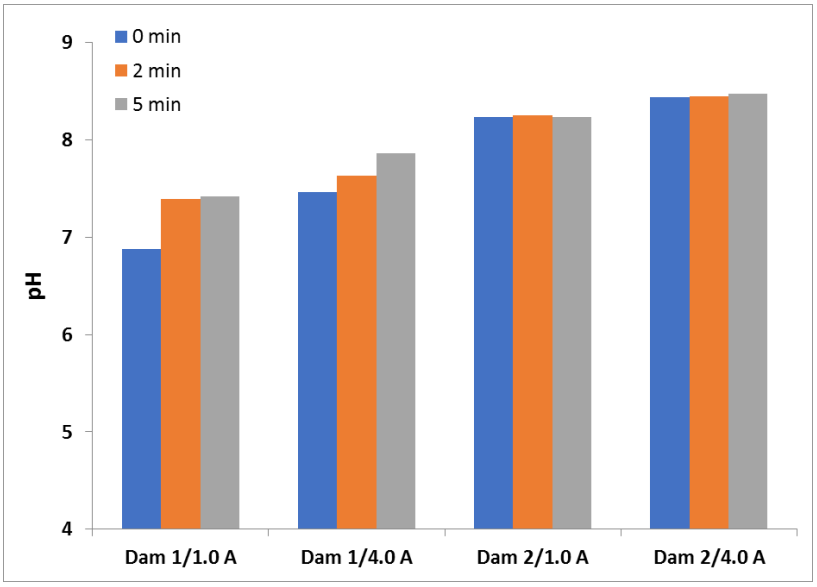


Fig. 5 Effect of Booster reactor treatment on pH of farm dam water over time.

4.2 Greenhouse trial results

4.2.1 Water properties

Water properties (free chlorine, ORP and pH) were monitored for each irrigation event, with the average values of these parameters presented in Table 1. The target values of 5 and 20 mg/L free chlorine were achieved by the single pass and recirculation Booster reactor treatments applied to the farm dam water. The ORP reflected the increased free chlorine concentration, with both low and high Booster reactor treatments showing a substantial increase in ORP when compared with the untreated water. There was a moderate increase in pH with Booster ractor treatment, especially with the recirculation treatment required to achieve ~20 mg/L free chlorine. The dissolved organic carbon content of the water was ~9 mg/L.

Table 1. Properties of untreated and Booster-treated water

Treatment	Free chlorine (mg/L)	ORP (mV)	pH
Untreated	0.01 ± 0.03	337 ± 90	8.48 ± 0.41
Low	5.71 ± 0.97	583 ± 69	8.62 ± 0.34
High	22.06 ± 2.97	643 ± 47	8.69± 0.32

4.2.2 Water microbial loads

Coliforms and total heterotrophs in untreated and Booster-treated water were monitored weekly during the plant growth period (Table 2). No *E. coli* were detected in the untreated water at any time point. Mean reductions of 2.7–3.0 $\log_{10}$ MPN/100 mL were obtained for coliforms in Booster-treated water (either Low or High). Mean reductions of 2.2–2.8 $\log_{10}$ CFU/mL) were obtained for total heterotrophs in Booster-treated water (either Low or High).

Table 2. Average microbial counts in the untreated and Booster-treated water. Coliforms reported as $\log_{10}$ most-probable-number (MPN) per 100 mL; total heterotrophs reported as $\log_{10}$ colony-forming units (CFU) per mL.

Treatment	Coliforms ($\log_{10}$ MPN/100 mL)	Total heterotrophs ($\log_{10}$ CFU/mL)
Untreated	3.17 ± 0.48	3.4 ± 0.4
Low (5 mg/L free chlorine)	0.42 ± 0.58	0.9 ± 0.2
High (20 mg/L free chlorine)	0.09 ± 0.18	0.3 ± 0.4

4.2.3 Plant leaf microbial loads

Plant leaf samples were processed to resuspend leaf-associated microorganisms and microbial counts were enumerated on selective or non-selective media (Tables 3 and 4).

Lettuce leaf microbial counts were substantially lower with the Booster-treated irrigation water, particularly for the High Booster reactor treatment. For coliforms, the Low treatment resulted in 0.4–2.6 (median 0.7) $\log_{10}$ reductions when compared with Untreated irrigation: High treatment resulted in 1.3–2.8 (median 2.6) $\log_{10}$ reductions when compared with Untreated irrigation water. For total heterotrophs, Low treatment resulted in 0–1.9 (median 1.3) $\log_{10}$ reductions when compared with Untreated, whereas High resulted in 0.4–2.8 (median 2.2) $\log_{10}$ reductions. The first sampling at week 2 showed limited effect of the Booster-treated irrigation water on plant leaf microbial counts, whereas weeks 4, 6, and 8 generally showed higher reductions for both coliforms and total heterotrophs. The reductions were reasonably consistent for the lettuce plants, except for the results from the first sampling at week 2, where there was limited effect of the Booster reactor irrigation treatments on both coliforms and total heterotrophs.

For spinach, the reductions were not as consistent as those found for lettuce. For coliforms, the Low treatment resulted in 0–2.5 (median 0.8) $\log_{10}$ reductions when compared with Untreated, whereas High resulted in 0.8–2.5 (median 1.2) $\log_{10}$ reductions. For total heterotrophs, the Low treatment mainly resulted in 0 $\log_{10}$ reductions, with 1.5 $\log_{10}$ reduction only observed for the sampling at week 8. For the High treatment, there were reductions of 0–1.7 (median 0.9) $\log_{10}$ when

compared with Untreated.

It is possible that aphid infestation contributed to the higher and more variable microbial counts on spinach plants, as once established early in the growth period, it was difficult to control the aphid infestation on these plants.

Table 3. Mean lettuce leaf microbial load during Booster reactor irrigation treatments. Coliforms and total heterotrophs are reported as $\log_{10}$ colony-forming units (CFU) per g wet weight of leaf material.

	Coliforms ($\log_{10}$ CFU/g WW[a] leaf material)			Total heterotrophs ($\log_{10}$ CFU/g WW leaf material)		
Week	**Untreated**	**Low**	**High**	**Untreated**	**Low**	**High**
2	2.73	1.83	1.37	4.17	4.24	3.77
4	3.13	2.69	0.76	5.08	3.50	2.54
6	3.79	3.24	0.94	4.96	4.03	3.06
8	3.75	1.17	0.88	5.11	3.24	2.28

[a]WW = wet weight. Low: 5 mg/L free chlorine; High: 20 mg/L free chlorine

Table 4. Mean spinach leaf microbial load during Booster reactor irrigation treatments. Coliforms and total heterotrophs are reported as colony-forming units (CFU) per g wet weight of leaf material.

	Coliforms ($\log_{10}$ CFU/g WW[a] leaf material)			Total heterotrophs ($\log_{10}$ CFU/g WW leaf material)		
Week	**Untreated**	**Low**	**High**	**Untreated**	**Low**	**High**
2	3.37	2.63	2.08	4.39	4.58	4.57
4	4.47	3.57	1.89	5.33	5.39	3.61
6	4.50	4.98	3.71	5.43	5.50	4.97
8	6.16	3.64	4.99	6.47	5.00	5.22

[a]WW = wet weight. Low: 5 mg/L free chlorine; High: 20 mg/L free chlorine

4.2.4 Soil properties

Soil properties (EC and pH) were assessed for the surface soil (0–2 cm) at the end of the plant growth period (Table 5).

Table 5. pH and electrical conductivity (EC) of Booster-treated irrigated soil planted with lettuce or spinach at the end of the growth period (n = 6, values are mean ± SD).

Treatment	**pH**		**EC (µS cm^{-1})**	
	Lettuce	**Spinach**	**Lettuce**	**Spinach**
Untreated	6.18 ± 0.23	6.01 ± 0.21	991 ± 211	1068 ± 123
Low	6.28 ± 0.38	6.03 ± 0.15	959 ± 249	1014 ± 275

High	6.42 ± 0.17	6.15 ±0.26	623 ± 241	683 ± 94

Low: 5 mg/L free chlorine; High: 20 mg/L free chlorine

The High treatment induced a reduction in the EC of soils in comparison with the Untreated and Low treatments for both plants. It is possible that this was related to the high free chlorine concentration in the High Booster-treated water, resulting in leaching of salts from the soil profile.

4.2.6 Plant leaf quality

4.2.6.1 Lettuce

Lettuce leaves did not show substantial effects of irrigation with any treatment, with all leaves showing good growth, with limited edge browning or leaf spots. Post-harvest analysis was not possible because of technical issues with the greenhouse experiment.

4.2.6.2 Spinach

Spinach leaves showed significant effects of irrigation with the different treatments. Spinach leaves showed significant effects of irrigation with the different treatments. Spinach leaves in the Untreated and Low Booster-treated irrigation water treatment groups showed white spots on many leaves, whereas leaves in the High Booster-treated irrigation water treatment group showed limited white spots and leaves generally looked green and healthy. This indicates the spots were not a phytotoxic reaction, but possibly caused by either a fungal disease or other pest.

Some spinach leaves were affected by aphids, showing curling and aphid eggs and adults on leaves (not shown). Leaves were sprayed with pyrethrum throughout the growth period to control aphid growth in the spinach and lettuce plants, however once established in the greenhouse the aphids were not able to be completely eliminated throughout the experimental period.

4.2.5 Images of Booster-irrigated plants in the greenhouse

Lettuce: Untreated irrigation water 	
Lettuce: Low Booster-treated water 	
Lettuce: High Booster-treated water 	

Spinach: Untreated water 	
Spinach: Low Booster-treated water 	
Spinach: High Booster-treated water 	

4.3 Field trial results

4.3.1 Water properties

The target free chlorine concentration was 5 mg/L in the Booster-treated water.

The free chlorine concentrations achieved ranged from 0.5–5.0 mg/L, with a mean concentration of 2.9 mg/L for water samples taken from a sampling port just past the Booster system, and from the first and last sprinklers on the irrigation line.

The pH of water samples did not change substantially with the Booster system treatment.

4.3.2 Water microbial counts

Coliforms and total heterotrophs were enumerated in control and Booster-treated water samples (Table 6).

Coliforms showed reductions of 2–3 $\log_{10}$ for all Booster-treated samples when compared with the untreated water. Total heterotrophs were reduced by 1–2 $\log_{10}$, with larger reductions observed for samples with longer residence time (i.e., further along the irrigation line), suggesting a beneficial effect of increased exposure time on the efficacy of the Booster-mediated disinfection process.

Table 6. Coliforms ($\log_{10}$ most probable number (MPN)/100 mL) and total heterotrophs ($\log_{10}$ colony forming units (CFU)/mL) in Booster-treated water

Weeks after planting	Coliforms ($\log_{10}$ MPN/100 mL)				Total heterotrophs ($\log_{10}$ CFU/mL)			
	1[a]	2	3	4	1	2	3	4
0	3.4	0.8	0.0	0.0	3.0	2.2	1.1	0.7
3	3.2	0.4	0.2	0.0	3.1	1.8	0.9	1.0
5	2.2	0.0	0.0	0.0	2.6	0.3	0.0	0.0
7	2.9	0.0	0.0	0.0	2.2	0.0	0.0	0.0

[a]1 = Pre-Booster; 2 = Post-Booster; 3 = first sprinkler along the irrigation line; 4 = last sprinkler along the irrigation line.

4.3.3 Leaf microbial counts

Plant leaves were assayed for coliforms and total heterotrophs (Table 7). There were reductions of 0.8-1.7 (mean reduction of 1.2) $\log_{10}$ coliforms per g wet weight leaf material after irrigation with the Booster-treated water when compared with control untreated water. There were no differences in total heterotrophs between Booster-treated irrigated plants and control plants.

Table 7. Mean lettuce leaf microbial load during Booster-treated and control irrigation treatments. Coliforms and total heterotrophs are reported as $\log_{10}$ colony-forming units (CFU) per g wet weight of leaf material.

Treatment	Weeks after planting	Coliforms	Total heterotrophs
Control	5	4.46	5.45
	8	3.53	4.95
	10	4.09	5.66
	12	3.97	5.79
Mean		4.01	5.46
Booster	2	2.88	5.09
	5	1.86	4.68
	7	2.63	5.90
	9	3.12	5.93
	12	3.63	5.95
Mean		2.82	5.51

Images of lettuce plants in the field are available in a separate document (Appendix 9: VG15068 Field Trial Images).

5. Discussion and Conclusions

5.1 Booster reactor testing

The Booster reactor provided electrochemical disinfection of farm dam water (replenished with recycled wastewater via the Virginia Pipeline Scheme) by the production of free chlorine from the naturally occurring salts in the water. This resulted in reductions of up to 6 $\log_{10}$ inoculated bacteria and 2–3 $\log_{10}$ coliforms in the Booster-treated water.

A slight increase in pH was observed for Booster-treated water when it was recirculated. This recirculation effect resulted in increased free chlorine concentrations and increased pH because of the generation of OH^- ions at the cathode.

Increasing the time of exposure to the active agent (free chlorine) is advantageous, which could easily be achieved by pumping, treating and storing water prior to irrigation.

5.2 Greenhouse trial

The greenhouse trial was initiated to test the Booster reactor irrigation treatment on lettuce and spinach under controlled conditions. Booster reactor treatments were applied to generate 5 mg/L free chlorine (Low: single pass through the Booster) and 20 mg/L free chlorine (High: recirculation required to achieve this concentration).

Coliforms in the irrigation water were consistently reduced by 2.7–3.0 $\log_{10}$ MPN/100 mL and total microbial load (total heterotrophs) was consistently reduced by 2.2–2.8 $\log_{10}$ CFU/mL.

Coliforms on lettuce leaves were reduced for both Low (0.4–2.6, median 0.7 $\log_{10}$) and High (1.3–2.8, median 2.6 $\log_{10}$) Booster reactor treatments. The reductions in total heterotrophs were variable for both Low (0–1.9, median 1.3 $\log_{10}$) and High (0.4–2.8, median 2.2 $\log_{10}$) Booster reactor treatments, but still generally showed a reduction when compared with the untreated irrigation water.

There were limited reductions in microbial counts on spinach leaves. For coliforms, there were 0–2.5 (median 0.8) $\log_{10}$ reductions for Low and 0.8–2.5 (median 1.2) $\log_{10}$ reductions for High; for total heterotrophs, there were 0–1.5 (median 0) $\log_{10}$ reductions for Low and 0–1.7 (median 0.9) $\log_{10}$ reductions for High when compared with the untreated control. Spinach leaf microbial counts might have been negatively affected by the aphid infestation that occurred during the growth period.

Irrigation of lettuce and spinach with Booster-treated water containing 5 and 20 mg/L free chlorine had no negative effects on the plant leaves. For spinach, in the early part of the experiment, the High Booster-treated leaves had better quality than those of Low and untreated irrigation treatments. There were no obvious differences in lettuce leaf quality among treatments.

Surface soil showed a reduction in EC at the end of the experiment with the High Booster-treated irrigation treatment for both spinach and lettuce, which might be related to the high free chlorine content in this treatment.

5.3 Field trial

Field testing of the Booster system resulted in generation of a mean value of 2.9 mg/L free chlorine in the irrigation water. Coliforms were reduced by 2–3 $\log_{10}$ and total heterotrophs were reduced by 1–2 $\log_{10}$ in the Booster-treated water when compared with the untreated water. Lettuce plants irrigated with this water showed no negative effects of the Booster reactor irrigation treatment, and reductions in coliforms on the leaf surface of 0.8–1.7 $\log_{10}$.

The field trial was conducted during the autumn/winter, which meant that there was substantial natural rainfall and low levels of irrigation required. We expect that the differences in leaf microbial load would be greater between untreated and Booster irrigated plants in spring/summer because of the higher rate of irrigation required.

5.4 Conclusions

A Booster reactor was tested as a more practical alternative to the production of EO water by the Water Disinfection System for irrigation water disinfection on-farm. Booster reactor treatment requires no addition of exogenous salts to the water sample to generate free chlorine. A Booster reactor/system can be installed within or close to the irrigation pumping station provided there is a power source available to supply the electrical current required.

Booster reactor treatment consistently reduced microbial counts, with the ability to tailor the current applied to deliver effective microbial reductions depending on the water properties and microbial load. There were limited changes in physicochemical properties of the Booster-treated water from the electrochemical treatment.

In both the field and greenhouse, irrigation water samples treated with a Booster reactor showed good reductions in total microbial load and target microorganisms (coliforms). Lettuce leaf samples showed consistent reductions in coliforms with Booster-treated irrigation, whereas results for spinach were more variable.

As part of a multi-barrier or hurdle approach to food safety, EO water or Booster reactor technology has the potential to reduce microbial contamination of minimally-processed foods throughout the growth period. In combination with post-harvest treatment, this pre-harvest treatment approach could result in enhanced food safety for leafy greens and other minimally-processed vegetables.

5.5 Future research

Several key limitations of this study could be addressed in the future:

- Booster reactor treatment of a wider range of irrigation waters
- Field assessment of Booster-treated irrigation water over several growth periods and seasons
- Field assessment for a range of different crops, especially including other leafy greens
- Assessment of the potential for Booster-treated water to reduce the application of pesticides/fungicides during the plant growth season through the reduction of the incidence and/or severity of plant pathogens
- Assessment of the potential for Booster-treated water to reduce biofilm build-up on irrigation pipes and equipment
- Cost analysis of Booster reactor implementation in the field
- Analysis of the potential for disinfection by-products to be produced by the Booster reactor electrochemical process and for plant uptake in the field

Case study: Improving safety of vegetable produce through on-farm sanitation, using Electrolysed Oxidising (EO) Water (available at https://fii.unisa.edu.au/our-research/current- projects-folder/improving-safety-of-vegetable-produce-through-on-farm-sanitation-using- electrolysed-oxidising-eo-water/

Submitted by **Dr David Ogunniyi, Professor Enzo Lombi and Dr Cathy Dandie**

Senior Research Fellow, Dean: Research and Innovation & Research Assistant

Foodborne diseases and illnesses due to microbial spoilage of fresh produce caused by foodborne pathogens are a widespread and growing public health and economic concern worldwide. Consequently, there are renewed calls globally for the development of safe, effective, inexpensive, easy to deploy and environmentally-friendly solutions to substantially reduce or eliminate illnesses and deaths linked to these pathogens.

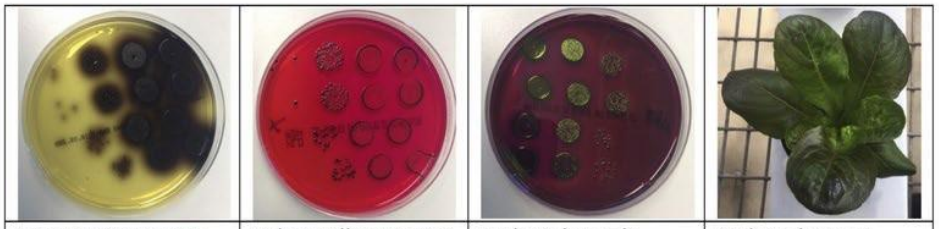
To address this need, our experts at FII and other UniSA collaborators are involved in a current Hort Innovation project examining the safety and efficacy of a pH-neutral electrolysed oxidising (EO) water produced by a South Australian company, Ecas4 Australia, to improve the overall quality and value of minimally-processed foods such as lettuce, spinach, broccoli, tomato, capsicum and melons.

The Molecular Biology Suite at FII, Mawson Lakes Campus | Red and green cos lettuce growing at Mawson Lakes Campus Greenhouse

We are comparing the efficacy of different doses and dosage regimes of EO water with those of industry standards such as sodium hypochlorite (through irrigation) in reducing/eliminating microbial contamination of these foods using quantitative culture and culture-independent molecular-based methods as well as shelf-life assessments.The molecular biology methods include quantitative culture, DNA and RNA analyses by qualitative and quantitative PCR, digital droplet PCR, metabolic activity measurements (using biosensors) and next-generation sequencing (16S rRNA gene metagenomics).

In addition, we are performing a detailed analysis of the mechanism of action and chemical composition (HOCl, OCl^-, Cl_2) of the various EO water delivery (liquid, fog, ice, gel, booster) systems, examining if any of these can eliminate biofilms and/or prevent the viable but nonculturable microbial state, and assessing the potential of the EO water to generate disinfection by-products.

The effects of EO water application on the physico-chemical parameters and microbial composition of different soil types are also being assessed. The results will provide recommendations for technology adoption by Industry, which should translate into health and economic benefit through reduction and/or eradication of food spoilage bacteria and job creation. It will also fortify engagement with industry and other stakeholders leading to translation of research outcomes into community benefit.



Listeria innocua 6a (ATCC 33090) on *Listeria* Selective Agar (Oxford)	*Salmonella enterica* serovar Enteritidis 11RX on Xylose Lysine Deoxycholate agar	*Escherichia coli* (ATCC 25922) on Eosin Methylene Blue agar	Red cos lettuce growing at Mawson Lakes Campus Greenhouse

Associated links

https://apps.who.int/iris/bitstream/handle/10665/199350/9789241565165_eng.pdf;jsessionid=F91E10EA8BA643F8316E903B6A34F6C6?sequence=1

http://www.foodstandards.gov.au/industry/foodrecalls/recalls/Pages/Woolworths-loose-leaf-lettuce.aspx

http://www.foodauthority.nsw.gov.au/_Documents/foodsafetyandyou/listeria_outbreak_investigation.pdf

https://www.foodstandards.gov.au/code/proposals/documents/P1015%20Horticulture%20PPPS%201FCS%20SD2%20Illness%20review.pdf

Zhu,Q., Cooneratne, R.and Hussain, **M.A.**(2017) *Listeria monocytogenes* in fresh produce: outbreaks,prevalence and contamination Levels. Foods 6(3), 21.

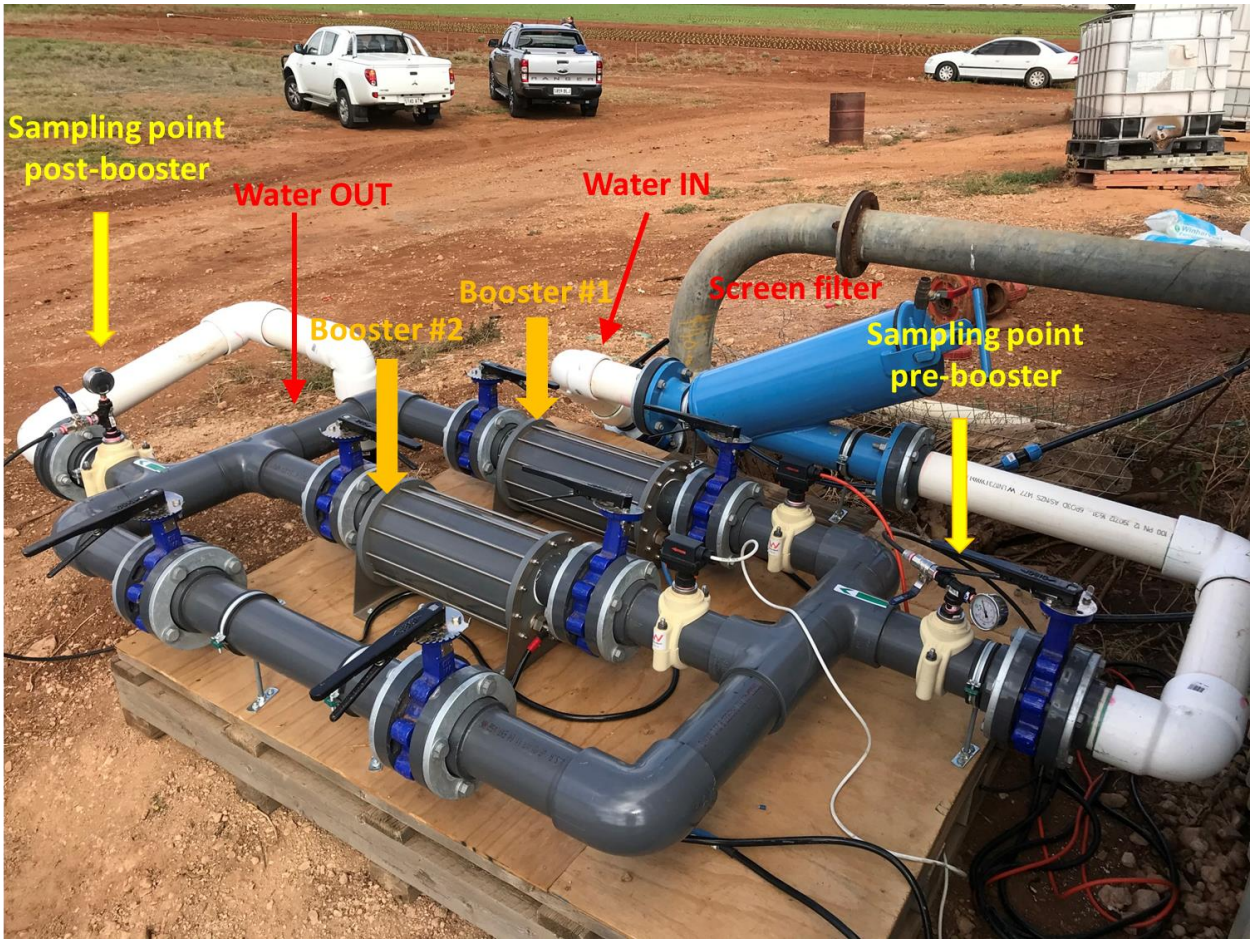
Field trial setup 21/04/2020

Control lettuce seedlings planted on 31/03/2020 and managed using standard practices, untreated irrigation water.
Booster-treated irrigation water used on Booster area, lettuce seedlings planted on 21/04/2020.

20 cm between plant spacing, 25 m between treatment area and control area

Booster-treated irrigation of lettuce plants at planting

The Booster installation on the irrigation system

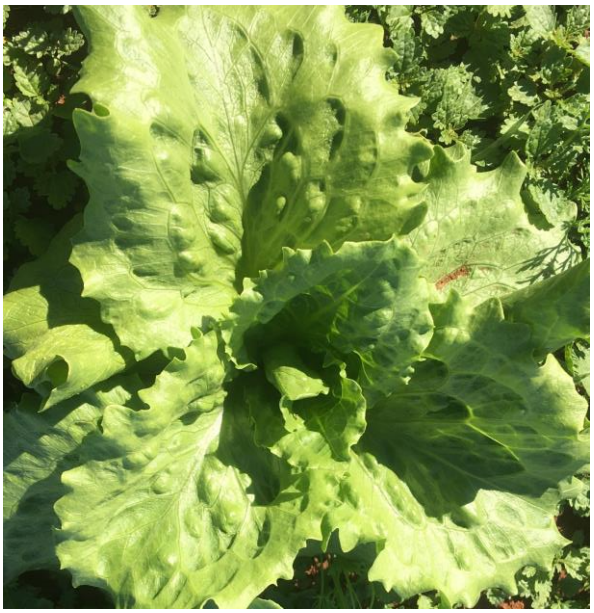


Control lettuce plants being irrigated 27/05/2020

Control lettuce plants

Booster treated lettuce plants

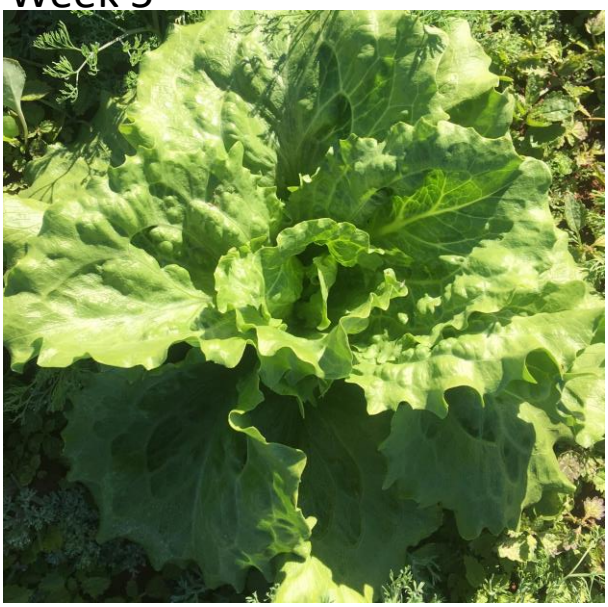
Week 2



Week 2 plants not available for control because of COVID-19 fieldwork restrictions

Week 5

Week 5



Week 8

Week 7

Control lettuce plants

Week 12

Booster treated lettuce plants

Week 12

OPTIMAL CONDITIONS FOR ELECTROLYSED OXIDISING WATER DISINFECTION OF IRRIGATION WATER

The objective of this Hort Innovation-funded project (VG15068) was to test whether Electrolysed Oxidising (EO) water could be used to increase the quality of vegetable irrigation water and facilitate safe use of microbiologically impaired waters.

Outbreaks caused by fresh produce contaminated by water and soil-borne pathogens are a serious human health issue and a threat to the Australian vegetable industry. There are numerous possible causes of microbial contamination, including the use of contaminated irrigation water. Various disinfection methods are available to treat irrigation water, including the use of EO technology.

This study compared the efficacy of EO water with that of other chlorine-based disinfectants for treating irrigation water contaminated by relevant water-borne pathogens.

EO water is a viable alternative to traditional chlorine-based disinfection processes.
The concentration of EO water can be adjusted to provide effective disinfection of irrigation water of varying quality.

CONCLUSIONS

- EO water treatment at **1 mg/L** free chlorine killed **>99%** of a mixed bacterial suspension of *E. coli*, *Listeria* and *Salmonella* in clean water in **<2 minutes**
- EO water produced using either **KCl or NaCl** had the same efficacy
- EO water effectively killed bacteria within the **pH range of 6.0–9.2**
- Organic matter content reduced the efficacy of EO water to kill bacteria but was overcome by increasing the free chlorine content of the EO water to **5–20 mg/L**
- EO water was **more effective** than sodium hypochlorite or chlorine dioxide in the presence of organic matter

Hort Innovation Strategic levy investment | VEGETABLE FUND

This project has been funded by Hort Innovation using the vegetable research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

OPTIMAL CONDITIONS FOR ELECTROLYSED OXIDISING WATER DISINFECTION OF IRRIGATION WATER

INTRODUCTION

Microbial contamination of fresh produce such as leafy greens by opportunistic and human pathogens continues to be a major source of foodborne illnesses and disease outbreaks worldwide. Pre-harvest water such as irrigation water has been identified as a potential source of contamination associated with human illness.

Current water disinfection processes involve either the use of chemicals (such as chlorine, ozone, peracetic acid, or hydrogen peroxide), or non-chemical disinfection methods such as ultraviolet irradiation and membrane filtration. However, these treatment technologies all have shortcomings in terms of efficacy and/or safety concerns. Consequently, there is a growing global focus on the deployment of safe, effective and environmentally-sustainable irrigation water sanitation technologies.

One approach being explored is the use of electrolysed oxidising water (EO water), which is generated through the electrolysis of chloride-containing water (generally in the form of sodium or potassium chloride (NaCl/KCl)) to form hypochlorous acid and reactive oxygen species that are toxic to microorganisms (Fig. 1).

The aim of this study was to identify the conditions under which EO water is effective in reducing microbial contamination of irrigation water, in comparison with other commonly available chlorine-based sanitisers.

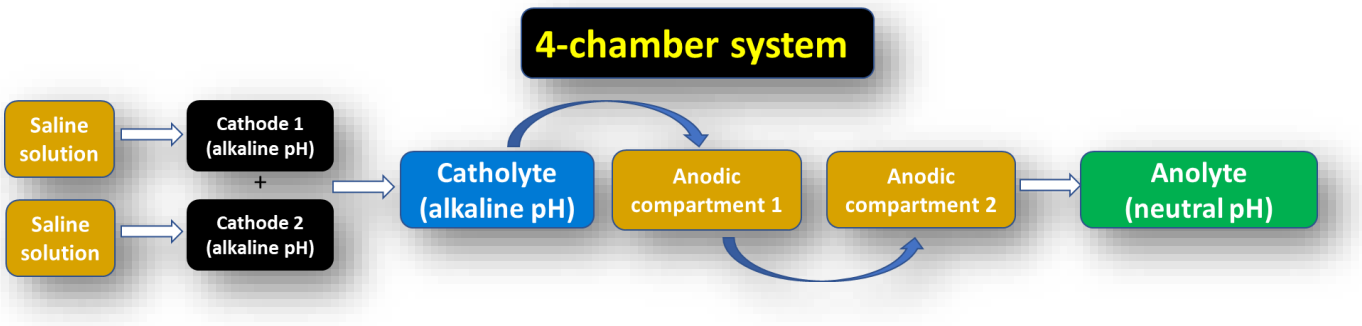


Fig. 1 Neutral EO water production process with a 4-chamber system as used in this study

OPTIMAL CONDITIONS FOR ELECTROLYSED OXIDISING WATER DISINFECTION OF IRRIGATION WATER

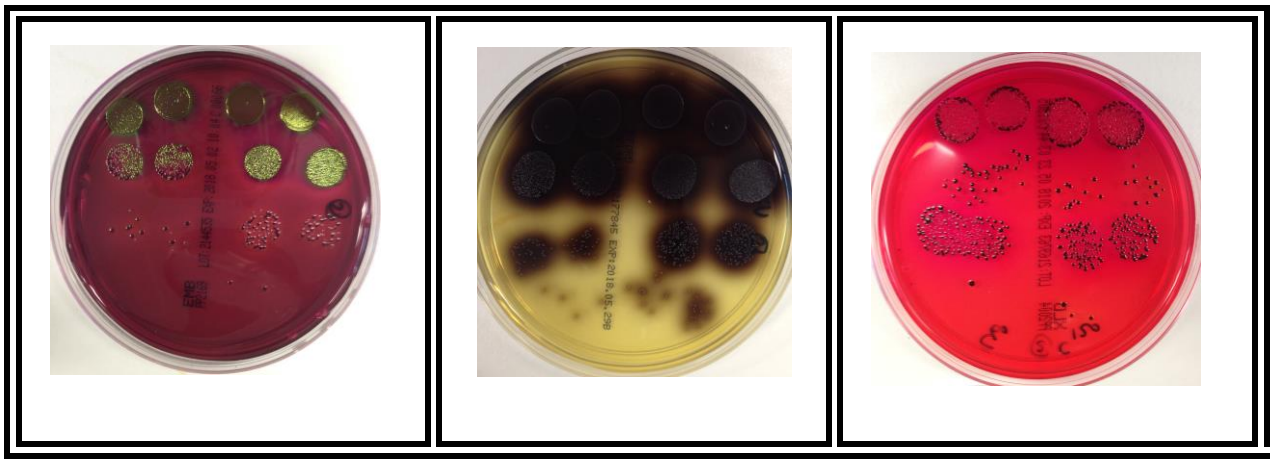
METHODS

The efficacy of EO water produced using potassium chloride (KCl) or sodium chloride (NaCl) was compared with two other disinfection treatments (sodium hypochlorite and chlorine dioxide).

Water samples were prepared with a range of pH, organic matter content, and pathogen loads. Three organisms, representative of important pathogenic bacteria, were inoculated into the test water: *Escherichia coli*, *Listeria innocua*, and *Salmonella enterica* serovar Enteritidis.

The kill rates were assessed using standard plating methods (Fig. 2) for viable microorganisms and molecular methods to detect viable but not culturable microorganisms for different disinfectant concentrations across the range of water conditions established. Residual total and free chlorine were measured for all treatments.

These results were used to define the range of water conditions within which EO water can effectively improve irrigation water quality.



Escherichia coli (ATCC 25922) growing on Eosin Methylene Blue agar

Listeria innocua 6a (ATCC 33090) growing on Listeria Selective agar (Oxford)

Salmonella enterica serovar Enteritidis 11RX growing on Xylose Lysine Deoxycholate agar

Fig. 2 Images of bacterial strains growing on selective agar

OPTIMAL CONDITIONS FOR ELECTROLYSED OXIDISING WATER DISINFECTION OF IRRIGATION WATER

RESULTS

- EO water treatment of a mixed bacterial suspension of *E. coli*, *Listeria* and *Salmonella* in clean water provided **99.99%–99.9999% (4–6 $\log_{10}$)** removal of the bacteria at **1 mg/L** and in **<2 minutes**
- EO water produced using either KCl or NaCl had the same efficacy
- EO water effectively killed bacteria within the **pH range of 6.0–9.2**
- Organic matter content reduced the efficacy of EO water to kill bacteria, but this could be overcome by increasing the free chlorine content of the EO water to **5–20 mg/L**
- **EO water was more effective than sodium hypochlorite or chlorine dioxide in the presence of organic matter**

CONCLUSIONS

EO water is a viable alternative to traditional chlorine-based disinfection processes.

The concentration of EO water can be adjusted to provide effective disinfection of irrigation water of varying quality. EO water treatment was more effective than traditional chlorine-based disinfection processes in the presence of contaminating organic matter.

For further information, please contact:

Professor Enzo Lombi (Enzo.Lombi@unisa.edu.au), Future Industries Institute, University of South Australia or see the associated publication (Ogunniyi et al. 2019, DOI: 10.1038/s41598-019-56248-7).

OPTIMAL CONDITIONS FOR ELECTROLYSED OXIDISING WATER DISINFECTION OF IRRIGATION WATER

VG 15068 IMPROVING SAFETY OF VEGETABLE PRODUCE THROUGH ON-FARM SANITATION, USING ELECTROLYSED OXIDISING (EO) WATER

Professor Enzo Lombi, Dr Catherine Dandie, Dr David Ogunniyi, Professor Erica Donner, Dr Barbara Drigo, Dr Baden Myers, Associate Professor Henrietta Venter and Dr Permal Deo, University of South Australia and Mrs Barbara Hall, SARDI.

August 2020

'Improving safety of vegetable produce through on-farm sanitation, using Electrolysed Oxidising (EO) water (VG15068)' was a three-year project (2017-2020) providing Australian vegetable growers with information on the effective application of EO water and electrochemical disinfection technology for the sanitisation of irrigation water.

VG15068 delivered new information on the conditions, efficacy and on-farm application of EO water and electrochemical disinfection technology for sanitisation of irrigation water and the effects on the microbial load in water and on the plant leaf surface.

This project has been funded by Hort Innovation using the vegetable research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

DEMONSTRATION TEST OF ELECTROCHEMICAL DISINFECTION OF IRRIGATION WATER UNDER GREENHOUSE AND ON-FARM CONDITIONS

Outbreaks caused by fresh produce contaminated by water and soil-borne pathogens are a serious human health issue and a threat to the Australian vegetable industry. There are numerous possible causes of microbial contamination, including the use of contaminated irrigation water. Various disinfection methods are available to treat irrigation water, including the use of electrochemical treatment. One promising application of this technology has been developed and named the 'Booster' reactor.

Electrochemical disinfection uses the naturally-occurring salts in water—when an electrical current is passed through the water, **reactive oxygen species and free chlorine** are produced. The salt content of the water, flow rate, current density and microbiological content determine the efficacy of the process. The Booster reactor can be installed in-line on the irrigation system, with a standard power supply needed to generate the current required.

The aim of this project was to assess the efficacy of Booster reactor electrochemical treatment under greenhouse and field conditions to reduce the microbial load of contaminated irrigation water and associated plant leaf microbial load.

Lettuce plants irrigated with Booster-treated water in both greenhouse and autumn field planting conditions showed no negative effects on plant growth and significantly reduced bacterial load on leaves.

CONCLUSIONS:

- Treatment with the Booster reactor successfully reduced the microbial load of contaminated water **by up to 99%**
- Increased contact time through recirculation or water storage resulted in increased disinfection efficacy
- Performance of the Booster reactor was easily adjustable depending on the water properties and power settings
- Lettuce plants irrigated with Booster-treated water under greenhouse conditions showed **no negative effects** of Booster-treated irrigation and had **reduced coliform load** with either low (5 mg/L) or high (20 mg/L) free chlorine concentrations
- Lettuce plants irrigated with Booster-treated water (~5 mg/L free chlorine) under autumn/winter field conditions showed **reduced coliform load and no negative effects**

Hort Innovation Strategic levy investment | VEGETABLE FUND

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DEMONSTRATION TEST OF ELECTROCHEMICAL DISINFECTION OF IRRIGATION WATER UNDER GREENHOUSE AND ON-FARM CONDITIONS

INTRODUCTION

Microbial contamination of fresh produce such as leafy greens by opportunistic and human pathogens continues to be a major source of foodborne illness and disease outbreaks worldwide. Pre-harvest water such as irrigation water has been identified as a potential source of contamination associated with human illness. Current water disinfection processes involve either the use of chemicals (such as chlorine, ozone, peracetic acid, or hydrogen peroxide), or non-chemical disinfection methods such as ultraviolet irradiation and membrane filtration. However, these treatment technologies all have shortcomings in terms of efficacy and/or safety concerns. Consequently, there is a growing global focus on the deployment of safe, effective and environmentally-sustainable irrigation water sanitisation technologies.

One approach being explored is electrochemical disinfection of irrigation water. This technology uses the naturally-occurring salts in the water—when an electrical current is passed through the water, reactive oxygen species and free chlorine are produced (Fig. 1). The salt content of the water, flow rate, current density and microbiological content determine the efficacy of the process.

The aim of this project was to assess the efficacy of an electrochemical Booster reactor under greenhouse and field conditions to reduce the microbial load of contaminated irrigation water and associated plant leaf microbial load.

DEMONSTRATION TEST OF ELECTROCHEMICAL DISINFECTION OF IRRIGATION WATER UNDER GREENHOUSE AND ON-FARM CONDITIONS

METHODS

This activity had two parts:

1. A **greenhouse trial** was conducted growing lettuce and spinach in soil taken from a Virginia field site used for lettuce production. Recycled wastewater on-site was supplied through the Virginia Pipeline Scheme. Preliminary characterisation of the Booster reactor was conducted to assess performance efficacy. Water for the greenhouse trial was obtained from the farm dam and transported to the greenhouse for use. Spinach and lettuce seedlings were planted in pots. Water was electrochemically treated to generate either 5 or 20 mg/L of free chlorine using a Booster reactor. Microbial counts of coliforms and total heterotrophs were assessed in the treated water. Plants were irrigated through an overhead irrigation system. Total bacterial counts and coliforms on the plant leaves were assessed fortnightly over 8 weeks.

2. A **field trial** was conducted at the same field site as described above. Water was treated with a Booster reactor system that comprised two in-line reactors to generate a target free chlorine concentration of 5 mg/L. Lettuce plants were grown according to standard practices, with the treated water applied through an overhead sprinkler irrigation system. The control plants were grown using untreated water. Microbial counts in the water and on plant leaves were assessed as described above for the greenhouse trial.

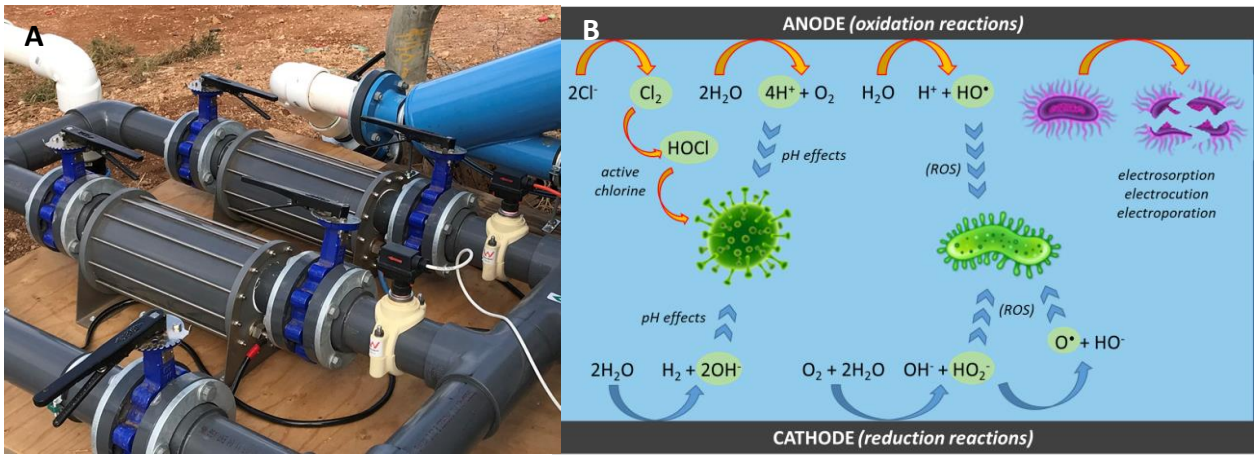

Fig. 1 A) Image of the installed Booster reactor system at Virginia field site and B) Mechanism of action of the electrochemical disinfection process

DEMONSTRATION TEST OF ELECTROCHEMICAL DISINFECTION OF IRRIGATION WATER UNDER GREENHOUSE AND ON-FARM CONDITIONS

RESULTS

Greenhouse trial

- **Preliminary Booster testing**: The Booster reactor provided electrochemical disinfection of farm dam water by the production of free chlorine from the naturally occurring salts in the water. This resulted in reductions of up to **99.9999% (6 $\log_{10}$) inoculated bacteria and 2–3 $\log_{10}$ coliforms in the Booster-treated water.**
- A slight increase in pH and an increase in free chlorine was observed for Booster-treated water when it was recirculated.
- The greenhouse trial was initiated to test the Booster irrigation treatment on lettuce under controlled conditions. Booster treatments were applied to generate 5 mg/L free chlorine (Low: single pass through the Booster) and 20 mg/L free chlorine (High: recirculation required to achieve this concentration).
- **Coliforms** in the irrigation water were consistently reduced by **99.8%–99.9%** (**2.7–3.0 $\log_{10}$)** most probable numbers/100 mL and **total heterotrophs** were consistently reduced by **>99.3%** (**2.2–2.8 $\log_{10}$)** colony-forming units/mL.
- Coliforms on lettuce leaves were reduced for both Low **(median 0.7 $\log_{10}$ reduction)** and High **(median 2.6 $\log_{10}$ reduction)** Booster reactor treatments. The reductions in total heterotrophs were variable for both Low (0–1.9 $\log_{10}$) and High (0.4–2.8 $\log_{10}$) Booster reactor treatments, but still generally showed a reduction when compared with the untreated irrigation water.
- Irrigation of lettuce with Booster-treated water containing 5 and 20 mg/L free chlorine had no negative effects on the plant leaves. There were no obvious differences in lettuce leaf quality among treatments (Fig. 2).
- In contrast, the effects of Booster-treated water and sodium hypochlorite-treated water (at the same free chlorine concentration) were compared in a preliminary trial: sodium hypochlorite treated water caused substantial leaf damage whereas Booster-treated water did not. This was attributed to the high pH of the sodium hypochlorite-treated water.

DEMONSTRATION TEST OF ELECTROCHEMICAL DISINFECTION OF IRRIGATION WATER UNDER GREENHOUSE AND ON-FARM CONDITIONS

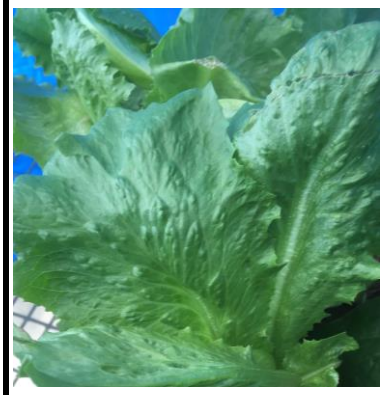		
Lettuce irrigated with untreated irrigation water	Lettuce irrigated with Booster-treated irrigation water with 5 mg/L of free chlorine	Lettuce irrigated with Booster-treated irrigation water with 20 mg/L of free chlorine

Fig. 2 Lettuce leaves irrigated with different Booster-treated irrigation water in the greenhouse

Field trial

- Field testing of the Booster reactor resulted in generation of a mean value of 2.9 mg/L of free chlorine in the irrigation water. **Coliforms** were reduced by **99%–99.9% (2–3 $\log_{10}$)** and **total heterotrophs** were reduced by **90%–99%** (**1–2 $\log_{10}$)** in the Booster-treated water when compared with the untreated water. Lettuce plants irrigated with Booster-treated water showed no negative effects this treatment (Fig. 3), and reductions in **coliforms** on the leaf surface of **0.8–1.7 $\log_{10}$**.
- The field trial was conducted during the autumn/winter, which meant that there was substantial natural rainfall and low levels of irrigation applied. It is expected that the differences in leaf microbial load would be greater between untreated and Booster-treated irrigated plants in spring/summer because of the higher irrigation rate required.

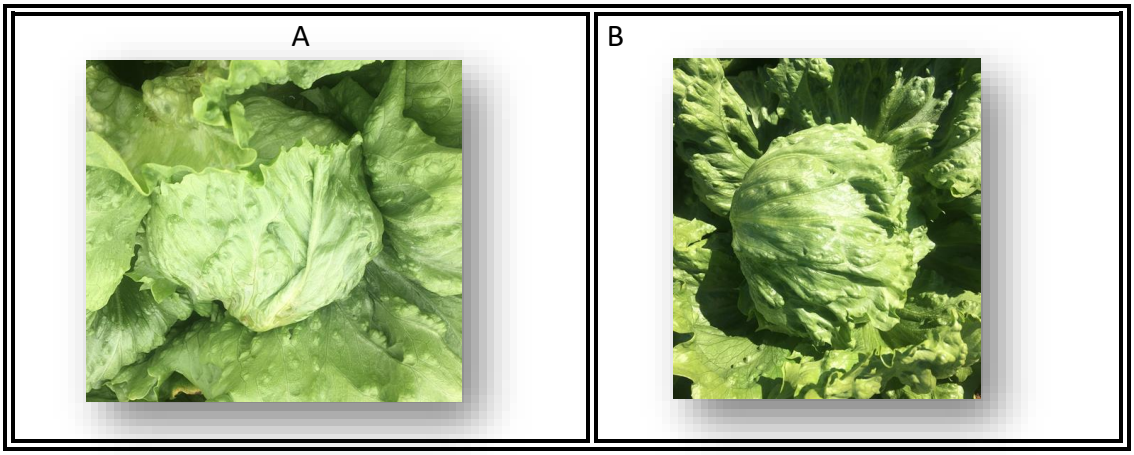

Fig. 3 Lettuce plants grown in the field with A) untreated and B) Booster-treated irrigation water.

CONCLUSIONS

The Booster reactor

The Booster reactor was tested as a practical solution to irrigation water disinfection on-farm. The Booster reactor requires no addition of exogenous salts to the water sample to generate free chlorine if enough salts are present in water and can be installed within or close to the irrigation pumping station with an available power source.

Treatment with the Booster reactor consistently reduced microbial counts, with the ability to tailor the current applied depending on the water properties and microbial load. There were minimal changes in physicochemical properties of the Booster-treated water from the electrochemical disinfection process. Increasing the time of exposure to the active agent (free chlorine) is advantageous, which could easily be implemented by storing booster-treated water prior to irrigation.

Greenhouse and field testing

In both the greenhouse and the field, irrigation water samples treated with the Booster reactor showed clear reductions in total microbial load and target microorganisms (coliforms). Lettuce leaf samples showed good reductions in coliforms with Booster-treated irrigation and no damage to the leaves.

Microbiological food safety

As part of a multi-barrier or hurdle approach to food safety, electrochemical treatment with the Booster reactor has the potential to reduce microbial contamination of minimally-processed foods throughout the growth period. In combination with post-harvest treatment, this pre-harvest treatment approach could result in enhanced food safety for leafy greens and other minimally-processed vegetables.

DEMONSTRATION TEST OF ELECTROCHEMICAL DISINFECTION OF IRRIGATION WATER UNDER GREENHOUSE AND ON-FARM CONDITIONS

Future field trials of this technology would be valuable to enable evaluation of the Booster reactor efficacy on a range of irrigation water of varying properties, during multiple growth periods and seasons, and testing on other leafy greens. Cost-benefit analysis and investigation of the potential for production of disinfection by-products is also warranted.

For further information, please contact:

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DEMONSTRATION TEST OF ELECTROCHEMICAL DISINFECTION OF IRRIGATION WATER UNDER GREENHOUSE AND ON-FARM CONDITIONS

VG 15068 Improving safety of vegetable produce through on-farm sanitation, using Electrolysed Oxidising (EO) water

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