



## RESEARCH

**Project:** Integrated post-harvest wash systems for fruit and vegetable decontamination for reducing water usage and decrease emission of disinfectant by-products in wastewater streams.

**Authors:** Mahdiyeh Hasani & Keith Warriner

**Date:** December 2020



DEPARTMENT OF  
FOOD SCIENCE



ONTARIO AGRICULTURAL COLLEGE  
Department of Food Science

**Date:** 5<sup>th</sup> December 2020

**To:** CPMA Members

**Cc.:** Ron Lemaire, President CPMA  
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Tracy Todd, VP, Finance & Operations  
Jeff Hall, Food Safety Specialist

**Project: Integrated post-harvest wash systems for fruit and vegetable decontamination for reducing water usage and decrease emission of disinfectant by-products in wastewater streams**

*Research overview and summary:* Post-harvest washing is commonly undertaken in fresh produce processing to remove soils and reduce the pathogenic (for example, *E. coli* O157:H7) loading along with spoilage microbes. However, research has shown that washing has limited efficacy in removing microbes from fresh produce and if the free-chlorine concentration is not maintained then contamination can be transferred between batches. Yet, free-chlorine is reactive with the organic and inorganic constituents (referred to as the chlorine demand) of wash water so is rapidly sequestered (neutralized).

There are several options available to overcome the problem of accumulating chlorine demand in fresh produce processing waters. For example, the processor can introduce volumes of fresh water (i.e. recharge) wash tanks thereby diluting the constituents contributing to chlorine demand. The main problem with this approach is the large of volumes of water required and to be disposed of. A further practice is to simply keep adding more chlorine to the wash tanks that ultimately satisfy the chlorine-demand allowing free-chlorine to exist. The problem with this approach is that high volumes of chlorine (hypochlorite bleach) results in “swimming pool” odor as the disinfection byproducts accumulate. A third option is to use alternative sanitizers to chlorine such as peroxyacetic acid or chlorine dioxide although these are expensive, in addition to being affected by organic and inorganic constituents.

The current research focused on an additional processing option based on reducing the chlorine demand of wash water by a continuous process referred to as Advanced Oxidation Process (AOP). The process is based on generating hydroxyl-radicals from the ultraviolet degradation of hydrogen peroxide. The radicals are highly oxidative thereby sequestering those constituents that add to the chlorine-demand of the water. The hydroxyl-radicals do not leave a residue and decompose to oxygen & water within seconds of reacting with water constituents. The actual process is routinely used in treating wastewaters from paper mills and mining industries but has yet to be applied for treating fresh produce water or as a continuous in-process treatment.

Baseline studies determined the characteristics (pH, chlorine demand, solids etc.) of spent wash water collected from shredded lettuce commercial processing lines. This facilitated the formulation of simulated spent wash water that was used in subsequent trials to develop and validate the AOP system.

Trials were performed on a lab-scale AOP reactor that was based in supplementing the water with low concentrations of hydrogen peroxide and a catalyst (copper or ferric chloride) prior to circulating around an ultraviolet lamp. Studies determined the effect of ultraviolet light, catalyst and hydrogen peroxide



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concentration with the hydroxyl-radicals being measure by oxidation of methylene blue dye or decrease in hydrogen peroxide. The maximal hydroxyl-radical production was found using 9 ppm hydrogen peroxide, 10 mg/l ferric chloride and ultraviolet dose of 667 mJ/cm<sup>2</sup>.

A pilot-scale AOP reactor was constructed that could operate at 60 liters/min. By applying this AOP treatment the chlorine demand of spent wash water was reduced from 130 ppm to 50 ppm due to sequestering of water constituents. When the spent water was acidified to pH 4 the chlorine demand of the spent water was 75 ppm and actually increased to 86 ppm when passed through the AOP reactor. This was likely due to the residual activity of ferric catalyst under the acidic condition.

Chlorine added to AOP treated spent wash water remained stable over 100 mins whereas the free-chlorine declined in non-AOP treated water declined as a consequence of the chlorine demand. AOP treated water did not improve the decontamination of inoculated lettuce but did prevent cross-contamination of pathogens between batches by preserving the free-chlorine levels. In contrast, spent wash water that had not been treated with AOP allowed pathogens to be transferred between lettuce batches as the free-chlorine became depleted. The research demonstrated proof-of-principal of a continuous AOP process to treat spent wash water that can be directly fed-back into the wash tank thereby saving water while maintaining free-chlorine levels.

#### **Publication Plans**

The research will be published in the Journal of Food Protection and presented at conference (IAFP, OMAFRA symposium) if the meetings are held. Patent protection will also be reviewed given the novelty of the approach.

#### **Related Publications**

Alharbi, K.K.D., Lau, V., Liang, C., Zytner, R.G., Shi, J., Warriner, K., 2017. Treatment of spent wash water derived from shredded lettuce processing using a combination of electrocoagulation and germicidal ultraviolet light. *Food Quality & Safety* 1, 147-152.

Barrera, M.J., Blenkinsop, R., Warriner, K., 2012. The effect of different processing parameters on the efficacy of commercial post-harvest washing of minimally processed spinach and shredded lettuce. *Food Control* 25. 745-751.

de Souza, V.R., Popovic, V., Warriner, K., Koutchma, T., 2020. A comparative study on the inactivation of *Penicillium expansum* spores on apple using light emitting diodes at 277 nm and a low-pressure mercury lamp at 253.7 nm. *Food Control* 110. 107039

Hadjok, C., Mittal, G.S., Warriner, K., 2008. Inactivation of human pathogens and spoilage bacteria on the surface and internalized within fresh produce by using a combination of ultraviolet light and hydrogen peroxide. *Journal of Applied Microbiology* 104, 1014-1024.

Hasani, M., Wu, F., Warriner, K., 2020. Validation of a vapor-phase advanced oxidation process for inactivating *Listeria monocytogenes*, its surrogate *Lactobacillus fructivorans*, and spoilage molds associated with green or red table grapes. *Journal of Food Science* 85, 2645-2655.



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Hasani, M and Warriner K., 2019. AOP for surface disinfection of fresh produce: From concept to commercial reality. UV Solutions. Available at: <https://uvsolutionsmag.com/articles/2019/aop-for-surface-disinfection-of-fresh-produce-from-concept-to-commercial-reality/>

Ho, J., Prosser, R., Hasani, M., Chen, H., Skanes, B., Lubitz, W.D., Warriner, K., 2020. Degradation of chlorpyrifos and inactivation of Escherichia coli O157:H7 and Aspergillus niger on apples using an advanced oxidation process. Food Control 109. 106920

Murray, K., Aldossari, H., Wu, F., Warriner, K., 2018. Dynamic changes in free-chlorine levels within a commercial post-harvest wash and prevention of cross-contamination between shredded lettuce batches. Food Control 85, 127-134.

Murray, K., Moyer, P., Wu, F., Goyette, J.B., Warriner, K., 2018. Inactivation of Listeria monocytogenes on and within Apples Destined for Caramel Apple Production by Using Sequential Forced Air Ozone Gas Followed by a Continuous Advanced Oxidative Process Treatment. Journal of food protection 81, 357-364.

Murray, K., Wu, F., Shi, J., Xue, S.J., Warriner, K., 2017. Challenges in the microbiological food safety of fresh produce: Limitations of post-harvest washing and the need for alternative interventions. Food Quality & Safety 1, 289-301.

Industry / academia collaborations are essential for the continuation of food safety research targeting the risks associated with fresh fruits and vegetables. This research creates the scientific foundation which leads to affordable, accessible and practical solutions to help reduce these risks.

We would like to thank the CPMA membership which made this work possible and we look forward to partnering with CPMA again on future projects.

Sincerely,

A handwritten signature in black ink, appearing to read "Keith Warriner".

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# **Integrated post-harvest wash systems for fruit and vegetable decontamination for reducing water usage and decrease emission of disinfectant byproducts in wastewater streams**

Start Date: May 2019

Final Report: December 2020

Version 3

**Author:** Mahdiyeh Hasani and Keith Warriner

## **Summary**

Advanced Oxidation Process (AOP) describes a water treatment based on the generation of hydroxyl-radicals to oxidize constituents with wash water that contribute to Chemical Oxygen Demand. In the following, AOP has been developed for reducing the chlorine demand of spent post-harvest wash water thereby enabling active levels of the sanitizer to be maintained to reduce cross-contamination between lettuce batches during the washing process. The bench-top AOP reactor was constructed using a quartz coil surrounding a UV-C lamp with the water sample, supplemented with hydrogen peroxide, being circulated via a peristaltic pump. A simulated spent wash water was prepared based on the composition of samples collected from a commercial lettuce processing line. The breakpoint of the simulated wash water was determined by the addition of hypochlorite followed by measuring the concentration of free-chlorine. Initial attempts to reduce the chlorine demand using AOP were unsuccessful due to the low generation of hydroxyl radicals from the hydrogen peroxide substrate. However, addition of copper catalyst enhanced the generation of radicals as detected by the degradation of hydrogen peroxide and oxidation of methylene blue. Although the AOP reaction could be supported by copper catalyst the rate of radical formation was slow and required treatment times of 30 mins. However, a more rapid AOP was achieved through substituting copper for ferric ion. Through optimization using Response Surface Modelling (RSM) it was possible to derive the optimal AOP processing parameters in terms of hydrogen peroxide and ferric ion concentrations, in addition to UV-C dose. Under the optimal conditions, the chlorine demand of simulated spent wash water (pH 6.9) was decreased from 160 ppm to 50 ppm. The generation of hydroxyl radicals from the action of UV and ferric chloride on hydrogen peroxide was enhanced at pH 4 compared to pH 6.9. However, the measured chlorine demand on the wash water adjusted to pH 4 was increased by AOP treatment which was likely due to the catalytic action of ferric ions on hypochlorite.

An pilot-scale AOP reactor was constructed that could treat 60 liters of spent irrigation water per min. The spent wash water at pH 6.9 or 4.0 was treated with the optimized AOP treatment (UV-C dose of 667 mJ/cm<sup>2</sup>, 9 ppm H<sub>2</sub>O<sub>2</sub> concentration and 10 mg/l FeCl<sub>2</sub>) then forwarded to trials to assess the efficacy for decontaminating lettuce, in addition to preventing cross-contamination. It was found that free-chlorine levels remained stable in spent wash water pH 6.9 treated with AOP but was sequestered in non-treated controls. The free-chlorine concentration in acidified (pH 4) wash water decreased to <1 ppm irrespective of receiving AOP treatment. The log reduction of model pathogens (*Listeria*, *E. coli* and *Salmonella*) inoculated onto shredded romaine lettuce were higher for *Listeria* in AOP treated water at pH 6.9 compared to non-AOP treated water. However, there was no significant difference in log reduction of *Salmonella* or *E. coli* on lettuce when washed in treated or non-treated AOP water at pH 6.9. The same trend was observed for spent wash water at pH 4 with the log count reductions of pathogens on lettuce being lower compared to treatments at pH 6.9.

The cross-contamination of pathogens between lettuce batches was negligible in AOP spent wash water but was significant with non-treated wash water at pH 6.9. However, no cross-contamination occurred between lettuce batches with spent wash water at pH 4.0 irrespective of AOP treatment. In conclusion, the AOP treatment developed in the study can be applied to reduce chlorine demand thereby preserving free-chlorine levels in water to prevent cross-contamination. The generation of hydroxyl-radicals by AOP was enhanced at pH 4 the ferric catalyst increased the chlorine demand although this did not result in cross-contamination of pathogens between lettuce batches.

### **Aims and Objectives**

The overall objective of the research was to develop and optimize an Advanced Oxidation Process to oxidize constituents within spent lettuce wash water that sequester free-chlorine leading to cross contamination events during the washing process. The research identified to optimal operating parameters of the process and verified performance in maintaining free-chlorine levels within lettuce wash tanks. The objectives are:-

- 1) Characterize spent wash water derived from commercial lettuce processing lines.
- 2) Optimize AOP to reduce chlorine demand and assess contribution of each working parameter of the AOP process through Response Surface Modelling.

- 3) Validate the AOP predictive model for reducing chlorine demand and supporting microbial inactivation.
- 4) Verification of AOP performance to reducing chlorine demand and maintaining sufficient free-chlorine to prevent cross-contamination events.

## Results

### Objective 1: Characterization of spent wash water from leafy green processing:

Spent lettuce wash water was collected from a commercial processing facility on three separate occasions and characterized with respect to ORP, chlorine demand, conductivity, turbidity, solids content, BOD and COD (Table 1). The processing line processed 2300 kg/hour of shredded iceberg lettuce over a period of up to 30 h. Sanitizer (50-200 ppm sodium hypochlorite) was automatically added by using a dual pH – ORP control system, the level of chlorine and pH in water were maintained in range of 600-850 mv and pH 6.5 – 6.9, respectively. The wash tanks were replenished with fresh water at a rate of 20-30% per hour.

The spent water characteristics was a relatively low BOD and COD with high turbidity. Although the solids content was also relatively low there was a high proportion of soluble solids indicative of low molecular weight species. The soluble solids likely contributed to the chlorine demand given the low amount of ammonia present. There were negligible amounts of free chlorine detected in the spent wash water confirming that the breakpoint was not reached in the course of commercial processing operations.

Table 1: Characteristics of spent wash water derived from a shredded lettuce processing facility.

| Parameter                          | Commercial Spent Wash Water |
|------------------------------------|-----------------------------|
| Turbidity (NTU)                    | 78±26                       |
| Oxidation Reduction Potential (mV) | 602±8                       |
| pH                                 | 6.9                         |
| Total Solids (mg/l)                | 544±87                      |
| Total Soluble Solids (mg/l)        | 104±98                      |
| BOD <sub>5</sub> (mg/l)            | 230±53                      |
| COD (mg/l)                         | 309±67                      |
| Ammonia (mg/l)                     | 0.56±0.12                   |

|                           |           |
|---------------------------|-----------|
| Free Chlorine (ppm)       | 0.43±0.21 |
| Total Chlorine (ppm)      | 21±2      |
| Chlorine Breakpoint (ppm) | 140±10    |
| Conductivity (μS/cm)      | 785±102   |

## 2) Optimize AOP to reduce chlorine demand and assess contribution of each working parameter of the AOP process through Response Surface Modelling

A simulated spent wash water solution was prepared by the addition a lettuce homogenate to give the corresponding solids content of the sourced samples from commercial processing. The chlorine demand was determined by sequential additions of hypochlorite then measuring the free chlorine after a 30 min wait period. The simulated wash water did not exhibit a “hump” which is reflective of the low ammonia content (ie low formation of chloroamines). The measured chlorine demand of the simulated wash water was 135±25 ppm which is within the required range and hence suitable for AOP optimization studies.

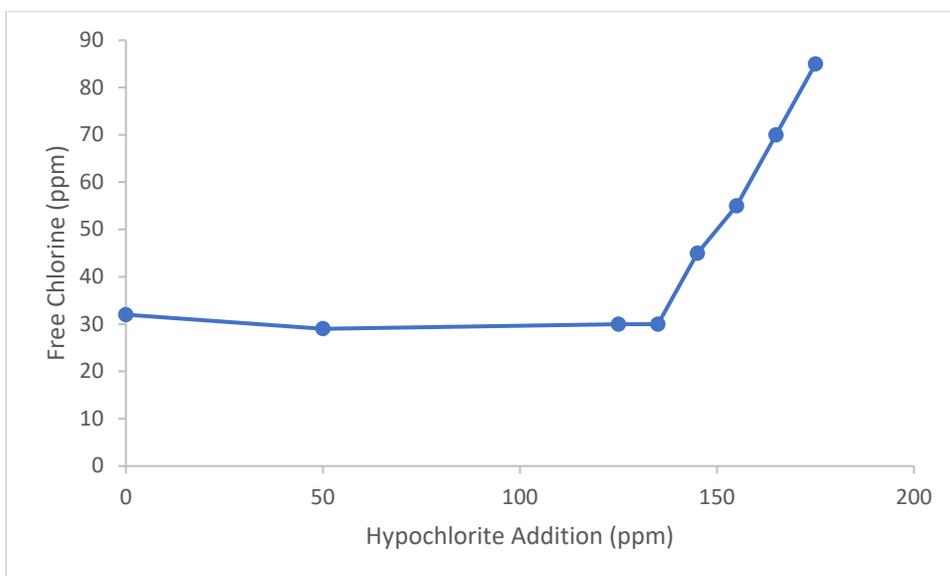


Figure 1: Chlorine breakpoint curve for simulated spent wash water prepared from diluted lettuce homogenate. Hypochlorite additions were made to the spent wash water and residual free-chlorine measured using DPD reagent after a 30 min wait period.

### Advanced Oxidation Process Reactor

The AOP system consisted of a UV lamp (602805 VIQUA, Guelph ON, CA) emitting at 254nm fitted inside a tube glass quartz coil with 1.25cm distance from the lamp to the coil (Figure 2).

The tube glass coil held the dimensions of 2.5mm ID, 5mm OD, and 21cm in length, which coiled around 21 times. At the ends of the coil, a tube (RK-06509-16 MASTERFLEX, Montreal

QC, CA) that feeds in and out of the coil, where the flowrate can be controlled by a peristaltic pump (RK-77202-60 MASTERFLEX, Montreal QC, CA) in rotations per minute. The peristaltic pump speed of 21rpm, which translated to a flow rate of  $2.8 \times 10^{-7} \text{ m}^3/\text{s}$  returned a Reynolds number of 90, indicating laminar flow. However, as the flow occurred in a coiled system, the presence of Dean vortices allows for mixing of the wash water. A radiometer (IL 1400A International Light, Newburyport MA, US) determined the UV-intensity at this distance to be  $9.2 \text{ mW}/\text{cm}^2$  to the outer diameter, which can be established as the average UV fluence rate at the outer diameter. Thus, by using UV fluence equation, the UV fluence of the system can be determined.

$$H = E_{avg} \cdot T_{GlassQuartz} \cdot t \cdot P$$

Equation 1. UV fluence equation

H is the absorbed dose by the wash water,  $E_{avg}$  is the average UV fluence rate in the wash water at any point of the cross section of the tubing,  $T_{quartz\ glass}$  is the transmittance of the quartz glass tubing (0.9), t is the residence time in the tube (5s) and P is the number of passes, with a pass time of 25s/pass. This was determined by multiplying the UV-intensity at the outer distance by the incidental cross-sectional area,  $9.2 \text{ mW}/\text{cm}^2 \cdot 1 \text{ cm} \cdot 0.25 \text{ cm} = 2.3 \text{ mW}/\text{cm}^2$ .

Baseline studies determined the influential parameters of the Advanced Oxidation Process reaction in the generation of hydroxyl-radicals via the UV-C mediated degradation of hydrogen peroxide in distilled water. Here an Response Surface Methodology established the contribution of UV-C dose (20 – 32  $\text{mJ}/\text{cm}^2$ ), temperature (3-40 °C) and concentration of  $\text{CuSO}_4$  catalyst (1-20 ppm). The AOP reactor consisted of a beaker containing the hydrogen peroxide and copper solution that was circulated around a Dean flow quartz coil with a central UV-C 23W lamp (Figure 2). The UV-C dose was controlled by the flow rate through the reactor with the temperature being adjusted via a hot plate or addition of ice.

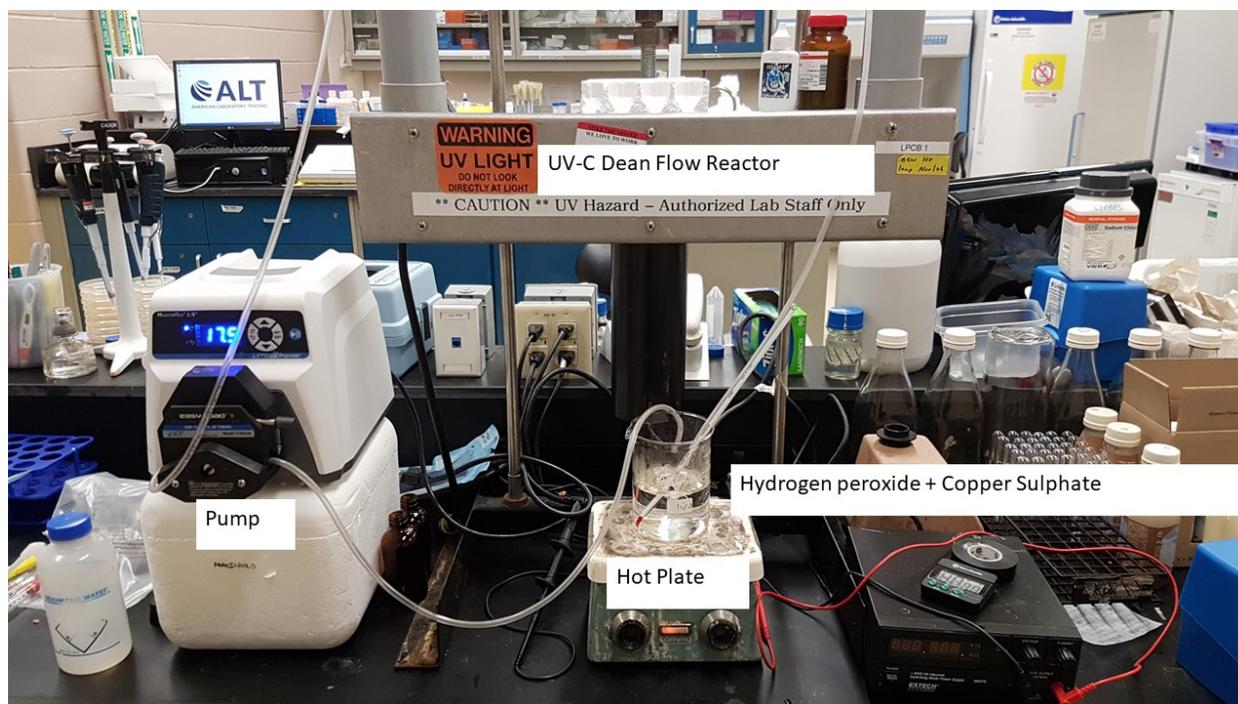


Figure 2: Advanced Oxidation Process Dean flow reactor. The hydrogen peroxide-copper sulphate solution is circulated through a quartz coil with a UV at 254nm being delivered via a central 23W lamp. The temperature of the solution was maintained via a hot plate (or cooled by addition of ice) with the UV-C dose being adjusted via the pump speed.

From the RSM analysis, the generation of hydroxyl-radicals was primarily dependent on temperature and copper catalyst concentration but less so on UV dose (Figure 3). Moreover, by increasing the catalyst concentration could compensate for low temperatures that would be expected to operate within a produce washing operation. Significantly, in the absence of copper catalyst there was a low conversion of hydrogen peroxide to hydroxyl radicals.

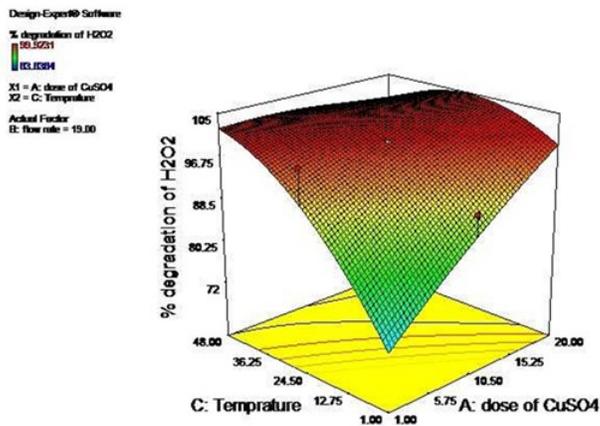
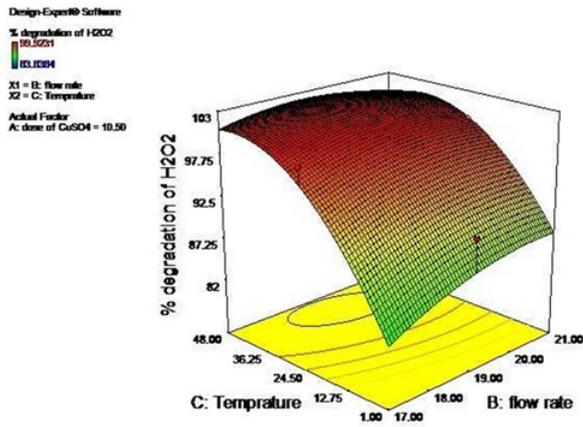
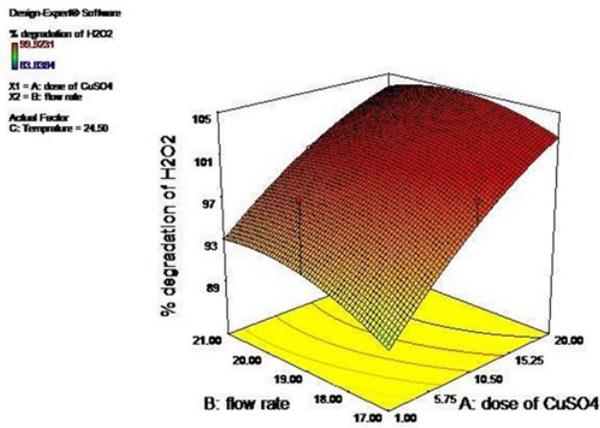


Figure 3: Contour graphs of RSM illustrating the interaction between UV-C dose (flow rate), copper catalyst and temperature on free-radical generation from the degradation of hydrogen peroxide.

## Methylene Blue Assay

The degradation of hydrogen peroxide is an indirect measure of free-radical formation. Therefore, further trials were performed using the methylene blue assay that is based on the oxidation of the reduced dye by the generated hydroxyl radicals.

The assay was performed using solutions of methylene blue supplemented with  $\text{CuSO}_4$  catalyst and different concentrations of hydrogen peroxide (Figure 4). In the absence of UV-C there was no oxidation of the methylene blue dye confirming the reaction required the generation of hydroxyl-free radicals. The highest rate of methylene blue oxidation was observed with AOP treatments with 10 – 60 ppm hydrogen peroxide (Figure 4). At higher concentrations of hydrogen peroxide the rate of dye oxidation decreased which is indicative of the generated radicals reacting (neutralizing) in termination reactions. However, 30 min treatment time resulted in an equivalent oxidation of methylene blue irrespective of the hydrogen peroxide concentration.

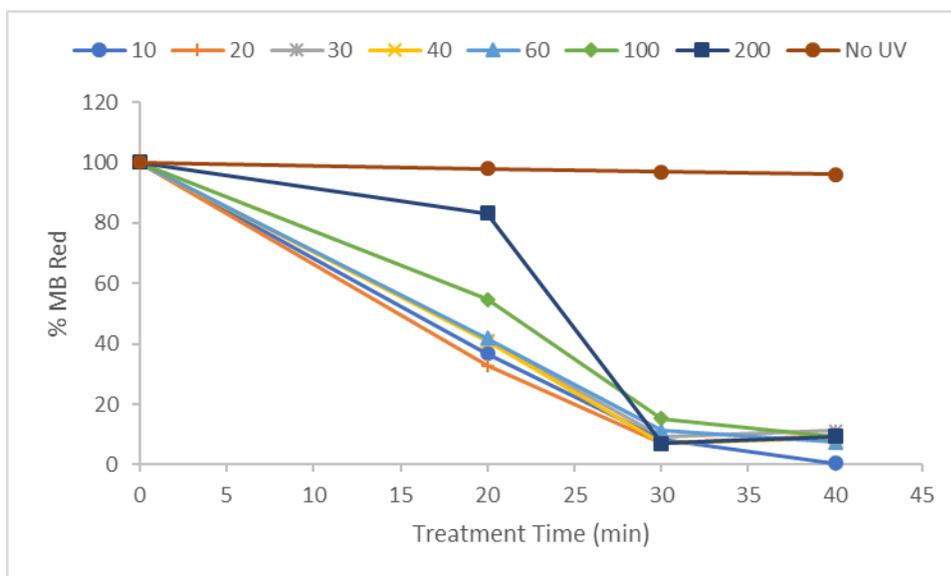


Figure 4: Oxidation of methylene blue by hydroxyl free radicals derived the UV-C degradation of different concentrations of hydrogen peroxide (10 – 200 ppm) containing 10 ppm  $\text{CuSO}_4$ . Solutions of methylene blue (6 mg/l) were supplemented with 10 ppm  $\text{CuSO}_4$  and the test hydrogen peroxide solution then circulated through the dean flow reactor with samples being collected at different time points. The absorbance at 664 nm of the samples was measured with the results being reported as % oxidation of methylene blue.

### Ferric-chloride catalyst

Although the AOP reaction could be catalyzed by copper ions the process was relatively slow and required extended treatment times. Therefore, ferric ion catalyst was evaluated as an alternative in a Fenton-photocatalytic AOP process. RSM was performed that applied UV-C dose, hydrogen peroxide and ferric ion concentration as the independent factors and extent of methylene blue oxidation being the dependent factor.

Table 1. RSM trials (20) that measured the oxidation of methylene blue (i.e decrease in absorbance at 664 nm) as a function of UV-C dose ( $\text{mJ}/\text{m}^2$ ),  $\text{H}_2\text{O}_2$  (ppm), and  $\text{FeCl}_2$  (mg/L) concentration.

| Run | UV-C ( $\text{mJ}/\text{cm}^2$ ) | $\text{H}_2\text{O}_2$<br>Concentration<br>(ppm) | Concentration of<br>$\text{FeCl}_2$ (mg/L) | % Methylene Blue<br>Oxidation |
|-----|----------------------------------|--|--|-------------------------------|
| 1   | 372.6                            | 5  | 5  | 25.7                          |
| 2   | 0                                | 5  | 5  | 6.8                           |
| 3   | 372.6                            | 10   | 5  | 28.5                          |
| 4   | 372.6                            | 5  | 5  | 31.0                          |
| 5   | 372.6                            | 5  | 0  | 27.9                          |
| 6   | 372.6                            | 0  | 5  | 11.4                          |
| 7   | 0                                | 10   | 0  | 9.2                           |
| 8   | 745.2                            | 10   | 0  | 31.0                          |
| 9   | 745.2                            | 5  | 5  | 32.0                          |
| 10  | 0                                | 0  | 10   | 14.2                          |
| 11  | 372.6                            | 5  | 5  | 29.3                          |
| 12  | 745.2                            | 10   | 10   | 39.0                          |

|    |       |    |    |      |
|----|-------|----|----|------|
| 13 | 0     | 10 | 10 | 14.8 |
| 14 | 0     | 0  | 0  | 0.1  |
| 15 | 372.6 | 5  | 10 | 28.3 |
| 16 | 372.6 | 5  | 5  | 29.0 |
| 17 | 372.6 | 5  | 5  | 29.4 |
| 18 | 372.6 | 5  | 5  | 23.9 |
| 19 | 745.2 | 0  | 0  | 13.2 |
| 20 | 745.2 | 0  | 10 | 14.7 |

## Response surface methodology

Factor Coding: Actual

**Average % Reduction (%)**

Design Points

● Above Surface

○ Below Surface

0.120734 39.0095

X1 = A: UV-C

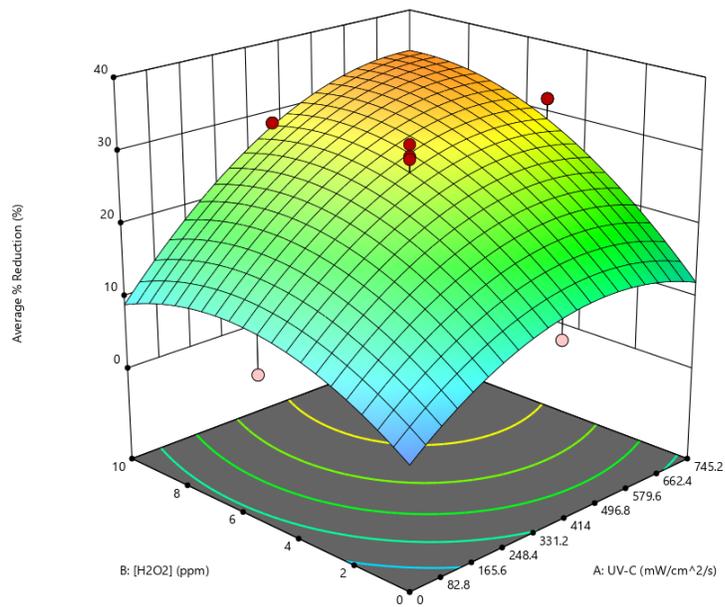
X2 = B: [H2O2]

**Actual Factor**

C: FeCl2 = 5



3D Surface



Factor Coding: Actual

**Average % Reduction (%)**

Design Points:

● Above Surface

○ Below Surface

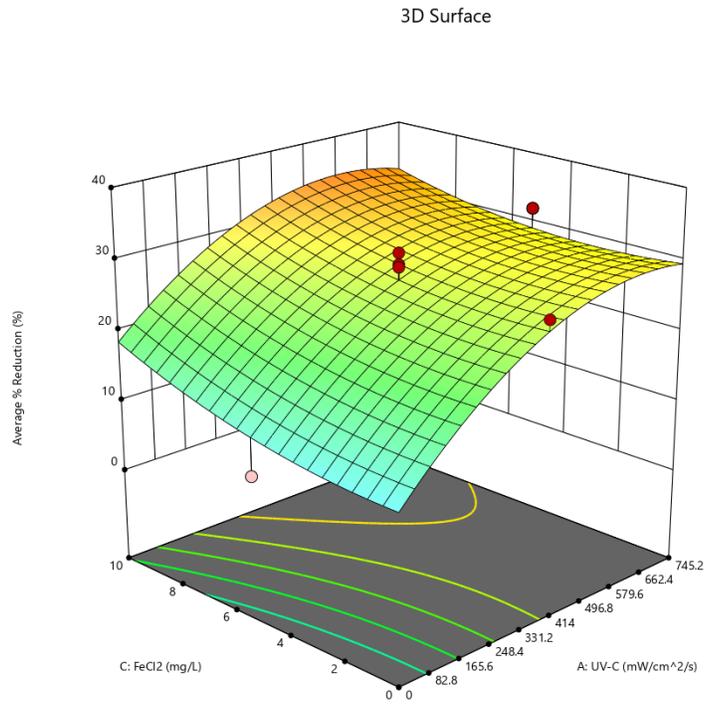
0.120734 39.0095

X1 = A: UV-C

X2 = C: FeCl2

**Actual Factor**

B: [H2O2] = 5



Factor Coding: Actual

**Average % Reduction (%)**

Design Points:

● Above Surface

○ Below Surface

0.120734 39.0095

X1 = B: [H2O2]

X2 = C: FeCl2

**Actual Factor**

A: UV-C = 372.6

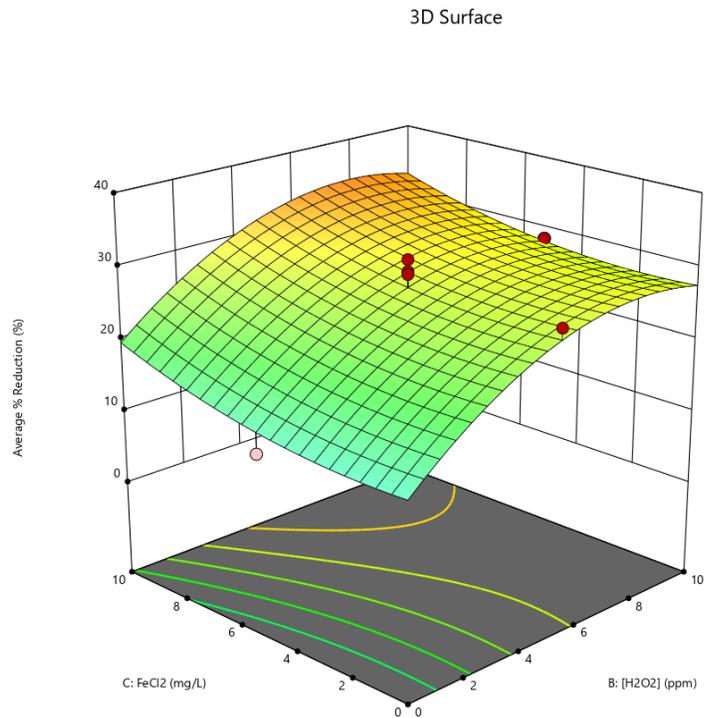


Figure 5. 3D Contour graphs of RSM illustrating the interaction between hydrogen peroxide and ferric ion concentration, along with UV-C dose in supporting the oxidation of methylene blue via AOP.

From the surface plots it was illustrated that the extent of methylene blue oxidation was dependent on the UV-C dose and hydrogen peroxide concentration (Figure 5). Although ferric chloride also contributed to the generation of free-radicals it was relatively independent of the catalyst concentration. This would suggest that ferric ions acted as a more effective catalyst compared to copper and minimized the neutralization of hydroxyl-radicals by rapid regeneration thereby sustaining the reaction.

From the data generated the optimal AOP working parameters were found to be a UV-C dose of 667 mJ/cm<sup>2</sup>, 9 ppm H<sub>2</sub>O<sub>2</sub> concentration and 10 mg/l FeCl<sub>2</sub>. The predicted oxidation of methylene blue was 37% which was verified from experimental trials.

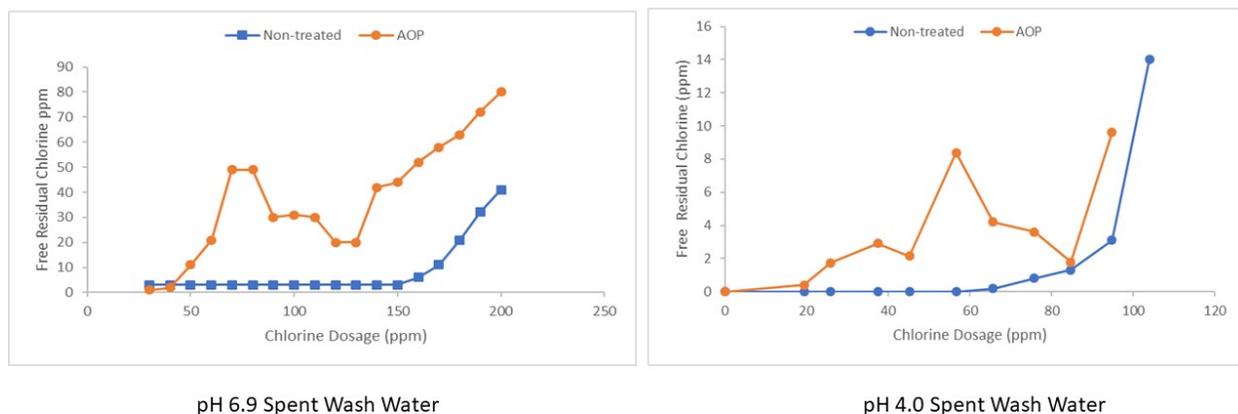
#### **Objective 4: Pilot-Scale AOP Reactor**

The pilot scale reactor was based on a UV-1500 flow through reactor operating at 68 liters per minute (Figure 6) that delivered a theoretical dose of 40 mJ/cm<sup>2</sup> as measured using the methylene blue reduction test. The simulated spent wash water (pH 6.9 or pH 4.0) sample was held within a 40-liter container and hydrogen peroxide (9 ppm) along with ferric catalyst (10 mg/L) added just prior to passing through the UV unit via a centrifugal pump.



Figure 6: Preparing simulated spent wash water for Advanced Oxidation Process using a UV-tube reactor.

Simulated spent was water was treated with the optimized AOP treatment (UV-C dose of  $667 \text{ mJ/cm}^2$ , 9 ppm  $\text{H}_2\text{O}_2$  concentration and 10 mg/l  $\text{FeCl}_2$ ) and changes in the chlorine demand of spent wash water at pH 6.9 or adjusted to pH 4.0.



the chlorine demand. This theory was supported by the fact that addition of chlorine (30 ppm) to AOP treated water resulted in depletion of free-chlorine to 0.27 ppm after 30 mins which was not significantly different from non-treated controls (0.34 ppm). In contrast, the chlorine concentration was stable in spent wash water at pH 6.9 after passing through the AOP unit with the free-chlorine being depleted in non-AOP treated controls (Figure 8).

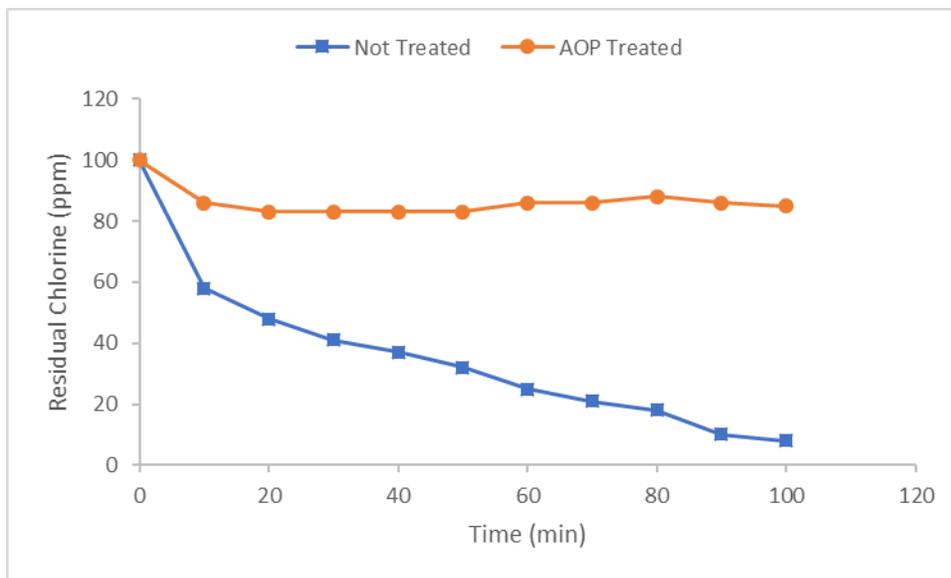


Figure 8: Residual chlorine concentration in simulated spent wash water treated or not treated by Advanced Oxidation Process.

### **Log Count Reduction of pathogens on lettuce and prevention of cross-contamination during washing in AOP treated and non-treated spent wash water.**

Trials were performed in determining the log count reduction on lettuce inoculated with *Listeria*, *E. coli* or *Salmonella* then washed in chlorinated spent wash water that had (or not) previously been passed through the AOP unit. Here, spent wash water was passed through the AOP unit the chlorine added to a final concentration of 30 ppm. The same volume of chlorine was added to the non-treated control spent wash water. The various wash water was subdivided into 500 ml volumes that were taken forward to cross-contamination evaluation trials (Figure 9).

Shredded romaine lettuce was prepared from whole heads and spray inoculated with a culture of *E. coli*, *Salmonella* or *Listeria monocytogenes* to a final cell density of approximately 7 log cfu/g. The bacteria were allowed 20 mins to attach before the lettuce was sub-divided into 50g lots. The inoculated lettuce lot was placed in the chlorinated spent wash water (non-treated or AOP treated) for 60s then removed. This was followed by adding 50 g of non-inoculated lettuce that was steeped for 60s and the process repeated with a third batch of non-inoculated lettuce

(Figure 9). The counts on the different lettuce samples was then determined using the appropriate agar.

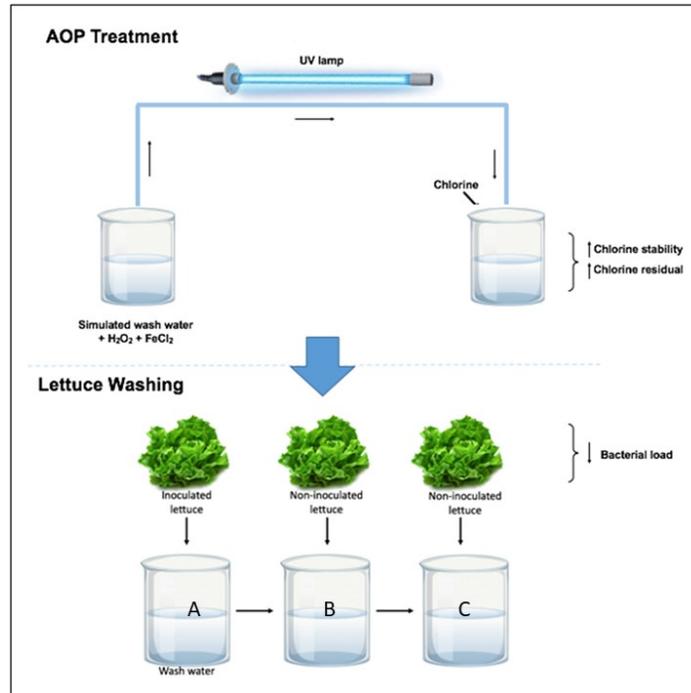


Figure 9: Schematic flow diagram of the trials performed to assess the efficacy of AOP treatment of water to maintain free-chlorine concentration at a sufficient level to prevent cross-contamination.

With AOP spent wash water at pH 6.9 it was found the log reduction of *Listeria monocytogenes* on lettuce was significantly higher compared to that supported by non-treated spent wash water (Table 2). The same effect was observed when pH 4.0 spent wash water was applied although the actual log reductions of *Listeria* were significantly ( $P > 0.05$ ) compared to water at pH 6.9. There was no significant difference ( $P > 0.05$ ) in *E. coli* or *Salmonella* counts on lettuce washed in AOP or non-AOP treated water irrespective of the pH (Table 2). Yet, the log count reduction supported by AOP treated water at pH 6.9 was significantly ( $P > 0.05$ ) compared to pH 4.

The results would confirm the limited efficacy of chlorinated washing was limited in log reduction of pathogens on lettuce. The limitation can be attributed to the pathogens residing in protective sties (for example, cut edges) leading to limited decontamination efficacy. The higher log reductions of AOP-treated spent water at pH 6.9 vs pH 4.0 it likely attributed to the free-chlorine concentration. The high free-chlorine of AOP water at pH 6.9 retained active chlorine whereas with the other samples the sanitizer had become sequestered. Nevertheless, the log reduction of pathogens was relatively low being  $< 1.64$  log cfu/g.

Table 2: Log count reduction of *Listeria*, *Salmonella* and *E. coli* on lettuce supported by washing inoculated lettuce in chlorinated simulated spent wash water that was treated with AOP compared to non-treated controls.

| Pathogen/Treatment              | Log cfu/g<br>(Log Count Reduction) |                        |
|---------------------------------|------------------------------------|------------------------|
|                                 | pH 6.9                             | pH 4.0                 |
| <i>Listeria monocytogenes</i>   |                                    |                        |
| Initial Load                    | 6.11±0.05                          | 6.34±0.08              |
| Non-treated Spent Water         | 5.40±0.03<br>(0.71)Aa              | 6.33±0.11<br>(0.02)Ba  |
| AOP-treated Spent Water         | 4.47±0.92<br>(1.64)Ab              | 5.97±0.16<br>(0.37)Bb  |
| <i>Salmonella</i>               |                                    |                        |
| Initial Load                    | 6.08±0.20                          | 5.93±0.23              |
| Non-treated Spent Water         | 4.79<br>(1.30)Ab                   | 5.03±0.11<br>(0.89)Bc  |
| AOP-treated Spent Water         | 4.61±0.31<br>(1.49)Ab              | 5.14±0.12<br>(0.79)Bc  |
| <i>Escherichia coli</i> O157:H7 |                                    |                        |
| Initial Load                    | 5.44±0.06                          | 6.91±0.04              |
| Non-treated Spent Water         | 4.45±0.73<br>(0.99)Aab             | 6.16<br>(0.75)Ac       |
| AOP-treated Spent Water         | 4.61±0.32<br>(1.23)Ab              | 6.37±0.15<br>(0.53)Bab |

Mean in rows with the same capital letter are not significantly different (P>0.05)

Mean within columns with the same lower case letter are not significantly different (P>0.05)

Cross contamination via wash water was assessed by initially washing inoculated samples then subsequently using the water to was non-inoculated lettuce samples. Pathogen of the different lettuce samples was then determined to assess the degree to which cells from the inoculated samples had been transferred to non-inoculated lettuce via the spent wash water (Table 3). It was found that pH 6.9 spent wash water that had not received AOP treated resulted in transfer of *Listeria* to the non-inoculated samples (Table 3). In comparison, negligible transfer occurred with the wash water that had been treated with AOP due to the active free-chlorine that had been sequestered in non-treated controls (Table 3). A similar patter was observed for *Salmonella* and *E. coli* O157:H7 with the prior treatment of spent wash water by AOP enabled free-chlorine concentrations to be maintained.

The AOP treatment of spent water at pH 4 was also proven effective in preventing cross-contamination of pathogens between lettuce batches (Table 3). However, the water receiving no AOP treatment also didn't show cross-contamination which contrasts to spent wash water at pH 6.9. The results could be explained by the acidic pH that inactivated to low number of pathogens released from the lettuce during washing.

Table 3: Prevention of cross-contamination between lettuce samples during washing in chlorinated water that had or had not received AOP treatment. The spent wash water at pH 4 or 6.9 was passed through the AOP unit the supplemented with hypochlorite to give 70 ppm free-chlorine. Inoculated lettuce was then washed in the water for 30s before removing. Non-inoculated lettuce was then added to the water and the process repeated for a third time as described in Figure 8.

| Pathogen/Lettuce Sample         | Log cfu/g<br>pH 4 |                   | Log cfu/g<br>pH 6.9 |                   |
|---------------------------------|-------------------|-------------------|---------------------|-------------------|
|                                 | Non-Treated Water | AOP Treated Water | Non-Treated Water   | AOP Treated Water |
| <i>Listeria monocytogenes</i>   |                   |                   |                     |                   |
| Initial Loading                 | 6.34±0.08         |                   | 6.11±0.03           |                   |
| Inoculated Lettuce              | 6.33±0.10         | 5.97±0.16         | 5.40±0.02           | 4.47±0.92         |
| T1 Non-inoculated Lettuce       | <1.00             | <1.00             | 2.32±0.30           | 1.33±0.58         |
| T2 Non-inoculated Lettuce       | <1.00             | <1.00             | 1.98±1.71           | <1.00             |
| <i>Salmonella</i>               |                   |                   |                     |                   |
| Initial Loading                 | 5.93±0.23         |                   | 6.13±0.29           |                   |
| Inoculated Lettuce              | 5.03±0.11         | 5.14±0.12         | 4.80±0.28           | 4.61±0.32         |
| T1 Non-inoculated Lettuce       | <1.00             | <1.00             | 1.86±0.31           | <1.00             |
| T2 Non-inoculated Lettuce       | <1.00             | <1.00             | 1.36±0.32           | <1.00             |
| <i>Escherichia coli</i> O157:H7 |                   |                   |                     |                   |
| Initial Loading                 | 6.91±0.04         |                   | 5.15±0.03           |                   |

|                           |           |           |           |           |
|---------------------------|-----------|-----------|-----------|-----------|
| Inoculated Lettuce        | 6.16±0.17 | 6.37±0.15 | 4.45±0.73 | 4.26±0.32 |
| T1 Non-inoculated Lettuce | <1.00     | <1.00     | 1.54±0.58 | <1.00     |
| T2 Non-inoculated Lettuce | <1.00     | <1.00     | <1.00     | <1.00     |

T1: Non-inoculated lettuce sample following washing of inoculated samples. The non-inoculated lettuce was removed from the wash water and a further non-inoculated sample added (T2) then washed for 30s. Residual pathogens on the different lettuce samples were then enumerated.

## Conclusions

- A simulated spent wash water was prepared based on samples collected from a commercial processing line. The chlorine demand of the spent wash water was between 135-180 ppm at pH 6.9 and 70-78 ppm at pH 4.0.
- An Advanced Oxidation Process that applied UV-C and hydrogen peroxide did not significantly decrease the chlorine demand on spent wash water unless a catalyst was added. Ferric catalyst supported the photo-degradation of hydrogen peroxide to produce hydroxyl-radicals as confirmed by methylene blue oxidation.
- The AOP process was optimized by using Response Surface Methodology that identified a treatment using 9 ppm hydrogen peroxide, 10 mg/l, Ferric catalyst and 667 mJ/cm<sup>2</sup> UV-C dose decreased the chlorine demand on spent wash water by 110 ppm at pH 6.9. Excess hydrogen peroxide concentration negatively affected the oxidative process through neutralizing reactions. With spent wash water at pH 4.0 there was an increase in the measured breakpoint from 78 ppm to 88 ppm when AOP treatment was applied that was presumably due to the ferri chloride catalyst.
- Under optimized conditions, the AOP treatment decreased the chlorine demand of simulated wash water (pH 6.9) from 160 ppm down to 50 ppm. The residual chlorine concentration of AOP treated water was maintained for over 100 mins although progressively decreased in non-treated controls. With spent water adjusted to pH 4 there was a rapid sequestering of free-chlorine and levels fell <1 ppm irrespective of the AOP being applied.
- When lettuce was washed in spent wash water at pH 6.9 there was extensive cross-contamination between lettuce batches due to the depletion of free-chlorine. However, AOP treated spent wash water where the free chlorine was maintained minimized cross-contamination events. The same observation was made for spent wash water at pH 4 but this was independent of AOP treatment being applied. This was presumably due to the low log reduction of pathogen from lettuce during the wash process and inactivation of low number of pathogens in water by virtue of the low pH.