CENTER for PRODUCE SAFETY

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FINAL PROJECT REPORT

Project Title

Enhancing the efficacy of fresh produce washing operations through establishing monitoring methods and water disinfection technologies based on a combination of filtration and UV

Project Period

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Principal Investigator

Keith Warriner
University of Guelph
kwarrine@uoguelph.ca
519-824-4120 x56072

Co-Principal Investigator

Objectives

1. Determine the efficacy of fresh produce washing within commercial processing facilities. Correlate the log count reduction achieved with wash water parameters (temperature, turbidity, conductivity, Oxidation Reduction Potential, oxygen consumption, impedance/capacitance).
2. Determine the cross-contamination events occurring during commercial wash processes.
3. Evaluate the efficacy of a combination of filtration and UV to recycle fresh produce wash water.
4. Perform verification trials on selected interventions and monitoring methods.

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FINAL REPORT

Abstract

The post-harvest wash process is considered a critical control point in leafy green processing whereby field acquired contamination can be removed thereby enhancing the microbiological safety of fresh produce. However, to represent a critical control point a means of monitoring the process is needed beyond simply measuring sanitizer concentration. The objectives of the research were to identify which wash water parameters could be used to predict the efficacy (i.e. Log Count Reduction) of post-harvest washing of leafy greens. To this end sampling trials were performed in three leafy green processors (two processing spinach and the other shredded lettuce). In each facility samples (pre-wash and post-wash, in addition to spent wash water) were taken over the course of a typical processing day for determination of water parameters and microbial counts. From the results it was found that the log count reduction achieved was independent of sanitizer concentration and microbial loading of the water. However, the log count reduction could be correlated to the initial loading on leafy greens at pre-wash (positive correlation), temperature (negative correlation) and conductivity (charged species; positive correlation). More over, although the log count reduction was independent of coliform loading in wash water, a correlation was found with respect to the carriage on post-harvest leafy greens. The latter indicated that the wash water process could result in cross-contamination events between batches of leafy greens. This was found to be the case when the microbial populations associated with produce and wash water were evaluated using a combination of 16S rRNA-DGGE and Substrate Carbon Utilization (SCU) Ecoplate profiles. Although the population was complicated by the relative homogenous population profiles encountered on incoming spinach or lettuce, the data indicated that microflora on pre-wash produce were deposited, acquired and directly carried through the process. Interestingly, it was found that the exchange of populations between water and produce (and visa versa) occurred to a greater extent with lettuce compared to spinach. More over SCU profiles indicated that microbial types differ in their ability to detach and persist in wash water. The results of the study supported the view that the wash process should be designed to prevent cross-contamination as opposed to be relied upon to remove field-acquired contamination. To this end a water recycling unit was developed that enabled continued treatment of spent wash water to minimize the accumulation of contamination within the tank. The technology was based on an initial coagulation step followed by filtration and UV disinfection. Each process was optimized within the laboratory and the final unit evaluated within a commercial processing facility. The water recycling unit was demonstrated to successfully decrease the organic loading of spent wash water and reduce microbial counts. Residual levels of coagulating agent (sodium aluminate) were below the regulatory level of 0.4 ppm. In addition to maintaining wash-water quality the recycling unit reduced water usage that would have a direct impact on costs incurred with down-stream waste treatment.

Background

The fresh-cut industry has experience rapid growth in recent years with an estimated 6 million bags of salads being produced daily (10). The consumption per capita of fresh-cut vegetables has grown about 30% from 1991 to 2006 with an average market growth of 1.7% per year (10). However, market growth has been mirrored by the increased frequency of foodborne illness outbreaks linked to fresh-cut produce (13). Indeed, fresh produce is the most significant source

of foodborne pathogens with more cases of illness linked to products such as leafy-greens compared to the traditional vehicles such as meat and seafood (12). Although a broad range of human pathogens have been linked to produce, *Salmonella*, *Escherichia coli* O157:H7 and enteric viruses remain the most significant (3). Leafy vegetables such as lettuce and spinach are the most common vehicle amongst fresh produce (10). One of the largest outbreaks reported was associated with Californian fresh-cut spinach contaminated with *E. coli* O157:H7 that resulted in over 200 confirmed cases and 3 deaths (4) .

A wash step is applied in fresh-cut processing in an attempt to remove field acquired contamination (5). A diverse range of sanitizers has been applied in produce washing although hypochlorite (100 – 400 ppm) remains the most commonly used (5). Because hypochlorite is rapidly sequestered by organic material it is common practice in industry to apply an Oxidation Reduction Potential (ORP) control system (9). Here, the ORP is maintained at a set point by the addition of hypochlorite and citric acid. Therefore, in theory at least the active hypochlorous acid concentration is held constant regardless of the organic loading in the wash water. Peroxyacetic acid is an alternative sanitizer with the advantage over hypochlorite in being more stable in the presence of organic material (11). Peroxyacetic acid is recommended to be used at levels in the order of 50 ppm although typically lower concentrations (10-30 ppm) are applied due to cost (11).

Although a range of sanitizing agents have been demonstrated in the laboratory to achieve high (3-4 log10 CFU) reductions in pathogen levels it is commonly acknowledged that Log Count Reductions (LCR) under commercial conditions are limited to <2 log10 CFU regardless of the sanitizer used (1, 2). The limitation of fresh produce washing is thought due to biofilm formation on the surface of produce and potential of microbes (including human pathogens) to become internalized (13). It is also possible that the accumulation of contamination within wash water could also lead microbes being transferred to leafy greens (5). This has led some to speculate that the washing process in fresh produce processing can represent a cross-contamination step as opposed to an intervention point (6, 7). It is known that the efficacy of fresh cut washing is a dynamic process and changes in the course of processing (8). Specifically, it can be expected that the LCR of fresh produce at the start of processing would be higher compared to towards the end of the processing activity when organics and microflora would have accumulated within the water. However, this has yet to be demonstrated experimentally.

Monitoring the microbiological quality of wash water by direct measurements s problematic. Although turbidity and conductivity are routinely used to assess municipal water quality there have been no studies to date on if such parameters can be used to estimate log count reduction or microbial loading of the water. Therefore, one of the objectives of the study will be to investigate the factors that effect the efficacy of the wash process in fresh-cut processing.

In addition to monitoring, it is possible to apply water re-cycling to ensure consistency of the wash process, minimize cross-contamination events and also derive cost savings through improved water management (in terms of usage and disposal) (3). Possible options for small-scale (<10, 000 liters) recycling include cross-flow filtration where the water is passed under pressure down a hollow tube lined with pores (for example >0.5µm). The water and other low molecular weight organics pass through the pores whilst the aggregated organics are retained

(i.e. retentate). The method is well established for water treatment and can reduced the organic loading by 90% (11). However, the initial costs of units are high, extensive fouling can occur and close monitoring is required to ensure that excessive pressure is generated that can negatively affect membrane integrity.

In the following, the basis for a low cost, low maintenance and efficient water recycling unit will be described. Although water recycling is a well developed area, current systems work on a batch operation that can take over 48h to complete the process. The sequential steps encountered in standard water recycling is a physical separation step where large debris are removed followed by the addition of coagulant that aggregates, and entraps, low molecular weight organics. The coagulation step can take in excess of 24h to complete with the aggregates settling-out under gravity of via passage through a filter. The separated water then can be passed through an activated carbon filter to remove residual low molecular weight, cationic, organics. The treated water then undergoes a disinfection step prior to being re-introduced into the process.

The key to developing a continuous water recycling system is to reduce the time required to achieve the water-recycling step. From the above the key step rate limiting step is the coagulation process and methods will be described in the following study how this was reduced to facilitate a continuous operation.

Research Methods

Monitoring Wash Water Process and Cross-contamination Events

Commercial facilities description:

Sampling trials were performed at 3 fresh cut processors (2 processing salad spinach and the other shredded lettuce). Each facility was visited on at least 4 different times over the course of a 3 month period. On each visit, samples were taken at different time periods during the processing activity. Each sample set consisted of 300 g leafy greens (pre- and post-wash) and 2 x 250 ml of wash water. The temperature of the produce (pre- and post-wash) and wash water was also taken. The conductivity, pH and ORP of the wash water samples were measured using XL20 meter fitted with the appropriate probe. Peroxyacetic acid concentration of Facility A (spinach processor) water samples was determined by using an Ecolab Peracid/Peroxide Test Kit. Turbidity of wash water was determined at 254, 450 and 600 nm using a spectrophotometer. Oxygen content was determined using a portable oxygen meter with the impedance being measured using a Solartron frequency response analyzer.

Microbiological analyses: Leafy green samples (25 g) were placed into 225 ml of 0.1% peptone water and stomached for 150 s. A dilution series was prepared and plated onto Aerobic Plate Count and *E. coli*/coliform (Health Canada MFHPB-43) Petrifilms. Aerobic Count Plates were incubated at 35˚C for 48±3 hours. The minimum detection limit (MDL) for both pre- and post-washed samples is 0.65 log10 CFU/g and the “too numerous to count” (TNTC) is 7.44 log10 CFU/g . *E. coli*/coliform Petrifilms were incubated at 35±1˚C for 24±2 hours. MDL for pre- and post washed products was 0.4 log10 CFU/g.

Heterotrophic plate counts of wash water was determined by spread plating onto plate count agar and incubated at 35°C for 48±3 hours. Total Coliforms and E. coli counts were determined by filtering 10 ml of water through 47 mm diameter, 0.45 □m pore size m was placed onto DC (Differential Coliform) agar plate and plate 0.2 ml aliquot spread plated onto the same medium. Plates were incubated at 36°C for 24 hours.

Experimental Design and Statistics. In total 416 leafy green and 256 water samples were screened over the course of the study. Facility A was visited six times with a total of 192 spinach and 96 water samples being collected. Facility B was visited four times with 128 spinach and 64 water samples being screened. Finally, Facility C was visited on three occasions with 96 lettuce and 48 water samples being collected. Each sample analysis was performed in duplicate with the bacterial counts being transformed into $\log_{10}$ values prior to statistical analysis using ANOVA and Tukey Test. Correlations between Log Count Reductions (LCR) of aerobic counts and coliforms were calculated using the Spearman's Correlation Coefficient. Relationship between LCR to the rest of the parameters required all data collected within the same visit and time to be treated as a continuous variable, and the covariance structure was taken into account using SAS® Proc Mixed for Windows, v.9.1, SAS Institute, Inc. (Cary, NC, USA).

16S rRNA DGGE and Substrate Carbon Utilization Profiling

Leafy green and water samples were collected on three separate processing days in Facility A (spinach) and C (lettuce) as described above. The lettuce or spinach samples (25 g) were placed in a sterile stomacher bag along with 100 ml of PBS and shaken vigorously for approximately 30s. The rinse was decanted into a sterile tube and placed at 4°C until required. Wash water taken from the first and second wash tanks at different times during the processing period. Microbes within water and rinse solutions were concentrated by passing 100 ml volumes through a 0.45µm filter. The filter was then transferred to 10 ml PBS and vortexed for 30 s to release microbes then taken forward to DNA extraction using QIAamp DNA Stool Mini Kit (QIAGEN, Mississauga, Canada).

The V2-V3 region of the 16S rRNA genes were amplified using primers HDA1-GC (5' CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAG T 3'; the GC clamp in boldface) and HDA2 (5'-GTA TTA CCG CGG CTG CTG GCA C-3') (Kumar et al. 2006). The reaction mixture (25 µl total volume) consisted of PCR reaction buffer (1x), 1.5 mmol l-1 $MgCl_2$, a 0.4 mmol l^{-1} dNTP mix, 10 pmol primer, 50 ng template DNA and 2U Taq DNA polymerase (Boehringer Mannheim, Mannheim, Germany). PCR was performed in 0.2-ml tubes in a DNA Engine® Peltier Thermal Cycler (PTC-200) (Genetic Technologies, Inc. Miami, US). The thermal cycle consisted of an initial denaturation step at 94°C for 4 min followed by 30 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s; with a final extension at 72°C for 10 min.

Denaturing Gradient Gel Electrophoresis (DGGE) analysis of the PCR amplicons was performed using the Bio-Rad DCode Universal Detection System (Bio-Rad Canada, Mississauga, ON). DGGE was performed using 10% (w/v) acrylamide gels containing 35-65% gradient of urea and

formamide increasing in the direction of electrophoresis. Electrophoresis was conducted in TEA buffer at 100 V for 18 h at 60˚C. DNA bands in gels were visualized by silver staining.

Microecology of tomatoes using Biolog Ecoplate

Aliquots (100 µl) water and rinse samples from above were dispensed into Biolog Eco plates (Biolog Ic, Haywood, CA, USA) that were subsequently incubated at 25°C. The A490nm and OD750nm of each well was recorded every 24 h for 5 days and the Average Well Development (AWD) determined. The SCU profiles generated were compared using Principal Component Analysis.

Statistical Analysis
Profiles generated using 16S rRNA-DGGE and Biolog plates were analyzaed using a combination of Principal Component Analysis and ANOVAR. For 16S rRNA-DGGE the profiles were based on the position and number of bands. Biolog profiles were compared on the basis of the number of AWD corrected positive wells.

Continuous Wash Water Recycling Unit

Jar Test
Jar Tests were performed to optimize and evaluated different coagulating agents to aggregate organics within a simulated wash water matrix. The simulation matrix was prepared by homogenating 5g/l of cos lettuce in distilled water to give the same turbidity and solids content measured in processing water. The Jar Test was performed by placing 500 ml of lettuce homogenate within a beaker along with the test coagulant and rapidly stirring (via a magnetic stirrer) for 30 min. The stirrer was then switched off and the aggregates allowed to settle over a period of 4h. Aliquots (5 ml) were then withdrawn and the absorbance/optical density was measured at 420, 600 and 680 nm.

The coagulants evaluated were sodium aluminate, aluminum sulphate, iron II sulphate, iron III chloride and were all obtained from Sigma-Aldrich (Oakville, ON, Canada). In addition, a cationic polymer preparation from Aquarius that is commonly used for treating swimming pool water was also evaluated. Where applicable, the pH of the homogenate-coagulant solution was adjusted using 0.1 M HCl or NaOH. Samples containing no coagulating agent ware run as a control.

Taylor-Couttee Reactor
The Taylor-Couttee reactor was evaluated as a means of accelerating the coagulation step through efficient mixing.

Taylor Couette reactor was constructed in collaboration with Trojan Technologies Inc. (London, Canada) (Figure 1). The Taylor Couette reactor was mounted on a drill machine platform that served to rotate an inner quartz cylinder with a diameter of 15.5 cm and a length of 14 cm. The inner volume of the gap between the rotating and stationary cylinders was 300 ml (Figure 1). The

stationary outer cylinder was constructed from polycarbonate and sat on a stainless steel platform. The liquid to be processed was delivered to the TC unit via a peristaltic pump (MasterFlex, Thermo Fisher Scientific, Ottawa, Canada) and entered the reactor at two points on the stainless steel base. Under operational conditions the inner cylinder sat on a lubricated rubber gasket positioned below the liquid inlet. The flow rate of the liquid (i.e. lettuce homogenate and sodium aluminate solution) was modulated via the pump speed and the rotation of the inner cylinder was controlled by the rate of rotation of the machine head. The samples were collected in a 1 liter beaker and allowed to stand for 10 mins at room temperature (23°C) before determining the absorbance/optical density at 680, 600 and 420 nm.

Construction of continuous water recycling unit

The unit was installed in Facility A that processed spinach. The wash tank had a volume of 4000 liters and spent wash water was withdrawn from a pipe place approximately 1 meter from the based (Figure 2). The water was circulated via a centrifugal pump operating at 20 liters per min. The recycling unit consisted of a coagulant holding tank (20 liters) containing the appropriate concentration of sodium aluminate (Sigma-Aldrich) then dosed the coagulant into the water flow stream at 100 ml/min via a peristaltic pump. The water then fed into a static mixer (7.5 cm diameter, 30 cm length with 14 elements) then into the base of a 470 liter holding tank (Figure 2). The water was then passed through 3 x 21 bag filtration units (300 µm mesh) prior to traversing through an 40 liter/min capacity activated carbon unit (Figure 2). The pressure within each unit was measured by gauges mounted on top of each filter. The final stage of water disinfection was achieved through passing the water through a 6 x 12W UV (254nm) unit before being returned to the wash tank. A sampling port was placed just prior to the water entering the tank and used to collect samples for analysis (turbidity, residual aluminum, conductivity, pH and microbial counts).

Results

Objective 1: Determine the efficacy of fresh produce washing within commercial processing facilities.

The main objective of this part of the study was to identify produce and/or wash water parameters that impact on the log count reduction of the wash process. A secondary objective was to determine if a single or combination of wash water parameters could be used to predict the log count reduction achieved by the post-harvest wash process.

Sampling trials have been performed at 3 fresh cut processors (2 processing salad spinach; Facility A & B; and the other shredded lettuce Facility C). Each facility was visited on at least 4 different times over the course of a 3 month period.

Facility A processed spinach which was washed in 5-10 ppm peroxyacetic acid which was not changed during the production period. Facility B processed spinach using an Oxidation Reduction Potential (ORP) controlled hypochlorite wash with constant re-charging of water.

Facility C processed lettuce using a hypochlorite ORP system with also constant re-charging of water. A common finding in all facilities was the strong correlation that existed between initial loading (Aerobic Colony Counts; ACC and coliforms) and Log Count Reduction. In Facility A the bacterial loading increased progressively throughout the processing period that led to high loading in the water.

From comparing all the microbiological data collected it was found that on average spinach had a higher TAC and coliform count compared to lettuce (Figure 3). The washing process supported a significantly ($P>0.05$) higher log reduction in bacterial counts with lettuce compared to spinach. This translated into spinach having a higher loading on the final product compared to lettuce.

Correlation analysis was performed to determine which (if any) of the measured parameters impacted on the Log Count Reduction achieved. There was a significant correlation between the TAC LCR achieved and the pre-loading on produce at pre-wash for both lettuce and spinach. However, the LCR of coliforms was not found to significantly correlate to initial loading on the leafy greens (Table 1).

Of the wash water parameters it was found that there was no significant correlation between the HPC and LCR in all three processing facilities visited. However, there was a significant correlation between the coliform counts associated with post-washed spinach and loading within the wash water. A negative correlation was found between TAC of the wash water and the pH (Facility A) or ORP (Facility B, C). This may have been expected given the pH (peroxyacetic acid) and ORP (chlorous acid concentration) are indirect measures of active sanitizer concentration within the wash water. However, there was no significant correlation between pH and ORP on the log count reduction in TAC. High temperature of the pre-wash spinach negatively affected the LCR in Total Aerobic Counts. No correlation was found between the LCR and impedance, oxygen consumption, conductivity or turbidity of the wash water. Yet, there was a correlation between the bacterial loading (heterotrophic plate count and coliforms) and the measured water conductivity.

Objective 2. Determine the cross-contamination events occurring during commercial wash processes

Studies were undertaken to determine the dynamics of microbial populations associated with produce (spinach and lettuce) and wash water over the course of processing with the aim of identifying diversity, persistence and cross-contamination events. The quantitative indexes of specific interest in population dynamics are the evenness (abundance of microbial types), diversity and richness (i.e. number of different microbial types). In general, the diversity of populations was not different between water, unwashed, prewashed and washed vegetable ($P>0.05$) at both facilities (Facilities A and C) except prewashed spinach at plant A five hours into production was significant different from that earlier in the processing period. The results would confirm that microbial populations were accumulating within the wash water as processing continued (Figure 4 and 7).

The diversity of raw lettuce entering the line of Facility C was constant throughout the processing activity indicating that microbiological populations were common to all units within

the batch (Figure 4). In some respects this could be expected given the lettuce was sourced from the same supplier and likely harvested at the same time. The diversity of microbial populations on pre-wash lettuce (i.e. lettuce that had been passed through the shredder unit) was lower than the raw material in the early part of processing but then increased towards to end of the processing activity (Figure 4). The results would suggest that the microflora was being transferred from the lettuce in the course of the shredding operation but then deposited on incoming material as contamination accumulated. The diversity of populations within the pre-wash tanks remained static over the initial period of processing but then increased towards the end of the activity. The diversity of microbial populations was greater than that of the pre-wash lettuce indicating that contamination accumulated in the tank despite operating a constant ORP system and re-charging the tanks continuously with 20% fresh water every hour. The biocidal wash tank harbored a lower diversity of microbial populations although this increased as the processing continued-a similar trend to the pre-wash tank (Figure 4). Interestingly, the increased diversity of microbial populations within the biocidal wash tank was mirrored to that of lettuce entering and leaving the tank. Such as trend in diversity indicates that microbes were being constantly introduced into the wash tank and being deposited on lettuce during the washing process. An alternative theory is that lettuce contaminated in the pre-wash tank was carried through the biocidal wash with microbial populations being deposited into the water from the leafy greens.

The different 16S rRNA patterns of the various samples were compared using Principle Component Analysis (PCA) that essentially provides a graphical representation of similarity (figure 5A). The PCA illustrated was constructed from comparing profiles from one days processing although the same trends were observed with sampling over multiple visits.

It was found the no specific clustering took place with a high degree of scattering between raw product, pre-wash and post-wash lettuce, in addition water within tank 2. However, it was noted that the profiles within Wash Tank 1 were different from the other profiles with additional bands observed on DGGE gels. The results would confirm that a proportion of the microflora on lettuce was carried through the process and an exchange in bacterial populations occurred with wash water (Figure 5A).

A further representation of the 16S rRNA population profiles is in the form of a dendrogram that clusters groups based on the degree of similarity. From comparing the different profiles taken from lettuce and wash water it can be observed that different clusters existed (Figure 6). In one such cluster there was a close relationship between profiles of washed lettuce, pre-washed lettuce and the wash tank water. The results provide strong evidence for deposition of bacteria from lettuce and persistence of microflora within the wash tank that subsequently is disseminated to subsequent batches of product. That is, bacteria deposited in the water persisted over extended time periods and could be recovered on washed lettuce (Figure 6). The other profiles presented within the dendrogram were less similar reflective of the different diversity of the samples. Yet, it can be observed that population profiles on starting material could be found subsequently recovered in water.

The main assumption of using diversity to demonstrate cross-contamination and persistence is that the bacterial populations are transferred as a collective. However, it is likely that different microbial types would exhibit a spectrum of persistence in wash water and attachment strength to produce.

Ribotyping (genetic typing) is a sensitivity and discriminating technique for establishing the diversity of bacterial populations. However, an additional technique referred to as Sole Carbon substrate Utilization (SCU) can be used to profile populations based on physiological traits. Here, Biolog Ecoplate plates are applied that have a panel of different substrates consisting of sugars, acids, amino acids, polymers and surfactants (Table 2). When inoculated with samples and incubated the ability of the microbial populations to utilize the substrates is illustrated by reduction of a redox dye. A profile of the sample is generated by determining which substrates are metabolized by the resident microflora. The profiles are compared using Principle Component Analysis (PCA) and graphically presented to illustrate the relatedness of the microbial populations present.

The SCU profiles obtained from the lettuce and water samples formed different cultures suggesting different populations existed (Figure 5). Specifically, profiles associated with the incoming lettuce exhibited a high similarity to those recovered from pre and post-washed lettuce (Figure 5). The results suggest that microbial populations were carried through the process. Yet, profiles on pre-washed lettuce were also recovered from those from water in the first wash tank. The result would confirm that a degree of exchange between lettuce and water occurred (Figure 5). Interestingly, the populations within the second wash water were distinct from those recovered from lettuce or the first wash tank. By reviewing the different substrates utilized by populations recovered from the water and lettuce it was noted the former metabolized organic acids, in addition to amino acids (Table 3). In comparison, the populations derived from lettuce samples had a preference for sugar alcohols that are commonly encountered in plant material. The significance of the different carbon utilization profiles between water and lettuce are unclear although may indicate that physiological factors may influence if a microbe is retained on leafy greens or released into the water.

Spinach

In Facility A the diversity of microbial populations based on 16S rRNA ribotyping, on the raw material was high but decreased up to the point of entering the wash tank (Figure 7). The results would suggest that microflora from the spinach as being deposited onto contact surfaces such as conveyors and the de-stoning unit. The diversity of populations within the wash water progressively increased during the processing period indicating that microflora was accumulating within the first wash tank over the processing activity (Figure 7). The same trend was observed for wash water in the second tank indicative of contamination being transferred from the first tank via the spinach. The actual diversity of microbial populations on the post-wash spinach was the same as observed for the pre-wash samples. The results would suggest that negligible exchange of microflora was occurring during the wash process.

The profiles obtained from 16S rRNA DGGE and SCU analysis were compared using PCA (Figure 8A). The 16S rRNA profiles indicated that populations associated with the incoming spinach clustered together confirming that the batches shared common bacterial populations. Inter-dispersed within the cluster were profiles recovered from pre-washed, post-washed spinach, in addition to wash water. The results would suggest that bacterial populations are carried through the wash process and deposited in the water. However, in the main the bacterial population profiles associated with wash water and post-washed spinach were dispersed and did not cluster to any great extent. Still, the similar profiles of wash water and post-wash spinach

would indicate an exchange of bacterial populations had occurred.

From SCU profiles it was again interesting to note that similar to lettuce, the profiles generated from wash water samples could be separated from those obtained from spinach (Figure 8B). From closer examination of the plates the populations in water metabolized a narrower range of substrates compared to those recovered from spinach. L-Arginine was the only carbon source that could be utilized by isolates in the water but not those recovered from spinach. Again the relevance of such differences remains unclear.

In summary, the diversity of bacterial populations associated with leafy greens and wash water revealed differences between spinach and lettuce. With lettuce it was evident that the diversity on post-washed lettuce was correlated to high diversity on the pre-wash samples and that encountered in wash water. In the case of spinach, the diversity of bacterial populations associated with post-wash samples appeared independent of that associated with the wash water. Specifically, the results suggest that bacterial populations were deposited in the wash water more than acquired.

Attempts were made to verify the findings through genotyping E coli recovered from produce and wash water. However, the prevalence of E coli recovered in the sampling trials were insufficient to generate DNA fingerprint patterns to establish a distribution pattern. Alternative markers were evaluated (i.e. *Aeromonas* and *Pseudomonas*) although neither proved reliable.

Objective 3 & 4
Developing and validating an effective wash water recycling system

Baseline studies evaluated a water treatment system composed to 3 x 40 liter capacity bag filters (300 µm pore size) that fed filtrate (250 liters/min) into a UV unit consisting of 6 x 12W lamps (254 nm). The spent wash water was fed from an inlet pipe position at mid-way depth of the tank (capacity 500 liters) and introduced the treated water at the base.

Preliminary trials were performed at the end of a 6 h processing period where approximately 3000 kg of spinach had been processed. The water entering the recycling unit had a Total Aerobic Count (TAC) of 4.2 cfu/100ml with a coliform count of 3.1 log cfu/100 ml. After passage through the recycling unit the TAC and coliform counts were reduced by 1.2 and 2.1 log cfu respectively. The limited log reduction was attributed to the relative high turbidity of the water that did not significantly change during the recycling process.

Further trials were undertaken to evaluate the efficacy of the recycling unit to maintain low microbial counts in the wash water. The TAC in the water over a typical processing period was 3.28±0.72 log cfu/100 ml with constant recycling was not significantly ($P>0.05$) different to compared to when the filtration/UV was not applied (3.0±0.63 log cfu/100 ml). The log count reduction of bacterial counts on spinach when the recycling unit was operating was not significantly different ($P>0.05$) compared to when the filtration/UV was not running being limited to <1 log cfu/g. The results of the trials clearly illustrated that using a combination of bag filtration and UV lights alone were insufficient to reduce counts in water or positively affect the log count reductions achieved. The limited efficacy of the recycling unit could be attributed to

the high solids content of the water that negatively affected the antimicrobial properties of the UV lamps.

With turbidity of the wash water identified as the key limiting factor steps were taken to enhance the filtration efficacy to facilitate the removal of organics. The common approach for removing organic content of water follows a sequence of coagulation with removal of the formed aggregates by filtration. There are a selection of coagulating agents available that vary in efficacy depending on the nature of the organic material and pH. The organics present in spent leafy green wash water consists primarily of latex and pectin, in addition to low molecular weight constituents, that form a colloid that initially requires to be disrupted prior to forming aggregates.

Coagulant selection
A series of experiments was undertaken to evaluate the efficacy of different coagulating agents to aggregate spent leafy green wash water. For this the Jar Test was performed using spent wash water from leafy green processing or a simulation matrix based on homogenated lettuce (5g/l) solution. The Jar Test is specifically designed to assess the efficacy of the test coagulation agent to precipitate organic material under different conditions.

In experimental terms, the Jar Test involves taking a sample of the wash water and supplementing different concentrations of the test coagulating agent. The solution is rapidly stirred for 30 mins then allowed to settle over a period of 3-4h. The progress of coagulation is followed by monitoring various parameters (BOD, COD, conductivity, TSS) which in the present case was turbidity.

The coagulating agents tested consisted of ferric and aluminum salts, in addition to proprietary polymer. From the results of the Jar Tests it was found that the addition of Iron III chloride at low concentrations (16.6 mg/l) was an effective coagulating agent but became less effective when added at higher amounts (Figure 9). It was also noted that the ferric chloride acidified the wash water below that typically operated in commercial operations (i.e. pH 5-7), hence was considered unsuitable for practical application. Ferric sulfate did not acidify the sample although was relatively ineffective at aggregating the organics unless added at high concentration (166 mg/l; Figure 9).

The polymer coagulant has the acknowledged advantage of being less dependent on pH compared to ferric or alum salts. This was found to be the case in the current study, however, only a negligible decrease in absorbance, indicative of low coagulation action (Figure 9).

Aluminum salts exhibited variation depending on the type tested. Al sulfate alkalized the water to pH 9.0 that did result in coagulation of organics although this was attributed to the pH as opposed to the action of Al. When the pH was adjusted to pH 5.0 (optimum for Al salts) the degree of coagulation was effective at low Al sulfate concentrations but decreased in efficacy at higher levels of the coagulant (Figure 9). Similar to ferric salts, at high concentrations of coagulant the aluminum sulfate stabilized, as opposed to dispersing the colloid. Sodium aluminate was found to be the most effective coagulating agent especially when the sample was adjusted to pH 6 (Figure 9). At more acidic pH (pH 5.3) a similar decrease in absorbance was

observed although illustrated a concentration dependency (Figure 9). Importantly did not result in colloidal stabilization when applied at high levels (Figure 10). Therefore, sodium aluminate was taken forward for further studies.

Reducing the time of the coagulation step

It is standard practice in water treatment to add the coagulating agent and allow the process of aggregation to take place over extended time periods (>24h). However, for continuous water recycling systems there is a need to reduce the time for coagulating organics given that extended settling times are unfeasible. To reduce coagulation times the two current options are to immobilize a layer of the coagulant on a multi-media filter surface then apply high pressure to pass the water through the filter and promote coagulation. As the water passes across the layer the organics present are coagulated and trapped within the filter matrix. An alternative approach is to ensure rapid mixing of coagulant into the water thereby enhancing the process through increasing the interaction between the developing aggregates. In terms of commercial feasibility, the latter technique is preferred given the expense and maintenance needs of high-pressure filter reactors.

Trials were performed by preparing the lettuce homogenate (5g/l) that was supplemented with sodium aluminate (0.22 mg/l) prior to passing through the Taylor Couette reactor operating at various rotation rates. The homogenate was collected in a beaker and allowed to stand at room temperature (23°C) for up to 10 mins with aliquots (1 ml) being withdrawn periodically for Absorbance (680nm) determination.

The decrease in absorbance (680nm) was found to be independent of the residence time (i.e. flow rate) within the reactor (Figure 11). The decrease in absorbance occurred with two minutes compared to over 4h when allowed to sediment out without prior mixing.

Rotation rate had an effect on the coagulation process with 150 rpm being optimal (Figure 11). The results can be interpreted on the basis of flow pattern within the reactor. At rotation rates below 150 rpm the flow is laminar that transitions into vortices and then into turbulent flow at >200 rpm. Therefore, it can be confirmed that efficient mixing results in accelerated coagulation compared to under static conditions.

Filtration Method

With the coagulation step optimized the next phase evaluated a range of filtration techniques for removing the formed aggregates, in addition to the low molecular weight constituents. To this end trials were performed using a multi-media sand filtration using layers of sand, carbon and gravel. Here, the sand acted as a physical matrix to filter out the aggregates with the carbon layer removing the charged low molecular weight compounds along with unbound coagulating agent (sodium aluminate). The gravel layer was simply to support the upper layers and prevent leaching of the bed into the filtrate flow stream.

When optimizing sand filtration a balance must be made between the efficacy to remove organics vs flow rate. Specifically, the greater the bed depth the more efficiently the organics are

removed but at the expense of slow filtration rates. Therefore, experiments were undertaken to optimize the sand filter configuration by varying the sand and carbon media depths.

The laboratory scale filter unit consisted of a measuring cylinder into which was added layers of drainage and separation grave. The carbon layer (6-14 mesh) of activated carbon was placed on top of the gravel bed followed by sand (50-70 mesh) (Figure 13). The bed was conditioned by flowing 10 liters of municipal water followed by 1 liter of homogenated lettuce (5g/l) previously treated with sodium aluminate.

From the results obtained from the different sand filter configurations it was evident the depth of both the carbon and sand layer within the range used did not significantly affect the efficacy of the filtration process (Table 3). The different absorbance readings corresponded to the constituents within the water. The insoluble aggregates were measured at 600 – 680 nm) with the soluble low molecular weight compounds being determined at 420 nm. As observed, the combination of sand and carbon achieved significant reductions of the organics present in the coagulated lettuce homogenate sample.

Conductivity related to the charged solutes and polymeric materials within the water. As expected the depth of carbon or sand did not have a significant effect on the conductivity of the filtrate given that both constitute ion exchange matrices as opposed to stripping ions from solution.

With the multi-media sand filtration conditions required to filter the aggregated organics out of the homogenated sample the aim was to scale-up the process to be compatible with the pilot plant trials. It was calculated that to treat the 4000 liters within the wash tank a filter unit of 60 cm diameter and 1 meter depth would have been required. However, to backwash a filter of this size would require at least 5000 liters that would have countered the benefits of implementing the recycling system. Consequently, alternative methods were investigated to achieve the rapid separation of coagulated organics from the wash water matrix.

Commercial trials

Based on the results of the laboratory trials a prototype water-recycling unit was installed in the collaborating partners processing plant. The unit could treat 20 litres/min and is depicted in Figure 2. The coagulation agent is introduced into the wastewater stream that then passes through a static mixer to a retention or holding tank (Figure 2). The coagulated materials sediment to the bottom of the tank with residual material being removed by a series of three mesh filters (300 μm). The filtrate is passed through a carbon filter primarily to remove non-reacted coagulant that can be potentially toxic at high levels. The final UV disinfection step is to inactivate microbes prior to returning to the wash tank (Figure 2).

Trials were performed following the end of processing given the safety risks of introducing alum coagulant into the wash tank that could have potentially contaminated the spinach product. The trials were initiated by pumping wash water through the system with samples being taken from the tank and at a sampling port leaving the UV unit prior to reentering the tank. Samples were taken over a 35 min period in which 700 litres of the 4000 liters within the tank was processed.

A series of trials were performed using different coagulant concentrations in the feed solution. The sodium aluminate was introduced into the water flow stream at 100 ml/min that equated to 0.022 mg/l, 0.002 mg/l and 0.0002 mg/l using a feed solution containing 25g/l, 2.5g/l and 0.25g/l respectively. When applied at the highest concentration the turbidity was decreased to below 10 NTU within 15 mins that was maintained for duration of the run (Figure 14). Lower coagulant concentrations also decreased to below 10 NTU although the starting turbidity half of that in the trials using 25 g/l sodium aluminate feed solution (Figure 14). The results suggested that the coagulation step was effective and disrupting and precipitating organic material within the wash water. This was confirmed by inspection of the residue retained in the holding tank and so captured by the mesh filters (Figure 15). The residual aluminum in the treated water was negligible when 2.5 or 0.25 g/l was used in the feed solution although did spike above the permitted level when applied at the higher sodium aluminate concentration (Figure 16). Yet, levels of Al residues decreased to basal levels after 35 min (Figure 16). The conductivity of the treated water was not significantly different regardless of the coagulant concentration used (Table 4). The pH of the treated water was more alkaline when 25g/l sodium aluminate was applied although within the neutral range to prevent the precipitation of Al (Table 4). The HPC within the wash water tank was reduced by 2 log cfu with no *E coli* or coliforms being detected (Table 4).

Outcomes and Accomplishments

- Identification of factors that effect the efficacy (log count reductions) of post-harvest washing of leafy greens.

- Confirmation of the carriage and cross-contamination events that occur during the post-harvest washing process.

- Water recycling technology that can be applied in SME operations to support continuous wash water treatment.

Conclusions correlating wash water parameters

- Lettuce harbors lower microbial loading and can be more efficiently washed compared to salad spinach.

- The LCR achieved by a wash process is largely defined by the initial loading on the incoming raw material.

- Although Facility B had better wash water quality (i.e. lower bacterial loading, maintaining ORP, re-charging tanks) the LCR obtained was not significantly different compared to Facility A (no ORP, high bacterial loading in the water, no replenishment).

- The heterotrophic plate count of the wash water did not significantly impact on the LCR obtained suggesting the HPC is a poor measure for predicting wash efficacy. High levels of coliforms within the water did correlate to lower LCR achieved by the wash process.

This would suggest the interaction (attachment) of coliforms to fresh produce differs from the other microflora found in wash water.

- Maintaining sanitizer concentrations (ORP, pH), maintaining cold temperatures and ensuring low bacterial loading on the raw material all act positively to enhance the efficacy of the wash process.

Cross-contamination events during leafy green processing

- The diversity of bacterial populations encountered on incoming lettuce was consistent between batches.

- The diversity of microbial populations decreased in the course of the shredding process by increases during the wash process.

- Evidence was obtained to suggest there was an exchange of microflora between shredded lettuce and wash water. Although the chlorine concentration was maintained to give an ORP of 650mV and the tanks were recharged with 20% fresh water, persistent populations existed in water, in addition to accumulating as processing continued.

- The results from Substrate Carbon Utilization (SCU) profiles indicated that a proportion of microbial populations were more likely to persist in wash water compared to those that are retained on the shredded lettuce.

- Bacterial populations present on incoming spinach were highly diverse but decrease up to the point of washing. In the course of washing the microflora of spinach leaves was deposited within the water more so that accumulating on the leaves.

- SCU profiles suggested that different microbial populations were encountered in the wash water compared to on spinach.

Water recycling of spent wash water from leafy green processing

- The organic material within spent wash water derived from leafy greens is colloidal in nature and readily blocks mesh filters unless a preliminary coagulation step is performed.

- From the coagulating agents tested, sodium aluminate was superior in terms of coagulating organics at low concentration (0.022g/l) at the normal pH of the wash tank (pH 5-6) and did not lead to high turbidity when added in high concentrations.

- Coagulation time to form aggregates of organic material by the addition of sodium aluminate could be accelerated by rapid mixing using either Taylor Couette reactor or a static mixing element.

- Commercial trials were performed using a unit combining an initial coagulation step, filtration and UV disinfection step. The unit could reduce the turbidity and microbial counts within wash water without resulting in excessive Al residue in the wash tank.

Summary of Findings

The overall objective of the research was to establish which parameters associated with the wash water process could be used as a metric to predict the log count reduction achieved, in addition to identifying cross-contamination events during the wash process and finally develop methods to ensure consistent washing of leafy greens by water recycling. The results from the study illustrated that the log reduction from the wash process was dependent on the initial loading on the leafy greens, low temperature and conductivity (charged solutes content). Although the LCR was independent on the microbial loading of the water it was noted that carriage of coliforms on the post-washed produce was dependent on the levels encountered within the wash tank. The results are consistent with the finding of the microbial population studies that illustrated that not all microbes are deposited in the wash water and disseminated to other batches via cross-contamination events. Although coliforms appear to be more readily disseminated via wash water it is unclear if the findings can be extrapolated to the behavior of pathogens such as *E. coli* O157:H7 or *Salmonella*.

It was noted that lettuce appeared to accumulate a greater degree of microflora from wash water compared to spinach despite the latter having a rough surface topography. There was tentative evidence that the different members of the leafy greens microbiota differed with respect to remaining on the leafy greens or being deposited in the water. Although not studied to any great extent, it was interesting to note the microflora residing on lettuce could utilize plant related sugar alcohols those in the wash water metabolized organic acids.

A water recycling system was developed based on an initial coagulation step followed by filtration and a final UV disinfection step. Through optimization trials the coagulant of choice was sodium aluminate and could rapidly coagulate the colloidal organics within spent wash water when combined with vigorous mixing. Commercial trials verified the performance of the water-recycling unit to decrease the organic loading in the wash water and microbial counts.

Recommendations

The study has illustrated that there is no one parameter that can be used to monitor or maximize the efficacy of the wash process. Importantly, the concentration of sanitizer within wash water was not found to be a reliable indicator to predict the log count reduction obtained. Yet, ensuring that the wash tank water is low and contains a degree of charged solutes (conductivity) enhance the wash process. Regardless of this fact it was evident that the wash process is limited in reducing populations on leafy greens but does pose a risk of cross-contaminating batches. Therefore, future research should focus on implementing effective decontamination technologies following the wash process. Cross-contamination events within the wash tank can be minimized by continually refreshing the water used in the wash process. To this end the water recycling technology developed in the course of the research has demonstrated strong potential. The only concern is the potential of high levels of aluminum accumulating in the wash tank. In this respect an addition cation exchange step should be integrated into the system.

APPENDICES

Publications and Presentations (required)

Publications

Barrera, M, 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.

Warriner K and Namvar A (2013). Post-harvest washing as a critical control point in fresh-cut processing: Challenges and solutions. In: A handbook of best practice, innovative commercial solutions and case studies. Ed J. Hoorfar. Woodhouse Publishing. *(In Press*).

Presentations

Warriner K. Water Management and recycling. Food Seminars International. May 2012.

Zahedi, H., Dunfield, K and Warriner K (2012). Identification of cross-contamination and prediction of log count reduction (LCR) in fresh produce washing operations. OMAFRA Food Safety Symposium. Guelph, Canada.

Zahedi, H., Dunfield, K and Warriner K (2012). Processing parameters that effect the efficacy of commercial washes in leafy green processing. CIFST National Conference. Niagara, Canada (May 2012); IFT, Las Vegas (Aug 2012)

Budget Summary (required)

See attached budget summary up to Sept 2012

Tables and Figures (optional)

Keith Warriner, University of Guelph

Enhancing the efficacy of fresh produce washing operations through establishing monitoring methods and water disinfection technologies based on a combination of filtration and UV

Figure 1: Taylor Couette reactor.

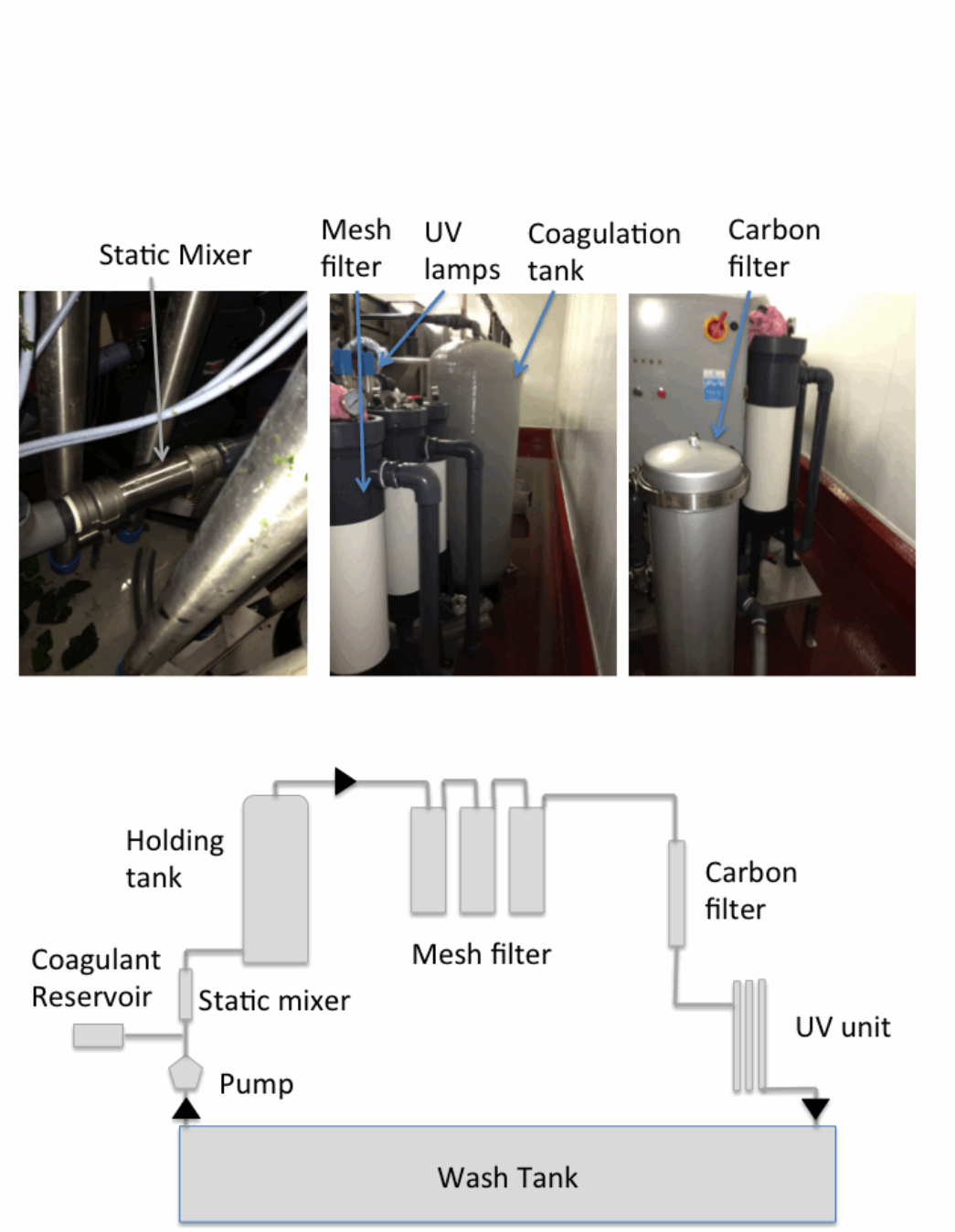


Figure 2: Schematic diagram of the water recycling system

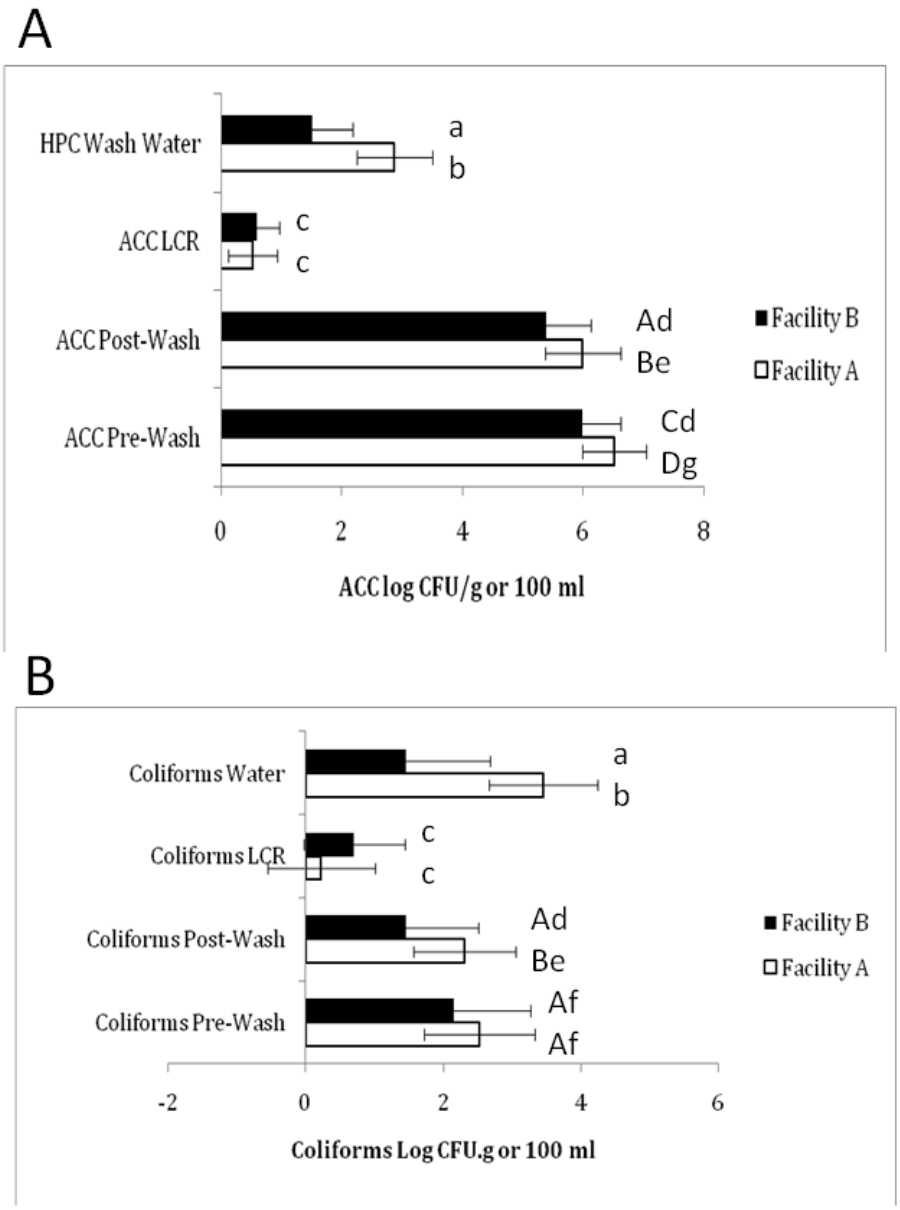
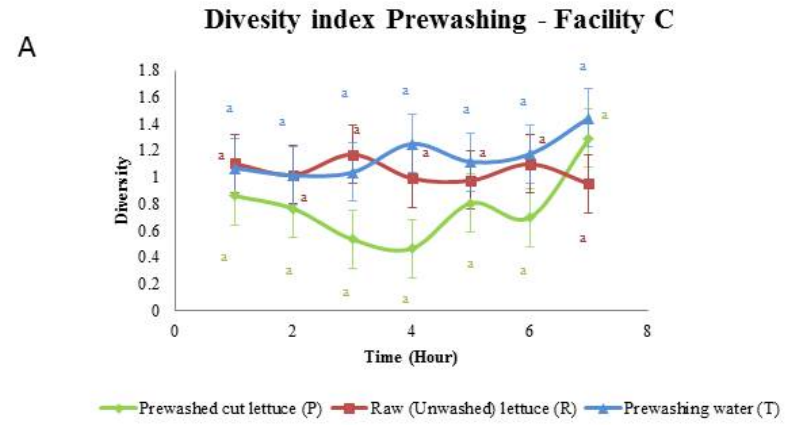


Figure 3: Average Total Aerobic Counts (TAC) and Heterotrophic Plate Counts (HPA) of produce and wash water samples collected from the participating facilities. (Facility A and B: Spinach Facility C: Shredded lettuce).

Table 1: Correlations between the measured parameters of spinach and wash water with the log count reduction in aerobic colony counts achieved by the wash process in Facility A and Facility B.

Parameter	**Facility A**		**Facility B**	
	Regression Constant	**Probability**	**Regression Constant**	**Probability**
Pre-wash produce count	0.40	0.008[a]	0.13	0.006[a]
Water Heterotrophic Count Plate	-0.04	0.79	-0.19	0.319
Water temperature	0.22	0.13	-0.14	0.488
Pre-wash produce temperature	0.18	0.26	-0.41	0.030[a]
Post-wash produce temperature	0.27	0.90	-0.52	0.005[a]
Water oxidation-reduction potential	-0.04	0.80	-0.19	0.324
Water conductivity	0.34	0.019[a]	-0.17	0.400

[a]Probability values in italics designates significant correlation between parameter and log count reduction



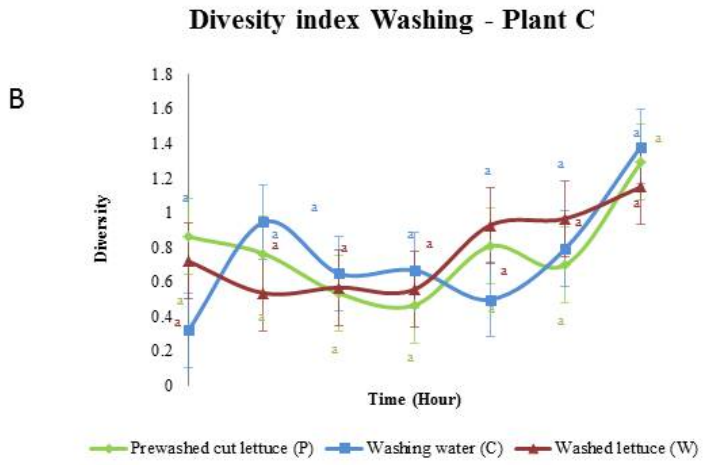


Figure 4: Diversity index of bacterial populations recovered from lettuce rinse and wash water samples as determined from 16S rRNA-DGGE profiles. Samples were taken up to (A) and following the biocidal wash step (B). Data points followed by the same letter do not significantly differ.

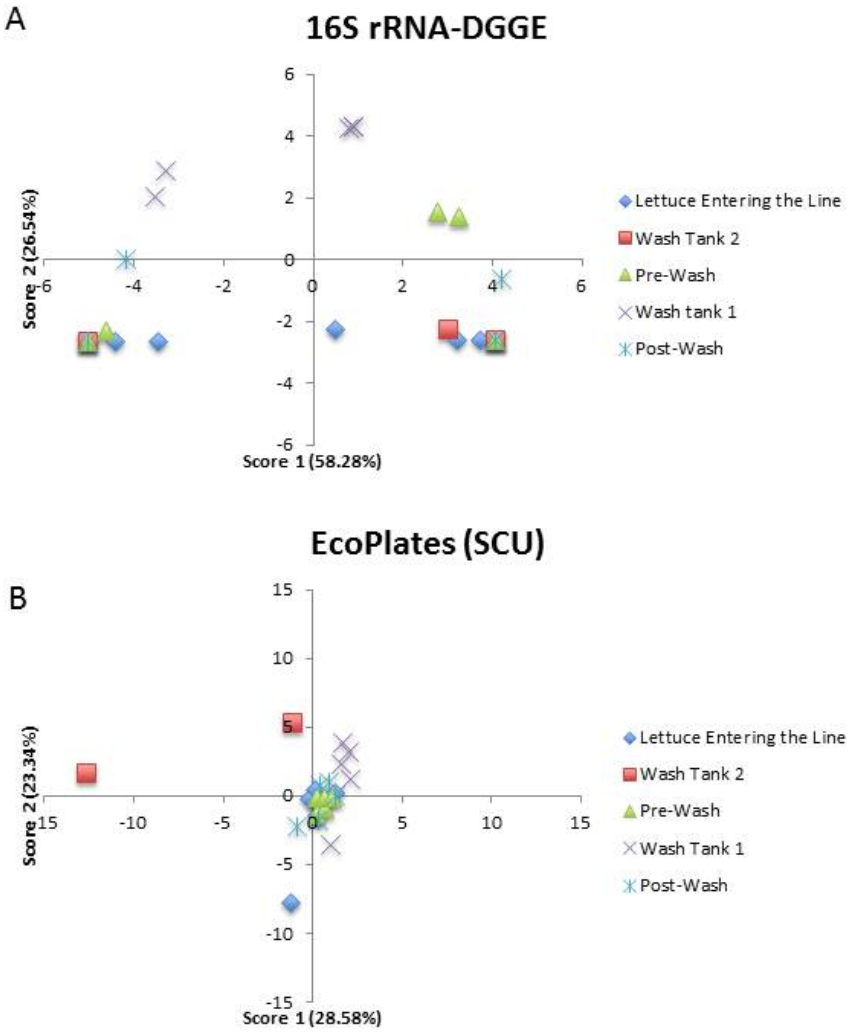


Figure 5: Principle Component Analysis (PCA) of 16S rRNA-DGGE profiles (A) and Substrate Utilization Profile (B) obtained from lettuce rinse and water samples taken at different points during processing.

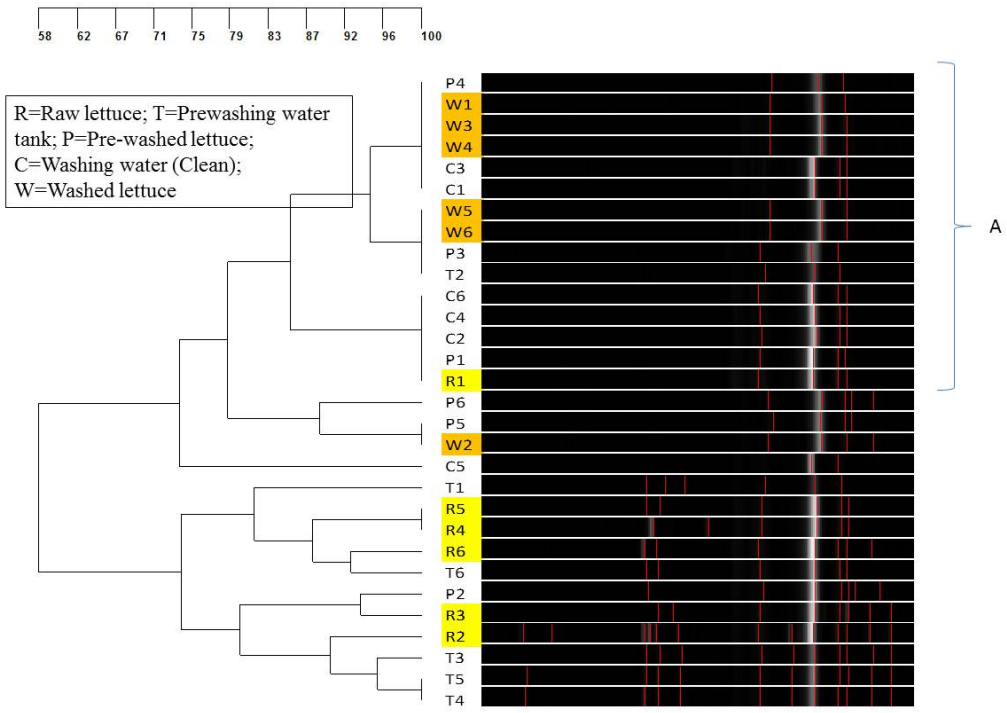


Figure 6: Dendrogram of 16S rRNA-DGGE profiles obtained from lettuce rinse and water samples taken over the course of a 7h processing period.

Table 2: Substrates used in the Ecoplate Substrate Carbon Utilization (SCU) profile.

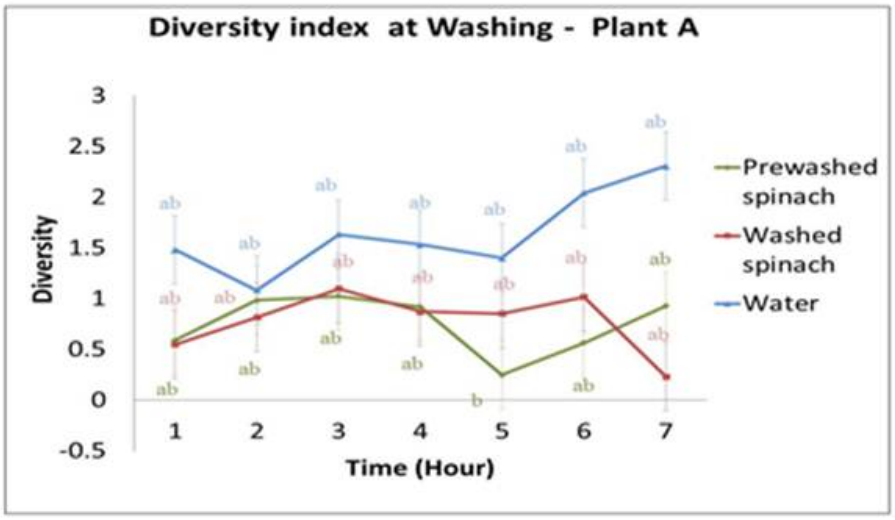
Carbon Source *
Methy-D-Glucoside1
D-Galctronic acid lactone (CA)2
L-Arginine (AA)3
Pyruvic acid methyl ester (CA)4
D-Xylose (CH)5
D-Galacturonic acid (CA)6
L-Asparagine (AA)7
Tween-40 (PO)8
L-Erythritol (CH)9
2-Hydroxy benzoic acid (CA)10
Phenylalanine (AA)11
Tween-80(PO)12 D-Mannitol (CH)13 4-Hydroxy benzoic acid (CA)14
L-Serine (AA)15
Cyclodextrin (PO)16
N-Acetyl-D-Glucosamine (CH)17
Hydroxybutyric acid (CA)18
L-Threonine (AA)19
Glycogen (PO)20
D-Glucosaminic acid (CA)21
Itaconic acid (CA)22
Glycyl-L-Glutamic acid (AA)23
D-Cellobiose (CH)24
Glucose-1-phosphate (CH)
Ketobutyric acid (CA)
Phenylethylamine (Am)
D-lactose (CH)
DL-Glycerol phosphate (CH)
D-Malic acid (CA)
Putrescine (Am)

*CH: Carbohydrate; PO: Polymer; CA: Carboxylic acid; AA: Amino acid; Am: Amine/amide.

Table 3: Differences in carbon source utilization by microbial populations in spent wash water and those on post-washed leafy greens.

Commodity	Carbon and Energy Source	
	Spent wash-water	Post-wash Produce
Shredded lettuce	Pyruvic acid methyl ester	Cellobiose
	Glycogen	Methyl-D-glucoside
	Glycerol phosphate	Xylose
	Galacturonic acid	Erythritol
	Hydroxybutyric acid	Mannitol
	Ketobutyric acid	4-hydroxy benzoic acid
	Malic acid	Phenylethylamine
	Arginine	Glucosamine
	Phenylalanine	
	Threonine	
Baby spinach	L-arginine	Tween 40
		Tween 80
		Cylodextrin
		Glycogen
		Cellobiose
		Lactose
		Methyl-D-glucoside
		Xylose
		Erythritol
		Mannitol
		Glucosamine
		Glucose 1-phosphate
		Glycerol phosphate
		Galacturonic acid
		4-hydroxy benzoic acid
		Hydroxybutyric acid
		Itaconic acid
		Asparagine
		Serine
		Glutamic acid

A



B

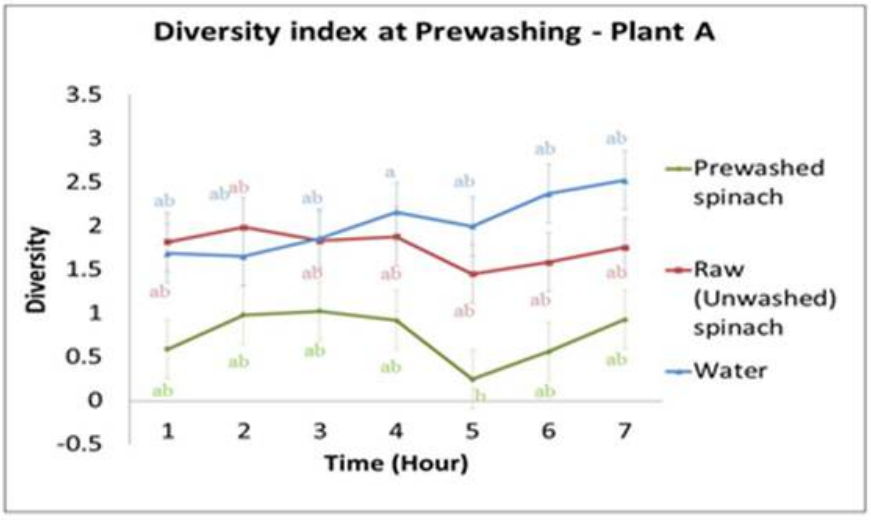


Figure 7: Diversity index of bacterial populations recovered from spinach rinse and wash water samples as determined from 16S rRNA-DGGE profiles. Samples were taken up to (A) and following the biocidal wash step (B). Data points followed by the same letter do not significantly differ.

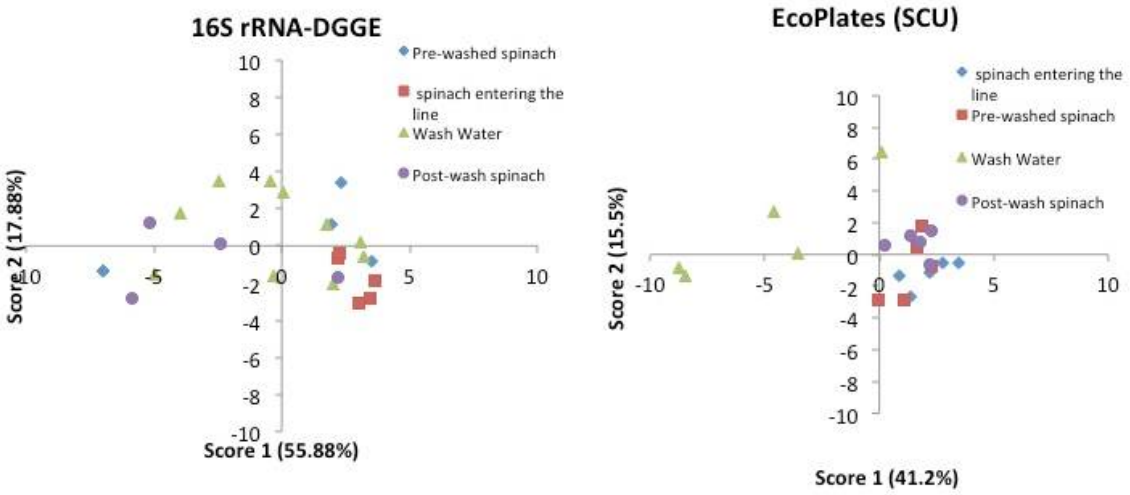


Figure 2: Principal Component Analysis of 16S rRNA-DGGE and SCU profiles obtained from the microbial profiling of samples collected at Facility A processing baby spinach.

Figure 8

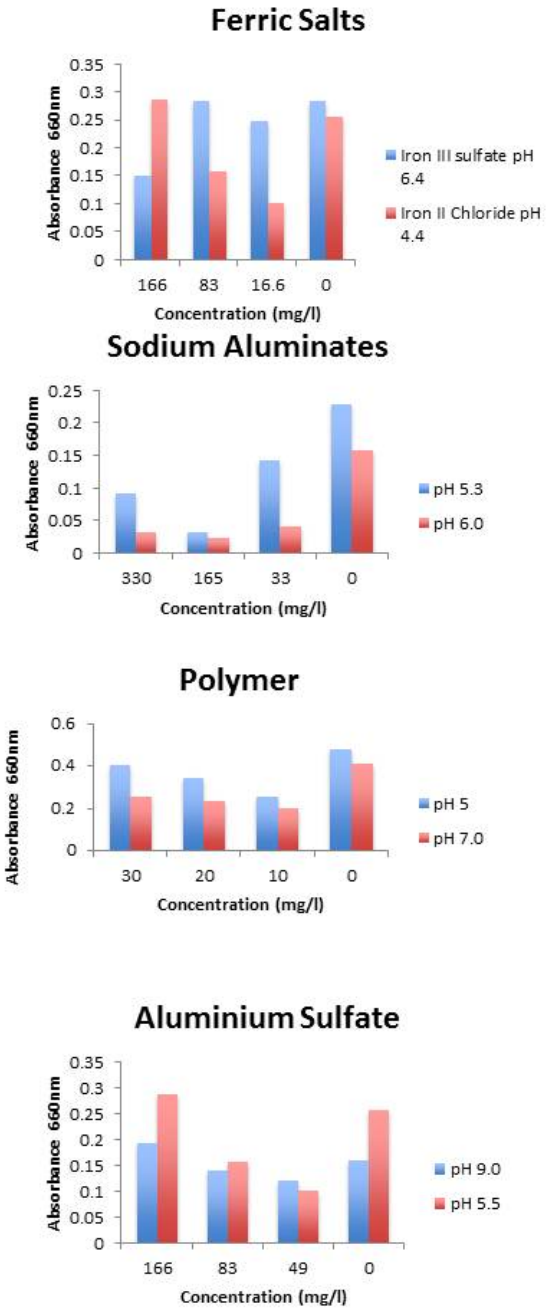


Figure 9: Efficacy of different coagulating agents to aggregate organics present in lettuce homogenate (5g/l). The Jar Test was performed by introducing the coagulating agent into 500 ml of homogenate and adjusting the pH if nessesary. The solution was stirred for 30 mins and then allowed to settle at room temperature for 30 min. Aliquots (3 ml) were then removed to determine the Optical Density at 660 nm.

Figure 10: Jar test illustrating the coagulation of organics within lettuce homogenate when treated with sodium aluminate.

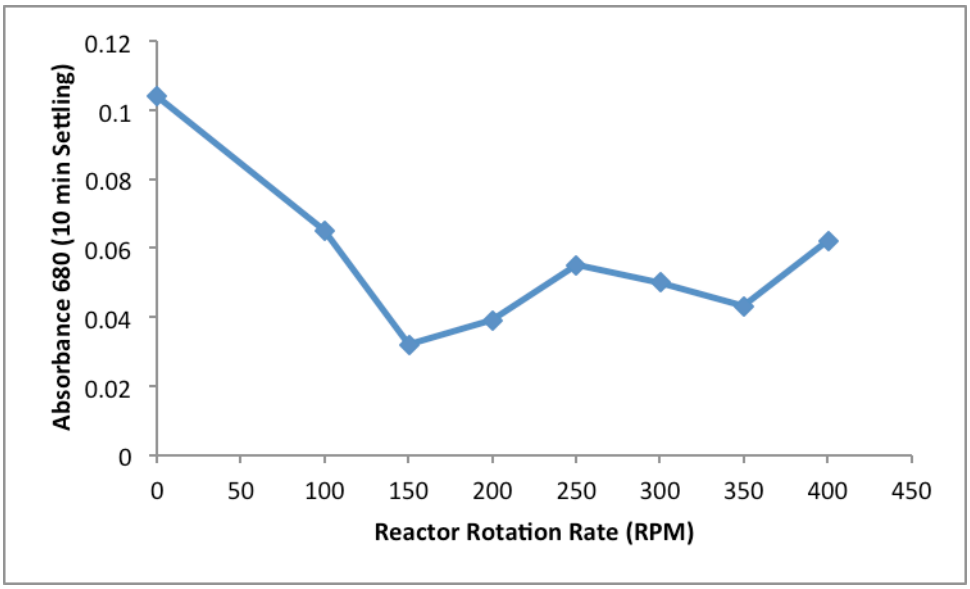


Figure 11: Effect of rotation rate of a Taylor Couette reactor on the aggregation of organics with lettuce homogenate treated with sodium aluminate. Lettuce homogenate (5 g/l) was supplemented with 0.022 g/l sodium aluminate and passed through the TC reactor (operating at different roatation rates) at 50 ml/min. The mixed homogenate was then left to sediment for 10 min before reading the optical density at 660 nm.

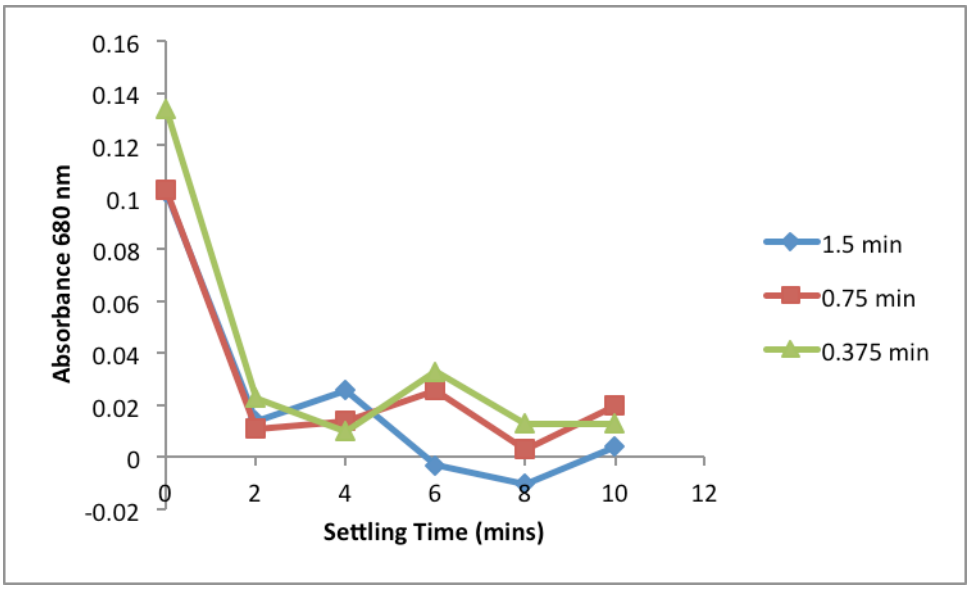


Figure 12: Effect of residence time within the Taylor Couttee reactor on the aggregation of organics within lettuce homogenate treated with sodium aluminate. Lettuce homogenate (5 g/l) containing 0.022g/l sodium aluminate was passed through a TC reactor (operating at 150 rpm) at different flow rates. The mixed homogenate was collected and allowed to stand for 10 mins prior to determining the optical density at 660 nm.

Figure 13: Laboratory scale multi-media sand filter. The filter bed consisted of a gravel layer overlaid with fine sand upon which was deposited activated carbon.

Table 4: Effect of sand and carbon depth on filtration efficacy for coagulated lettuce homogenate solution. Lettuce homogenate was prepared by homogenating 5g of lettuce per liter of distilled water. The homogenate was treated with sodium aluminate (33 mg/l) and allowed to settle over a 30 min period. The liquid was decanted and passed through the sand filter with the filtrate being collected for analysis.

Carbon Depth(cm)[a]	**A 680nm**	**A 600nm**	**A 420nm**	**Conductivity (µS)**
Homogenate sample	0.749	0.829	1.159	1611
2.4	0.014	0.021	0.031	1692
4.0	0.011	0.010	0.028	1454
6.0	0.002	0.092	0.023	1720
9.0	0	0	0	611
Sand Depth (cm)[b]				
9	0.010	0.015	0.047	1354
15	0	0	0	1360

[a]Sand filter depth (8 cm)
[b]Carbon filter depth (4 cm)

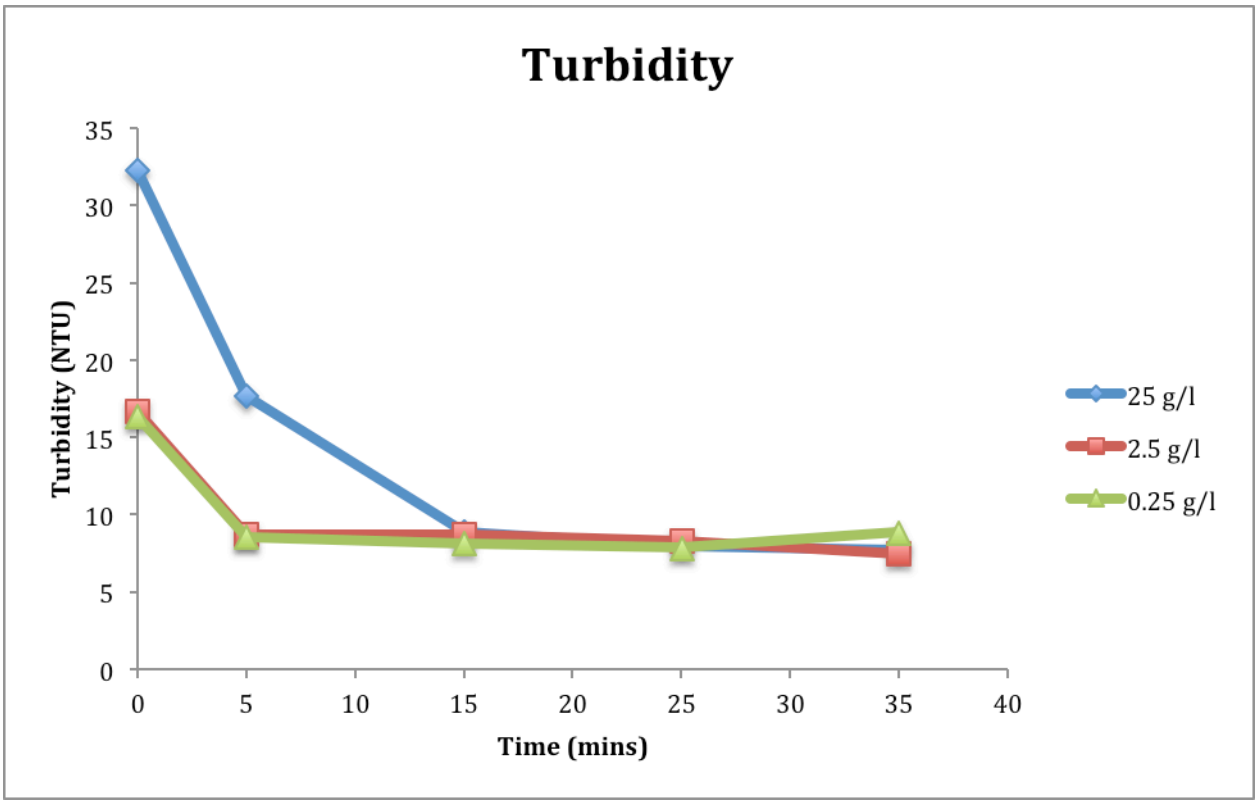
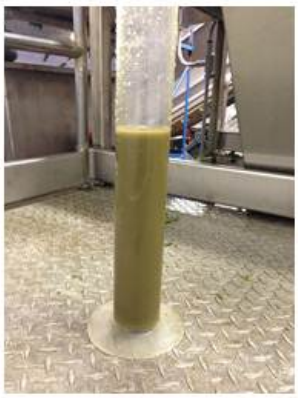


Figure 14: Turbidity (NTU) of spent wash water passed through the water recycling unit as described in the Legend of Figure 2. The sodium aluminate concentration within the holding chamber was varied between 0.25 – 25 g/l and introduced into the water stream at 100 ml/min. The water went through a static mixer into a holding tank and then through a series of mesh filters. The filtrate was passed through a carbon filter before a UV unit before returning to the wash tank. Samples were collected at the entry point of the wash tank.

Wash Water Tank



Retentate within the holding tanks

Mesh filter at the end of filtration run

Visual appearance of original water from wash tank (right) and (left) water after passing through the recycling unit

Figure 15: Pictures of the water recycling unit installed within Facility A. Illustrated is the wash tank water following a 7h processing of spinach. The water was passed through the recycling using at 20 litres/min for the trial period of 35 min. At the end of filtration run the coagulated material in the holding tank was examined (top right) along with the mesh filter (bottom left). Bottom right shows the spent wash water before and following recycling.

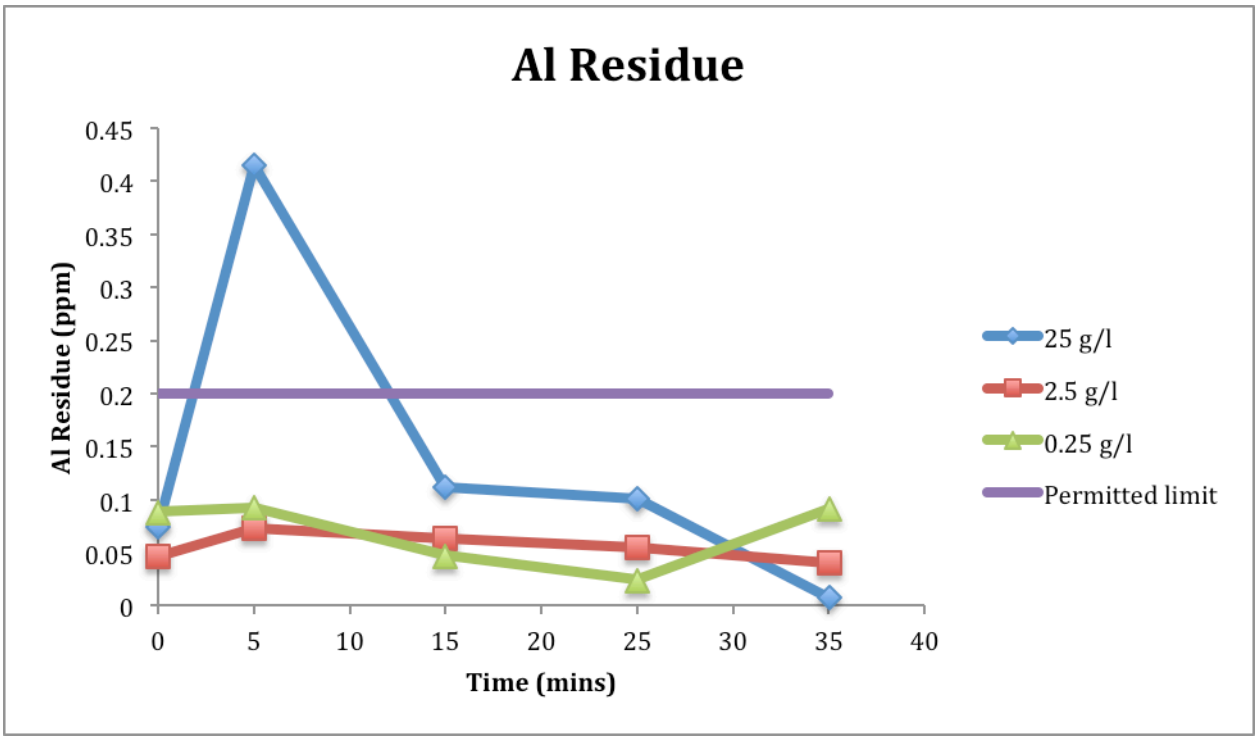


Figure 16: Residual aluminium levels within recycled water treated within the recycling unit using different concentrations of sodium aluminate coagulating agent.

Table: 5: HPC, pH and conductivity of spent processing water before and after recycling.

Sodium aluminate in feed solution	pH		Conductivity (µS/cm)		Heterotrophic Plate Count Log cfu/100 ml	
	Water Tank	Post-Treatment	Water Tank	Post-Treatment	Water Tank	Post-Treatment
25	6.02	7.04	553	454	3.82	1.68
2.5	6.44	6.29	402	425	3.59	1.76
0.25	5.65	6.61	391	454	4.12	1.99

Suggestions to CPS (optional)

We gratefully acknowledge financial support from the Center for Produce Safety to undertake the project. Through such research the challenges facing the fresh produce industry can be identified and addressed. The CPS enables such fundamental to be undertaken and provides a direct route for findings to be translated to real applications.

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