CENTER for PRODUCE SAFETY

CPS 2019 RFP
FINAL PROJECT REPORT

Project Title
Post-harvest fresh produce wash water disinfection by submerged cold plasma non-chemical continuous treatment system

Project Period
January 1, 2020 – December 31, 2021 (extended to April 30, 2022)

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Objectives
1. *Construct and install a 100-gallon flow-through water tub for lab-scale testing.*
2. *Modify reverse vortex gliding arc plasmatron electrode to fit the 100-gallon tub.*
3. *Optimize existing 3-kW power supply for newly constructed electrode.*
4. *Using the microbial "cocktail" (E. coli strains: ATCC 25922, 35218, 11229, and 8739), validate disinfection efficiency and generate the operating parameter space for the plasma system.*
5. *Using increasing amount of organic load, validate plasma disinfection efficiency.*
6. *Optimize plasma system for industry-level testing, and perform testing at SmartWash Solutions facility in Salinas, CA.*
7. *Generate intellectual property, schematics, etc. for commercial prototype system.*

Funding for this project provided by the Center for Produce Safety through:
CDFA 2019 Specialty Crop Block Grant Program & CPS Campaign for Research

FINAL REPORT

Abstract
Cold plasma water treatment is a possible solution for non-thermal disinfection washing of minimally processed fresh-cut produce. The reverse vortex gliding arc plasma systems created by scientists of Nyheim Plasma Institute, Drexel University, can be used to disinfect delicate fresh produce with no adverse quality effects, low-cost operation, and no added chemicals. Drexel used its experience in commercialization of plasma water treatment for this project. Drexel optimized the existing reverse vortex gliding arc plasmatron for the specifics of the food processing plant, designed, fabricated and tested this new system in the lab, and validated the developed plasma technology with specific fresh produce (romaine lettuce) provided by an industry collaborator.

Cold plasma is proven to be effective at rapidly inactivating pathogens in water. In our study, plasma-activated water (PAW) can inactivate *Escherichia coli* O157:H7 in water for at least 7-log reduction. Higher power or longer treatment time of PAW leads to higher efficiency of inactivation. Washing spinach, kale, iceberg lettuce and romaine lettuce in PAW achieved 0.7-log to 2.7-log reductions of *E. coli* and reduce the cross-contamination between batch to batch compared to washing in tap water. PAW not only inactivated bacteria in wash water, the inactivation of *E. coli* on the surface of fresh produce also was found by staining and observing under fluorescence microscope. In addition, plasma improved the wash-out ability of water by changing the physical properties of water. In the food industry, 50–200 ppm chlorine is used as disinfectant to reduce the cross-contamination between fresh produce. Our study found plasma can improve the inactivation effect of chlorine. Higher log reduction was achieved when washing *E. coli*-inoculated spinach in PAW with 10 ppm chlorine compared to washing in 200 ppm chlorine water without plasma.

Background
Cold plasma is proven to be effective at rapidly inactivating pathogens in water. Drexel Plasma Institute team has developed a number of plasma-based water treatment technologies to meet the needs of different sources of water contamination and purification requirements. The goal of this project was to design a plasma-based wash water management system for minimally processed fresh produce to eliminate cross-contamination and reduce the aggressive and toxic chemicals usage. Specific to reuse of produce wash water system is the interference from organic load. Plasma offers oxidative and non-oxidative pathways to achieve pathogen inactivation and, as organic load increases, inactivation mechanisms switch from the dominant oxidative pathways to the non-oxidative ones.

The target of our technology is the minimally processed fresh produce industry. Specifically, we are looking to replace or reduce the use of aggressive chemicals in water washing and chilling tubs because of the associated short-comings. Chlorine (NaClO), the most commonly used chemical disinfectant, needs to be replenished periodically because its concentration declines in the presence of organic matter. Additionally, there are quality (color bleaching and off-odors) and safety concerns. Chlorine dioxide (ClO_2) has high oxidative capacity and lower organic matter reactivity and has no carcinogenic byproducts, but it is very unstable and highly explosive as a concentrated gas. It also decomposes readily in the presence of sunlight. Chlorine and ClO_2 are also inconsistent in controlling pathogen cross-contamination.

Even with emerging growth technologies such as vertical farming, hydroponics, aquaponics, etc., the threat of cross-contamination persists and thus the need for produce washing remains.

As efficacy of chlorine and other aggressive chemicals (like peracetic acid) is affected with increased organic load, new technologies for produce washing are being explored, for example UV in combination with H_2O_2, combined with surfactants or the use of "natural" antimicrobials such as curcumin or gallic acid/propyl gallate (in combination with other methods). Cold plasma is unique in being a non-chemical and continuous treatment system that does not need constant monitoring and replenishing of chemical concentration. The challenge is to provide sufficient mixing of the water treated by plasma and of bulk water in the produce washing system.

Research Methods

Based on preliminary lab-scale experiments with disinfection and activation of water, we designed an integrated 3-tank plasma washing system for disinfection of fresh produce (**Fig. 1**). This integrated system included:

- Gliding arc–based water tank for disinfection and activation of washed water;
- Fresh produce washing tank (sink) optimized for use of plasma-activated water (PAW) produced in gliding arc–based water tank;
- Sedimentation tank optimized for operation with dirty PAW used for fresh produce washing.

The system operates in the following way:
The gliding arc plasmatron is connected to the tank filled with tap water (**Fig. 2**). Air is injected tangentially in the gap between 2 cylindrical electrodes and creates vortex. Power supply applies high voltage between high voltage electrode and ground electrode. Plasma discharge starts between 2 electrodes and the air vortex stretches and rotates gliding arc and produces plasma zone inside the plasmatron. Water injected by water pump into the plasmatron is passing through the plasma zone and collected at the exit of plasma system. Processed in the gliding arc plasmatron water obtains the sterilization properties due to production in plasma different kinds of active species such as OH radicals, hydrogen peroxide, NOx etc. During plasmatron operation the air coming out of plasma zone reacts with the tap water producing plasma-activated water (PAW). Usually, pH of PAW is 3–3.5 compared to 6–6.5 of tap water. The PAW after finishing its preparation is pumped to the washing tank (sink) for fresh produce washing and disinfection. During this process the plasma-activated water gradually become dirty, contaminated with organic load and losing its activity. Dirty water then transported into sedimentation tank for sediments separation. After that, water from sedimentation tank is pumped back into PAW preparation tank (**Fig. 3**).

<u>Plasma-activated water production</u>
Plasma-activated water (PAW) was produced by the installed plasma system. The plasma power, water flow rate, plasma air flow rate, wall protection air, and the water atomization air were adjusted based on different experiment needs. After PAW was generated, a chiller was used to cool the water down to 3–5 °C.

<u>Water chemistry measurement</u>
Temperature, conductivity and pH were measured by a pH meter after cooling. NO_3^-, NO_2^- and peroxide were measured by test strips. Sucrose was added to PAW to increase the organic load to 1000 and 2000 mg/L.

<u>Bacteria culture</u>
Rifampicin-resistant strain of *Escherichia coli* O157:H7 (ATCC 700728) was kindly provided by Dr. N. Nitin at University of California at Davis. Fresh *E. coli* was cultured in Tryptone Soy Broth

(Becton Dickinson, USA) containing 100 mg/L of rifampicin (Sigma Aldrich, USA) at 37 °C overnight. The final bacteria concentration in colony forming units per mL (CFU/mL) was adjusted for each experiment and was confirmed by plating on Tryptone Soy Agar (TSA) with 20 mg/L rifampicin. *E. coli* inoculum was prepared before inoculated on the fresh produce. To prepare the inoculum, 10 mL of fresh cultured *E. coli* was centrifuged at 3000 rcf for 15 min. Pellets were re-suspended in 10 mL of sterile phosphate-buffered saline (PBS). Then bacterial suspension was centrifuged and re-suspended again to remove extra organic in culture broth. The bacterial suspension was then diluted 1:10 to obtain a concentration of 7−8 log CFU/mL.

Inactivation of PAW

PAW generated with different plasma parameters was used for inactivation test. Sucrose was added to PAW to increase the organic load to 1000 or 2000 mg/L. 1 mL of fresh *E. coli* culture was added into tubes contained 9 mL of PAW and sit for a period. After the reaction for different time, 0.5 mL of the solution were plated on rifampicin contained TSA and cultured overnight for quantification.

Inoculation on fresh produce

Baby spinach, chopped kale, and iceberg lettuce were purchased from local grocery store and kept at 4 °C until test. Romaine lettuce was shipped on ice from an industry collaborator and kept at 4 °C in lab until test. Kale, iceberg lettuce and romaine lettuce were cut into pieces of about 1 gram. For small scale washing test, each spinach leaf or each piece of other fresh produce was inoculated with 0.1 mL of 10^6 CFU/mL *E. coli* inoculum. For large scale washing test, 15 mL of inoculum was added into a plastic bag with 200 g of spinach and shaken until well mixed. Inoculated produce was stored in unsealed plastic bags and kept at 4 °C for 20 h to allow *E. coli* attached to the surface of leaves.

Washing procedure

1) Small scale wash by using teabags (**Fig. 5, left**)

After overnight storage, 10 g of fresh produce pieces were separated into 3 tea bags and washed by dipping into PAW that was cooled to 3–5 °C. The wash time was 1 minute. Produce to water ratio was 1:20 (10 g leaves: 200 mL of water). Two batches of romaine were washed in the same beaker of water (**Fig. 6**). Batch 1 was inoculated with *E. coli,* and batch 2 was not inoculated. PAW or tap water was added between the two batches for replenishing. Fresh produce was also washed in tap water as a control experiment.

2) Small scale wash by using steel basket (**Fig. 5, right**)

5 g or 10 g of inoculated romaine lettuce pieces were placed in a steel mesh basket with holes on the side and bottom. The basket was placed in a 250 mL beaker with 200 mL of PAW that was cooled to 3–5 °C on a shaker. The speed of the shaker is 150 rpm. The contact time of fresh produce and PAW was 1 minute.

3) Large scale wash (**Fig. 4**)

After overnight storage, 200 g of inoculated spinach and 400 g of non-inoculated spinach was weighed. Total 3 batches of spinach were washed in the same tank of water to examine the cross-contamination between batch to batch. The first batch was 200 g of inoculated spinach. The second and third batch were 200 g of non-inoculated spinach. The produce to water ratio for large scale washing test was 1:40 (200 g leaves: 40 L). When wash started, the first batch of spinach was dumped into the sink with 40 L of PAW. Three pumps and nozzles were turned on to help mix the spinach and water. After 1 minute contact time, spinach was taken out and dewatered. Batch 2 and 3 were washed in the same way. The gap between batch to batch was

4 to 5 minutes. The cross-contamination between batch to batch was determined by the following equation:

$$\% \text{ of } E.\ coli \text{ remained on the surface} = A/A_0 \times 100\%$$

where A is the concentration of *E. coli* on spinach after wash, CFU/g; and A_0 is the concentration of *E. coli* on spinach without washing, CFU/g.

Different wash water was prepared for experiments included PAW (pH 3), PAW (pH 7 adjusted by adding sodium hydroxide), 200 ppm, 100 ppm, 10 ppm chlorine, 10 ppm chlorine combined with PAW, and tap water. All chlorine solutions were prepared by diluting 13% sodium hypochlorite to appropriate concentration. The pH of all sodium hypochlorite wash water was adjusted to 6.5–7.5 by adding sodium hydroxide or hydrochloric acid.

Bacteria recovery and quantification

After wash, the fresh produce was dewatered by a salad spinner (OXO) and stomached in 10 mL of sterile PBS to extract *E. coli*. *E. coli* was quantified by plating on rifampicin contained tryptic soy agar (TSA) overnight at 37 °C.

Decontamination efficiency of *E. coli* on fresh produce washed in PAW, PAW with organic and tap was determined by the following equation:

$$\text{Wash out efficiency} = \log(A_0/A)$$

where A_0 is the initial concentration of *E. coli* on leaves; and A is the concentration of *E. coli* that remained on leaves after washing.

The % of *E. coli* remained on leaves was determined by $\% = A/A_0$.

Dead cell staining

Propidium iodide (PI) is a fluorescent intercalating agent that can be used to stain cells and nucleic acids. PI is not membrane-permeable, making it useful to differentiate live and dead cells based on membrane integrity. A working solution of 50 µg/mL PI was prepared by diluting 1 mg/mL PI solution (Sigma-Aldrich). 200 µL of working solution was dropped on inoculated romaine lettuce. A fluorescence microscope was used to observe the cells on romaine lettuce.

Quantification of remained DNA

The DNA of *E. coli* that remained on lettuce after wash was extracted from the *E. coli* inoculated pieces of lettuce via QIAamp Fast DNA Stool Mini Kit (Qiagen). The qPCR primer sets targeting *E. coli* O157:H7 (F 5' TAAATG- GCACCTGCAACGGA - 3'; R 5' - GTCATCTTACGGCTGCGGAT- 3') was ordered from IDT DNA and used for qPCR analysis. A fast SYBR Green qPCR assay was applied to obtain the concentration of *E. coli*.

The QuantStudio 3 Real-Time PCR System and Applied Biosystems Fast SYBR Green Master Mix were used to conduct all qPCR assays. Total qPCR reaction volume was 20 µL. Each reaction mixture contained 6µM of the forward and reverse primers, 2 µL of template DNA and 1 0µL of fast SYBR Green Master Mix. The program employed: pre-incubation for 20s at 95 °C; 40 amplification cycles of 1s of denaturing at 95 °C and 20s of annealing at 60 °C; and, finally, 1s of 95 °C, 20s of 60 °C, and 1s of 95 °C for melt curve. All assays were conducted in triplicates of each sample with negative controls and positive controls.

Research Results

1. PAW has strong ability to inactivate *E. coli* in water. Higher power or longer treatment time of PAW leads to higher efficiency of inactivation.

Table 1 shows the water properties of PAW generated by different power. PAW shows at least 4-log reduction of *E. coli* since all samples were below detection limit (60 CFU/mL) after reaction. As the power increase, the water is more acidic, and the inactivation ability is stronger. The addition of organic load in PAW may lower the inactivation effect but can prolong the effective time of PAW (**Fig. 7** and **Fig. 8**).

2. PAW achieves 0.7-log to 2.7-log reduction of *E. coli* on different fresh produce in small scale of test and reduces the cross-contamination between batch to batch.

Typical properties of PAW that was used to wash fresh produce is shown in Table 2. And Table 3 shows the log reduction of *E. coli* on spinach, kale, iceberg lettuce and romaine lettuce washed in PAW and PAW with additional organics by dipping with teabags. Spinach inoculated with *E. coli* washed in PAW had 2.7-log reduction while washed in tap water had 1.9-log reduction. The lower log reductions happened on romaine lettuce washed in PAW and tap water (0.7-log in PAW and 0.5-log in tap) may be due to the different surface complexity.

Table 4 shows the results of romaine lettuce washed by shaking in steel basket, and also shows that PAW has higher decontamination efficiency than tap water. Meanwhile, comparing the results of washing in basket to washing in teabags, the washing method itself has impact on the decontamination efficiency besides the wash water used. So, the log reductions of *E. coli* on fresh produce are not comparable when washed in different methods, especially washed in small scale experiments and large experiments.

In industry, multiple batches of fresh produce are washed in the same tank of water. Therefore, we washed 2 batches of romaine lettuce in the same water to exam if cross-contamination happened between batch to batch. The first batch of romaine was inoculated with *E. coli* and the second batch was not inoculated with *E. coli*. The results in **Fig. 9** show the batch 2 romaine (no *E. coli*) washed in tap water has more residual *E. coli* compared to washed in PAW, which means PAW can reduce the cross-contamination between these 2 batches.

3. Plasma improves the wash-out ability of water.

Microbial decontamination of fresh produce consists of bacteria inactivation on the surface and bacteria being washed out from the surface. The decontamination efficiency shown in previous sections was determined by comparing the plate count of washed and unwashed samples. Therefore, the decontamination of lettuce washed in water includes inactivation of *E. coli* on the surface of lettuce and wash-out to the water. The total amount of DNA of *E. coli* that remained on the romaine lettuce after washing in PAW or tap water was measured by qPCR. These numbers included both alive and dead *E. coli* on the surface of lettuce. More total DNA was found after washing in tap water than after washing in PAW. It means that 50% more *E. coli* (either alive or dead) was washed out from the surface of lettuce when the PAW was used.

4. PAW can inactivate *E. coli* on the surface of fresh produce.

PI is a fluorescent dye can bind with dead cell DNA and shows red color under microscope. **Fig. 10** shows PAW can also inactivate *E. coli* on the surface of romaine lettuce.

5. PAW can improve the inactivation efficiency of sodium hypochlorite.

A series of experiments of washing spinach were conducted in large scale system. The PAW generated in this system was close to room temperature. The pH was around 3, NO_3^- and NO_2^- was 500 mg/L and 40 mg/L, respectively. The inactivation efficiency of *E. coli* in PAW is more than 0.7 log.

Fig. 11 shows the results of *E. coli* inoculated spinach washed in tap water, PAW (pH 3), PAW (pH 7), 10 ppm, 100 ppm, and 200 ppm chlorine, and 10 ppm chlorine with PAW. The *E. coli* concentration on batch 1 spinach before washing was 7 log CFU/g. After washing, spinach washed in tap water had 0.22-log reduction and 0.53-log reduction by washed in PAW. Spinach washed in different concentration of chlorine reached higher reduction (0.92 and 0.93-log for 100 and 200 ppm, respectively). When 10 ppm chlorine was added to PAW and adjusted pH to 7, the decontamination efficiency of spinach achieved 1.6-log, while 0.6-log reduction was achieved when washed in only 10 ppm chlorine. Therefore, PAW can improve the decontamination ability of low concentration sodium hypochlorite.

In addition, PAW reduces the cross-contamination between batch to batch (Table 5). After washing the first batch (with *E. coli*), second batch and third batch (no *E. coli*) of spinach in tap water, 1% of *E. coli* transferred to the second batch and 0.3% transferred to the third batch from the first batch. After washing in PAW, 0.08% and 0.02% of *E. coli* transferred from batch 1 to batch 2 and 3. Chlorine was effective to reduce the cross-contamination. When wash in 200 ppm chlorine, the percentage of *E. coli* remained on batch 2 and 3 were 0.03% and 0.08%, respectively. When PAW combines with 10 ppm chlorine, the transition reduced to 0.002% and 0.001% for batch 2 and 3.

Outcomes and Accomplishments

Outcomes and accomplishments of the project can be summarized in accordance with major objectives of the project:

1. 100-gallon flow-through water tub has been constructed, installed and successfully applied for lab scale testing.
2. Reverse vortex gliding arc plasmatron electrode has been modified to fit the 100-gallon tub for lab testing.
3. Existing 3-kW power supply has been modified for newly constructed gliding arc plasmatron electrode.
4. Disinfection efficiency has been validated to generate the operating parameter space for the plasma system. For this purpose, instead of using *E. coli* cocktail, rifampicin-resistant strain of *E. coli* O157:H7 (ATCC 700728) was used in this project. Because first, the plasmid of this strain is modified to make it rifampicin resistant, which means it is more accurate when we quantify by plate count. And second, strain O157:H7 are similar to those strains in the cocktail based on the information from ATCC. There is no research that states the disinfectant resistance ability are different between them.
5. Plasma disinfection efficiency has been additionally validated in the presence of organic load. For this purpose, sucrose was added into PAW to increase COD up to 2000 mg/L. The presence of COD caused limited reduction of the inactivation efficiency in PAW but can prolong the effective time of PAW.
6. The washing results data has been validated with specific fresh produce (romaine lettuce) provided by an industry collaborator.
7. Intellectual property (patent disclosure), and schematics for commercial prototype system has been generated.

Summary of Findings and Recommendations

1. PAW has strong ability to inactivate *E. coli* in water. Higher power or longer treatment time of PAW leads to higher efficiency of inactivation.
2. PAW achieves 0.7-log to 2.7-log reduction of *E. coli* on different fresh produce and reduces the cross-contamination between batch to batch.
3. Plasma improves the wash-out ability of water.
4. PAW can inactivate *E. coli* on the surface of fresh produce.
5. PAW can improve the inactivation efficiency of sodium hypochlorite.
6. High efficiency of fresh produce washing using PAW has been demonstrated not only in pure water but also in water containing significant organic load.
7. High efficiency of fresh produce washing using PAW has been demonstrated not only on the lab scale but also scaled up to industrial demonstration level of 100 gallons.

APPENDICES

Publications and Presentations

1. Patent disclosure Docket # 22-2415 “Controlling Physical Properties (Viscosity, Thermal Conductivity, Diffusivity, Surface Tension and Capillary Effect, Wettability, Surfactancy e.a.) of Water and Other Liquids Using Non Equilibrium Plasmas”
   Alexander Fridman, Alexander Rabinovich, Gary Nirenberg, Mobish Abraham Shaji, Dmitri Vainchtein, Christopher Sales, Jinjie He. (Date of submission: February 21, 2022)
2. Effects of plasma on physical properties of water: nanocrystalline-to-amorphous phase transition and improving produce washing. J He, A Rabinovich, D Vainchtein, A Fridman… 2022 - arxiv.org
3. International Symposium on Plasma Bioscience ISPB10, A. Fridman, Applications of Plasma Activated Water, Seoul, South Korea, 2021.
4. International Symposium on Plasma Bioscience ISPB11, A. Fridman, Atmospheric Pressure Plasma for Biological and Agriculture Applications, Seoul, South Korea, 2022.

Budget Summary

This project was awarded a total of $302,776 in research funds, and the majority of funds were spent.

Figures 1–11 and Tables 1–5 (see below)

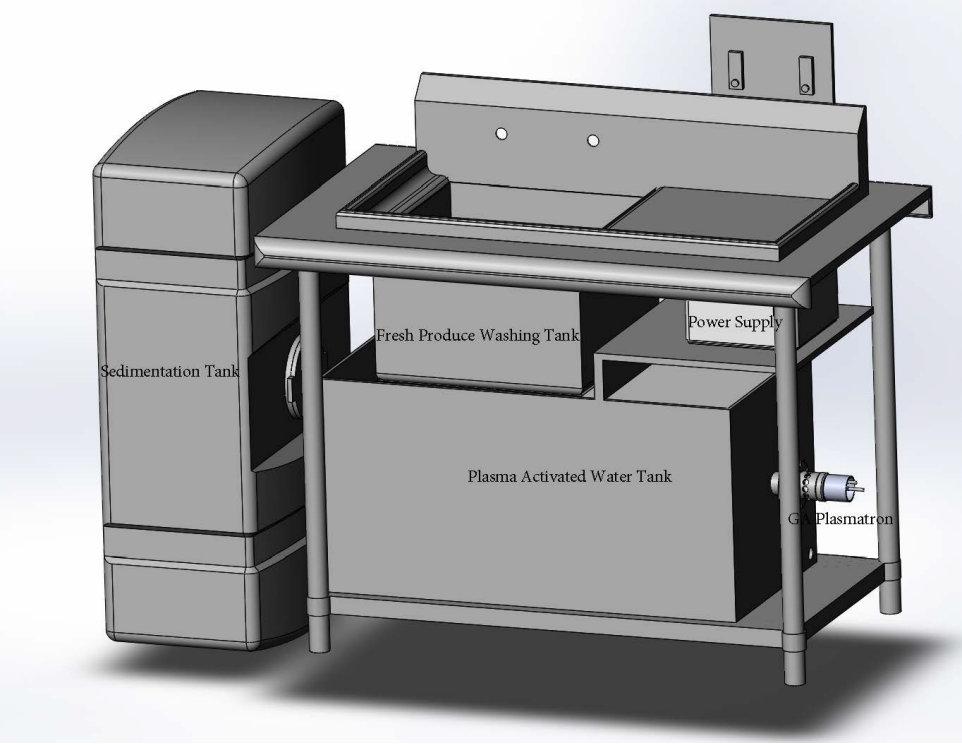

Figure 1. Schematic of integrated plasma washing system for disinfection of fresh produce

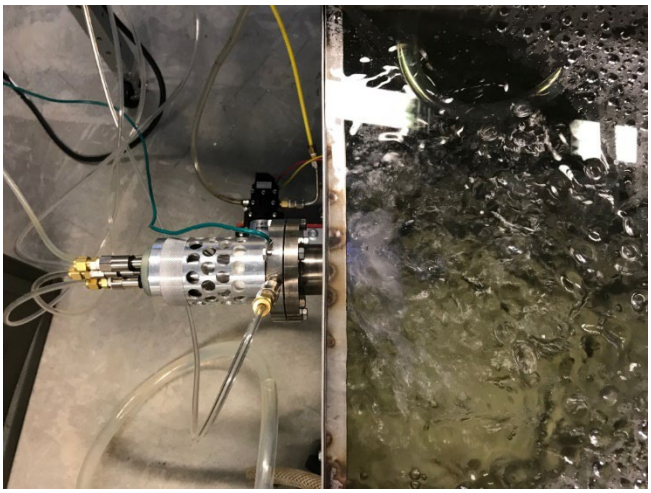

Figure 2. Operation of gliding arc plasmatron in a water tank during PAW production

Figure 3. Integrated plasma washing system

Figure 4. Large scale washing system

Figure 5. Small scale washing methods. Left is washing in teabags; right is washing in steel basket

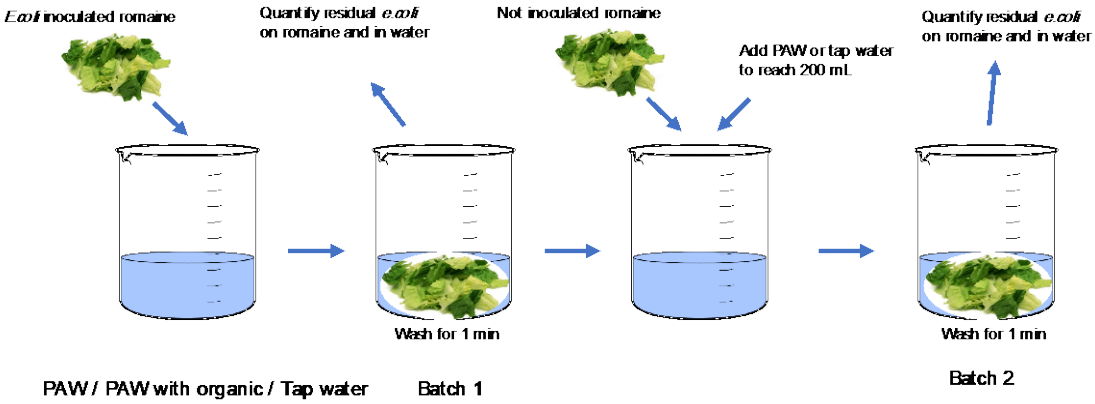

Figure 6. Washing procedure of two batches of romaine lettuce

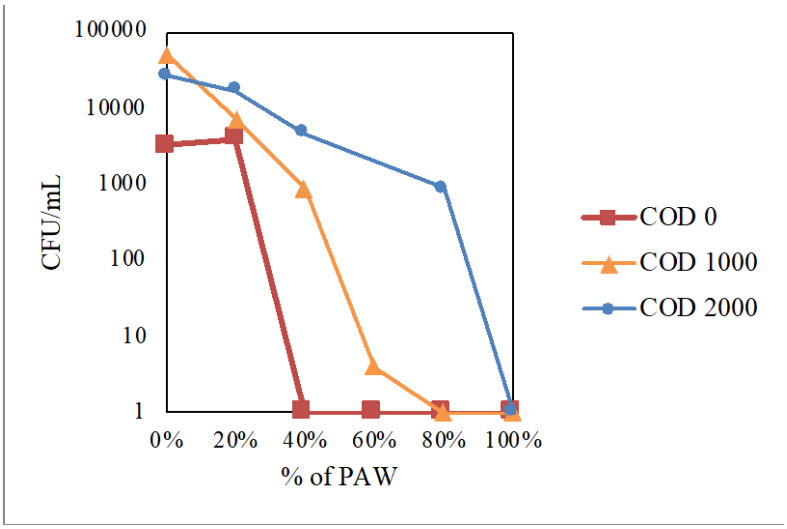

Figure 7. *E. coli* remained in PAW that was diluted to different ratio with distilled water. (i.e. 20% is 2 mL of PAW added to 8 mL of distilled water; 100% is no dilution of PAW)

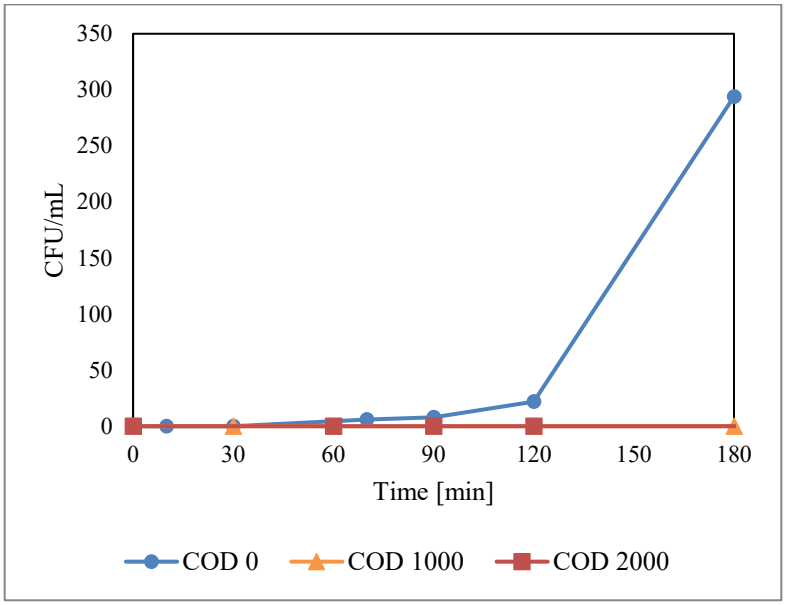

Figure 8. *E. coli* remained in the PAW with organic loads after 30 min reaction. The initial concentration of *E. coli* is 5 log CFU/mL. *E. coli* culture was added into PAW after curtain of time and then sit for 30 minutes before quantification.

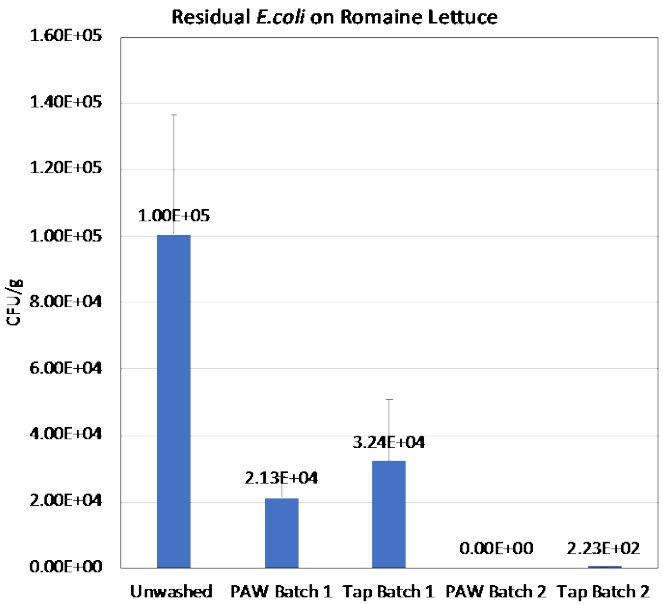

Figure 9. Residual *E. coli* on romaine lettuce washed in PAW and tap water. Batch 1 romaine was inoculated with *E. coli* and Batch 2 was not inoculated with *E. coli*

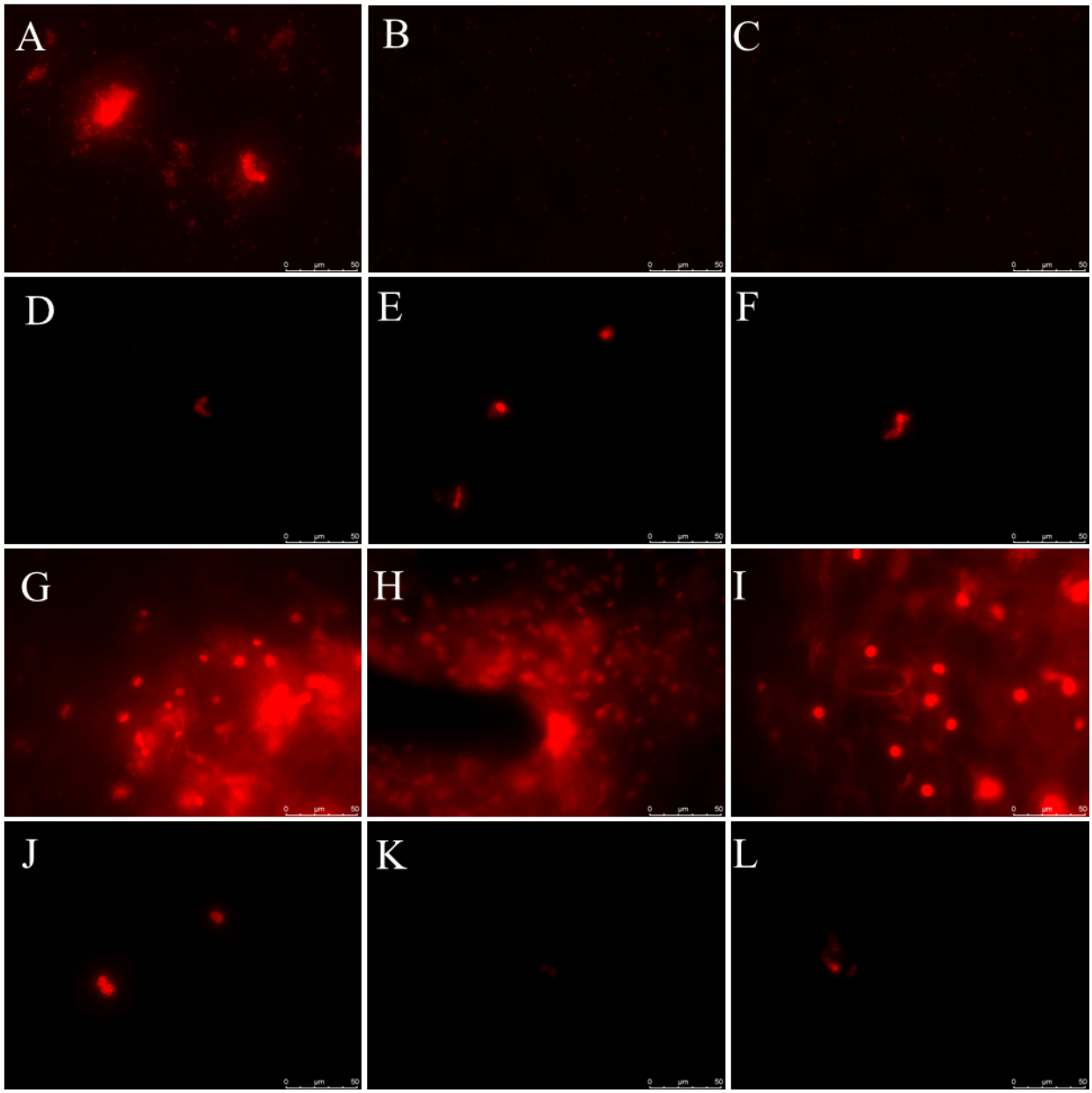

Figure 10. Dead cells staining by PI on washed romaine lettuce. ABC, positive control (dead *E. coli* cells obtained by heating on heat block); DEF, romaine lettuce inoculated with *E. coli* and did not wash; GHI, inoculated romaine lettuce and washed in PAW; JKL, inoculated romaine lettuce and washed in tap water

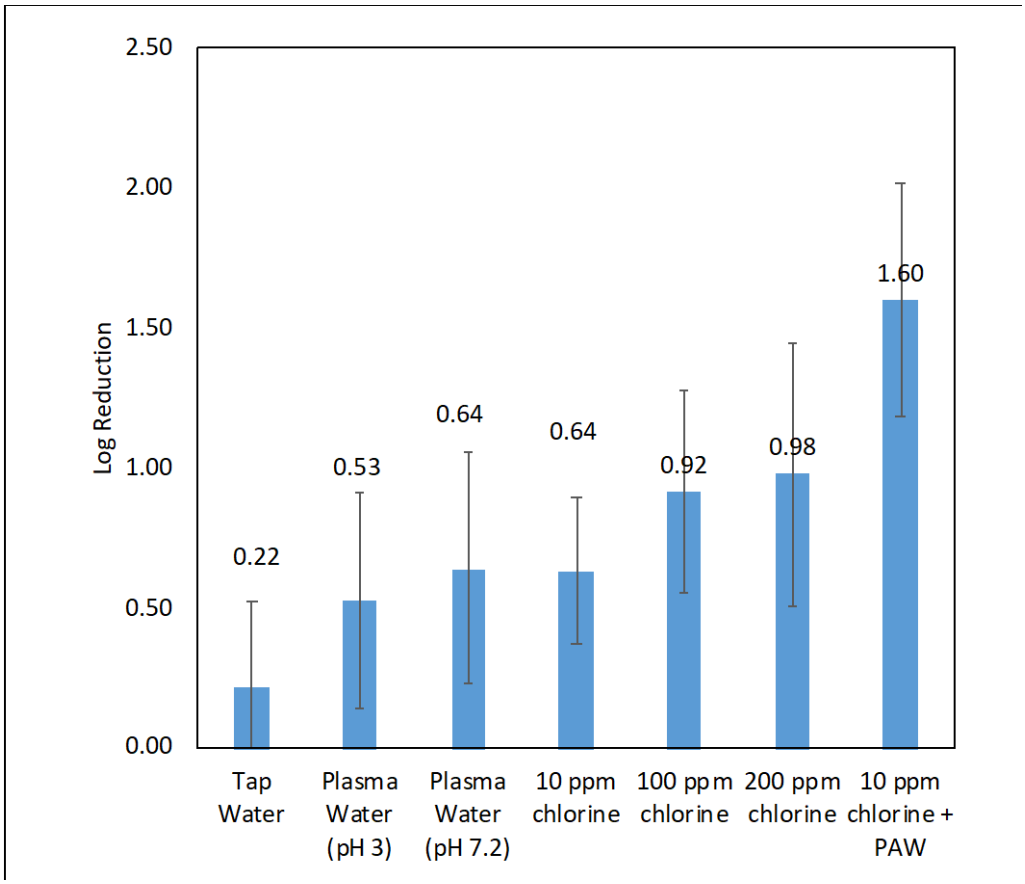

Figure 11. Decontamination efficiency of spinach with *E. coli* washed in large scale system

Table 1 Properties and inactivation efficiency of PAW generated by different power. Log reduction calculated based on the concentration of *E. coli* added into PAW because all samples were below quantification limit of 60 CFU/mL after reaction.
* sucrose added to distilled water to increase COD, then this water was treated by plasma; ** sucrose was added into plasma treated water

Power [W]	**COD [mg/L]**	**pH**	**conductivity [mS/cm]**	**Temperature [°C]**	**NO_3^- [mg/L]**	**Peroxide [mg/L]**	**Log reduction**
1125	0	2.56	2	34.4	250	2	>3.47
1320	0	2.3	2.11	29.6	500	5	>3.20
1320	0	2.29	2.03	44.8	500	5	>4.16
1320	1000 (*)	2.3	2.55	30.4	500	2	>4.41
1320	1000 (**)	2.42	1.87	29.4	500	2	>4.20
1320	2000 (**)	2.37	1.85	39.7	500	<0.5	>4.13
1600	0	2.24	2.17	46.9	500	5	>4.16

Table 2 Typical water properties of PAW and tap water used for washing fresh produce in small scale test

	Cooled PAW	**Tap water**
pH	2.61	6.93
EC mS/cm	1.62	0.43
Temperature °C	3.9	6
Peroxide ppm	5	0
NO_3^- mg/L	500	0
NO_2^- mg/L	0	0

Table 3 Log reduction of *E. coli* on fresh produce washed in PAW and tap water by dipping in teabags. Mean log reduction ± S.D.

	plasma water COD 0	**tap water**	**plasma water COD 2000 mg/L**	**tap COD 2000 mg/L**
spinach	2.70±1.09	1.90±0.79	2.36±0.49	2.09±0.28
kale	1.92±0.51	1.22±0.67	2.17±0.59	1.26±0.45
iceberg lettuce	1.21±0.43	0.63±0.27	0.73±0.2	0.80±0.21
romaine lettuce	0.67±0.21	0.55±0.32	0.70±0.33	0.91±0.16

Table 4 Log reduction of *E. coli* on romaine lettuce washed in PAW and tap water by shaking in steel basket. Mean log reduction ± S.D.

Produce: water	**1:20**	**1:40**
PAW	0.32 ± 0.29	2.09 ± 0.19
TAP	0.13 ± 0.24	1.84 ± 0.16

Table 5 Percentage of *E. coli* remained on the surface of spinach washed in large scale system

		tap water	PAW (pH3)	PAW (pH7)	10 ppm chlorine	100 ppm chlorine	200 ppm chlorine	10 ppm chlorine + PAW
Before Treatment	Batch 1	100%	100%	100%	100%	100%	100%	100%
	Batch 2	0%	0%	0%	0%	0%	0%	0%
	Batch 3	0%	0%	0%	0%	0%	0%	0%
After Treatment	Batch 1	64.679%	21.613%	19.420%	23.045%	14.772%	13.228%	3.631%
	Batch 2	1.040%	0.078%	0.037%	0.017%	0.001%	0.029%	0.002%
	Batch 3	0.257%	0.019%	0.009%	0.008%	0.0002%	0.079%	0.001%

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