Evaluation of Low-Pressure Cold Plasma for Disinfection for ISS Grown Produce and Metallic Instrumentation

Paul E. Hintze[1]
National Aeronautics and Space Administration, Kennedy Space Center, FL, 32899

Carolina Franco[2]
University of Space Research Administration, Kennedy Space Center, FL, 32899

Mary E. Hummerick, Phil R. Maloney, Lashelle E. Spencer[3]
Engineering Services Contract, Kennedy Space Center, FL, 32899

Cold plasma (CP) cleaning is a dry, non-thermal process, which can provide broad-spectrum antimicrobial activity yet reportedly causes little to no damage to the object being sanitized. Since cold plasma uses no liquids, it has the distinct advantage when used in microgravity of not having to separate liquids from the item being cleaned. This paper will present results on an effort to use low pressure CP to disinfect or sterilize materials for in space applications. Exposure times from 0 to 60 minutes and pressures ranging from 10 to 100 Pa were used to optimize plasma parameters to achieve acceptable kill rates for 3 bacteria, *Bacillus cereus, E. coli and Bacillus pumulis SAFR-32* and one fungi, *Aspergillus niger*. These tests were done on produce and metal coupons to simulate medical equipment. Produce testing was not successful, with unacceptable kill rates and the produce being negatively impacted by exposure to the plasma. The plasma caused a 5 log reduction in the number of viable bacteria on metal coupon tests, which placed the number of viable bacteria below the detection limit. This is a very promising result showing that sterilization of medical equipment with cold plasma is feasible. Scanning Electron Microscope images were taken before and after exposure. The images after plasma exposure shows that the bacteria spores have been physically affected, as their size has gotten smaller and the appearance has changed.

Nomenclature

APCP	=	atmospheric pressure cold plasma
CFU	=	colony forming units
CP	=	cold plasma
DI	=	deionized water
ISS	=	international space station
MPN	=	most probable number
SEM	=	scanning electron microscope
TSA	=	trypticase soy agar

I. Introduction

Cold plasma is increasingly under research for sterilization of medical instrumentation and foods, specially fruits and vegetables. Several research suggests that its effect on produce remains under research; however, it has been demonstrated to effectively inactivate microbes. Low-pressure cold plasma cleaning is a dry, non-thermal process. Because it uses no liquids, it has the distinctive advantage when used in microgravity of not having to separate the liquids used for cleaning from the goods. Currently, produce grown at the International Space Station (ISS) are sterilized by using antibacterial wipes, which have been effectively removing organisms form its surface; however, because of the waste the process is not practical. So far, literature suggested the use of atmospheric cold plasma; though, no studies have been done for low-pressure cold plasma effects on sterilization of medical devices and inactivation of microbes in foods.

Foodborne outbreaks are often associated with produce quality and emerging pathogens are becoming more resistant to existing disinfection methods. Also, consumer demand for high quality and safe-nutritious food has led

to the development of novel liquid free, non-thermal technologies. Lacombe et al. used atmospheric cold plasma to treat blueberries inoculated with *Escherichia coli* and *Salmonella* at different time periods, up to 120 s. Blueberries were taken for plate count of yeast/molds immediately after treatment at 1, 2, and 7 days showing a considerable reduction in plate count after treatment, concluding to have complete inactivation of the microbes. Qualitative studies were also performed and blueberries exposed to more than 60 s of plasma, showed several damaging characteristics, such as firmness, color, and nutritious value (A Lacombe, 2015). Misra et al. used atmospheric cold plasma to study its effects within sealed packages of cherry tomatoes to observe the physical quality parameters and respiration rates at different storage period times. Changes in pH, color, weight loss, and firmness implicated that cold plasma could be use as means of decontamination while retaining good quality (N N Misra, 2014). Bermudez-Aguirre et al. used atmospheric cold pressure plasma in argon to treat carrots, lettuce, and tomatoes inoculated with *Escherichia coli* for periods between 30 s and 10 min. Results of the plate counts demonstrated the inactivation of bacteria, especially with the produce treated for longer time. Tomatoes and lettuce were easier to disinfect than carrots; however cell structure showed disruption of the membranes and deformation after treatment (D Bermudez-Aguirre, 2013). On the other hand, Klampf et al., showed promising results for the sterilization of sensitive medical devices. They observe a 4 to 6 $\log_{10}$ reduction with most of the microbes tested (different strains of *Bacillus* and Geobacillus) with the exception of *C. albicans*. They also observed that the high inactivation rates obtained were independent of the material of the test specimen (T G Klampfl, 2012).

Based on the positive results obtained with the different studies already performed with foods and medical instrumentation, it is believed that low-pressure cold plasma could be as well an optimistic treatment method for disinfection and inactivation of microbes. This paper will present results on an effort to use air low pressure cold plasma to disinfect and/or sterilize materials contaminated with microbes for space applications, such as produce and metal coupons to simulate medical instrumentation. Three different strains of bacteria were used, *Bacillus cereus, E. coli and Bacillus pumulis SAFR-32* and one fungi*, Aspergillus niger.*

II. Experimental Methods

A. Inoculum Preparation

Produce was inoculated with *Escherichia coli* ATTC# 11775 grown to early stationary phase. Standardizing the growth conditions and phase helps to maintain consistent treatment effects. The time to reach stationary phase under specific incubation conditions, was determined by sampling a culture grown in nutrient broth hourly, reading the absorbance at 540 nm on the spectrophotometer (Spectronic) and plating onto trypticase soy agar (TSA) to determine colony forming units (CFU). Cultures were grown in triplicate and incubated at 37° C on a rotary shaker at 212 rpm. From these experiments it was determined that stationary phase for this *E. coli* strain was reached in 8-9 hours. Subsequent cultures used for inoculation were therefore grown under the same conditions for this length of time.

B. Produce inoculation with Escherichia Coli
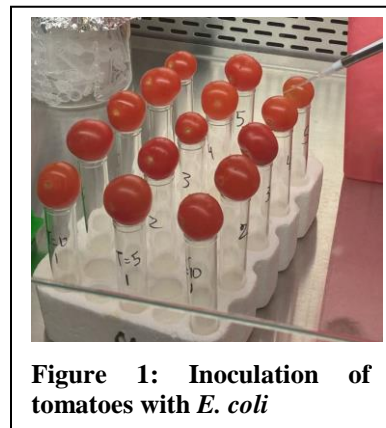
Figure 1: Inoculation of tomatoes with *E. coli*

Tomatoes, peppers and radishes grown in controlled environment chambers as described were used for testing. Preliminary tests were done to determine the recovery of *E. coli* from the surface of the produce after inoculation and drying. Desiccation can affect the viability of *E. coli*. Two methods, blending and vigorous shaking in buffer for removal of the bacterium from the surfaces were also tested to achieve the highest level of recovery. Initially tomatoes were inoculated with approximately 10^5 cells per gram by applying several spots using a pipette holding approximately 10ul. (Figure 1). After inoculation and drying, individual tomatoes were placed into sterile sample bags. Buffered peptone water was added at 1:10 wt/volume. Three tomatoes were blended for 2 minutes using the bag blender which macerates the tomato while two were shaken vigorously for two minutes. Buffer solutions from the sample bags were diluted and plated onto media selective for *E. coli*. Recovery of the bacterium from the blended tomatoes was below the detection limit of 100 CFU/gram. Recovery from the shaken tomatoes was 7 x 10^4 per gram. Similar testing on another plasma system is

being performed at Johnson Space Center. Microbiologists there confirmed similar results with tomato inoculation and recovery of *E. coli*. A 45-minute drying time was also recommended to minimize the effects of desiccation on the viability of the bacterium. Based on these results, all subsequent testing was done using the shaking method.

Tests on pepper revealed a recovery of approximately 3 orders of magnitude less than the amount of *E. coli* initially inoculated on the surface (4×10^5). Further testing was not done on plasma treatment of the pepper as the fruit quality was drastically effected.

Before inoculation, radishes were first submerged in a solution of 1% Prosan, a food grade produce sanitizer, to reduce the background microbial numbers. Testing showed a reduction by 3 orders of magnitude of background flora with a 30 second exposure. After washing with Prosan, radishes were rinsed with sterile DI water and dried in a biological safety cabinet until no moisture could be seen on the surface (approximately 1 hour). The inoculum was administered to the surface of the radish by pipetting 10 µl drop by drop of the culture diluted to 10^9 bacteria/ml in nutrient broth to achieve an inoculum of approximately 10^7 bacteria/radish. The inoculum was allowed to dry for approximately 45 minutes before placing the radishes into the plasma chamber. Tomatoes and peppers were inoculated with a lower number of bacteria /mL (10^5) using the same methods as radish with the exception of the Prosan rinse since the microbial load on the surface of these fruits is low. It was determined from the tomato and pepper recovery testing that a higher inoculum density (10^7/radish) should be used in order to detect a > then 3 log reduction.

C. Coupon inoculation with Bacillus pumilus

Aluminum 1.0 x 2.0 cm coupons were heat sterilized at 150 °C for 18 hours and stored in a sterile container. Previously harvest endospores of *B. pumilus* were used for inoculation. The stock spore suspension contained 10^9 spores/mL. Each coupon was inoculated with 10 µl divided into about 9 drops. Coupons were allowed to dry at least 24 hours in a biological safety cabinet before use in testing. Dried coupons were stored in a desiccator. To confirm the inoculum density and spore pattern on the coupons, a most probable number (MPN) test and SEM imaging were done. The inoculum density was determined by MPN to be approximately 5×10^7 /coupon and SEM images showed mostly a single layer of spores with the exception of the edge of the spot.

D. SEM parameters and sample test matrix

All scanning electron microscope (SEM) images were captured using a JEOL JSM-7500F Field Emission SEM. The microscope was operated in LEI (lower secondary electron image) mode over a range of magnification depending on the characteristic being observed (i.e. spore density, surface morphology, etc.). Generally, at least two areas of inoculation per coupon were examined and imaged to check for consistency across the coupon. For most experiments, four of the six coupons tested were set aside for SEM imaging. Experimental samples were prepared in two batches, hence the need for two sets of controls. The first batch included Control A, Vacuum 15, Plasma 5, Plasma 10, and Plasma 15. The second included Control B, Vacuum 60, Plasma 30, and Plasma 60. Sample descriptions corresponding to coupons in multiples of two, indicate multiple experiments under those conditions (i.e. six coupons equates to three experiments using the same parameters). Coupons for two experimental conditions (Control B and Plasma 60) have been prepared, but were not imaged in time to be included in this report. These images will be included in subsequent reports.

III.   Results and Discussion

A. Optimization parameters for produce disinfection under plasma

The produce selected for plasma disinfection test were cherry tomatoes (cultivar Red Robin), radishes (cultivar Cherry Bomb), peppers (cultivar Pompeii), and cabbage (cultivar Tokyo Bekana). A sample of each type of the produce was tested before inoculation to set parameters that will work without observing any apparent damage of the good. Since pressure was the intrinsic factor to be modified as the disinfection was being done under vacuum, different pressure values were tested in each of them. Once it was determined that vacuum itself will not affect the physical appearance of the vegetable, plasma under vacuum was applied. As expected, the high percentage of water in vegetables greatly affected the low pressure plasma performance. When moisture was present in the chamber, the pressure setting had to be increased as the power of the plasma and the gas supply could not be maintain to the set point. Even though coupons were run under 0.10 mbar pressure, none of the produce was able to achieve such low pressure as a consequence of the presence of water. Table 1 gives a summary of the optimized parameters for each of the goods used in plasma disinfection trials.

Table 1: Optimized parameters for each of the inoculated produce used in plasma disinfection trials

Produce	Pressure (mbar)	Plasma Radiation	Number of goods
Coupons	0.10		4 to 8
Tomatoes		Bright	5
Peppers	0.80		2
Radishes			3

The first trials were done with the cherry tomatoes, were a 0.60 mbar minimum pressure was achieved. If the pressure was set lower, the gas supply did not allowed the power of the plasma to turn on. Five tomatoes were placed on the rack expecting them to release a large amount of water; however, the skin of the tomatoes seem to be strong enough to keep its moisture within. This veggie was the only one to achieve such low pressure with bright plasma radiation and without any skin damage, see Figure 2. Other pressure that worked for the tomatoes was 0.80 mbar where five, four, and three of tomatoes were tested at a time observing no effect on the pressure setting. Based on these trials, the optimization parameters for the tomatoes were either 0.60 or 0.80 mbar, five tomatoes at a time, and a maximum exposure time of 15 min.
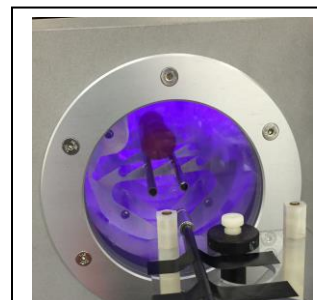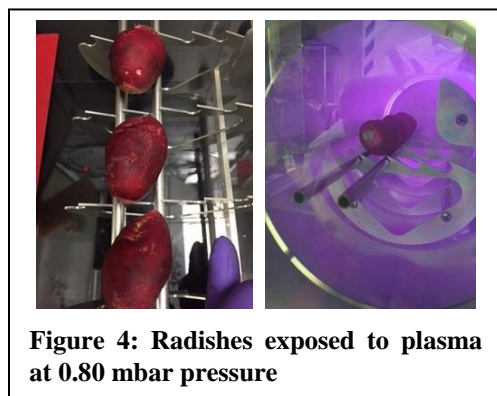

Figure 2: Cherry tomatoes inside plasma chamber at 0.80 mbar

The next produce under optimization for pressure and time were the peppers. Peppers were subject to 1.0, 0.80, and 0.60 mbar pressures for a maximum of 15 min. Peppers were tested as a whole, split in half, and with small holes. The 1.0 and 0.80 mbar pressures worked with two and three (maximum amount that designed rack was able to hold) whole peppers, the plasma light was bright through the 15 min and no physical damage was observed. Then, the 0.60 mbar pressure was tried with two peppers showing a very dim light and a low gas supply. Three peppers were also tested at 0.80 mbar but this time the chamber could not pumped down to the pressure set point and test was not possible. Since it was known that moisture content will affect pressure conditions, a pepper with poked holes was also tested at 0.80 mbar and proved to be unsuccessful. The only pressure that worked for the poked pepper was 1.0 mbar but the plasma light was once again very dim and physical damage to the skin was observed around the holes. One last test was done under the 1.0 mbar pressure with one pepper cut in half displaying the same results as with the poked pepper. Based on these trials, the optimization parameters for the peppers were 0.80 mbar, two whole peppers at a time, and a maximum exposure time of 15 min. Figure 3 shows a picture of the different trials done with the peppers.
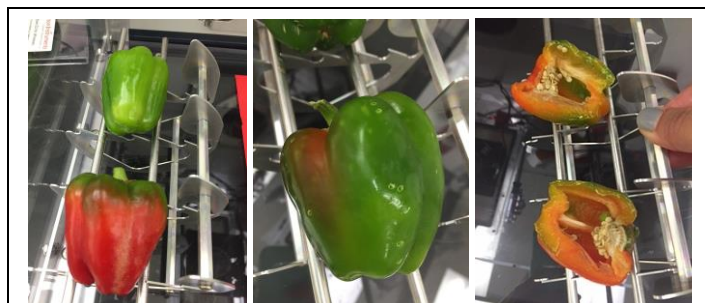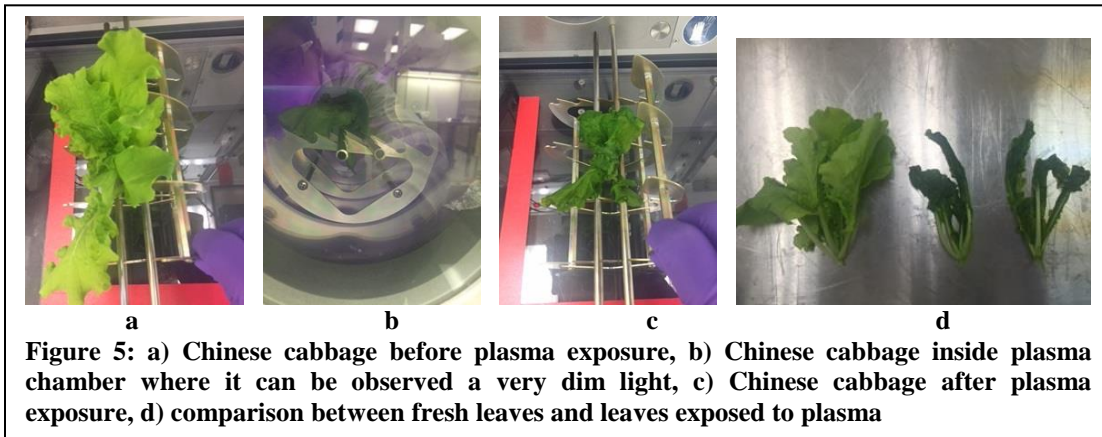

Figure 3: Whole (left), poked (center), and cut (right) peppers exposed to plasma and vacuum at 0.80 mbar

The next produce for testing were the radishes. Since the one of the goals for low pressure plasma produce

Figure 4: Radishes exposed to plasma at 0.80 mbar pressure

disinfection is to set the same parameters for any vegetable in use, the initial pressure tested was 0.80 mbar; this pressure previously worked with tomatoes and peppers. When four medium size radishes were picked and introduced inside the chamber at this pressure, the chamber's pressure did not pumped down and conditions did not work. Therefore, the same was tried with 1.0 mbar, where it could be observed that the instrument took a while reaching the set pressure and only a very dim plasma light was observed. Consequently, both pressures were tested again but only with three radishes instead of four. In both cases the conditions worked and plasma was carried for 5 min (Figure 4). Based on these trials, the optimization parameters for the radishes were 0.80 mbar, three medium size radishes at a time, and a maximum exposure time of 15 min.

The last set of produce tested was the Chinese cabbage. The expectations for this vegetable were not optimistic because of the fragility of its leaves and the great water content. As mentioned above, the presence of moisture inside the chamber was affecting the ability for the plasma to go down to lower pressures; the higher the water content, the higher the expected pressure. Nonetheless, a test under 0.80 mbar was started with one bundle of cabbage leaves placed on the rack. The instrument was able to pump down to the set pressure; however, only seconds after the plasma started, the water content inside increased also increasing the set pressure. As a consequence the instrument shut off the gas supply shutting the plasma radiation as well. Other trials were done at higher pressures, 1.0 and 1.20 mbar, and the same behavior was observed. Once the cabbage was removed from the chamber, the leaves were very cold with pronounced tissue damage. Figure 5 exhibits several pictures of the cabbage leaves before and after treatment, where it can be observed the damage caused after plasma exposure. Based on these results, the Chinese cabbage will be eliminated from the inoculation procedure as no disinfection plasma will be possible.


a   **b**   **c**   **d**

Figure 5: a) Chinese cabbage before plasma exposure, b) Chinese cabbage inside plasma chamber where it can be observed a very dim light, c) Chinese cabbage after plasma exposure, d) comparison between fresh leaves and leaves exposed to plasma

B. Inoculated produce after plasma treatment

Two types of produce were tested for plasma disinfection after inoculation with bacteria, the cherry tomatoes and the radishes. As previously mentioned all produce was inoculated with *E. coli,* and treated within an hour of bacteria inoculation. One of the remarkable characteristics of the tomatoes that was observed right before placing them inside the plasma chamber, was that they were very ripen and some water was detected through the removed stalk. The preliminary studies with tomatoes were the parameters were determined, were done with tomatoes that were not as ripen as this new set. Immediately it was believed that this could lead in pressure problems and even damage of the good. Most of the trials done with the tomatoes were unsuccessful. As expected, when the pressure was lowered to the 0.80 mbar set point, the fruits that showed presence of water burst open and even exploded inside the chamber;

Figure 6 illustrates some pictures of the failed trials showing the tomatoes completely damaged. Only one trial for the tomatoes was successful as the pressure was maintained during the 15 min with bright light radiation and steady pressure.

Figure 6: Damaged inoculated tomatoes after exposure to plasma disinfection
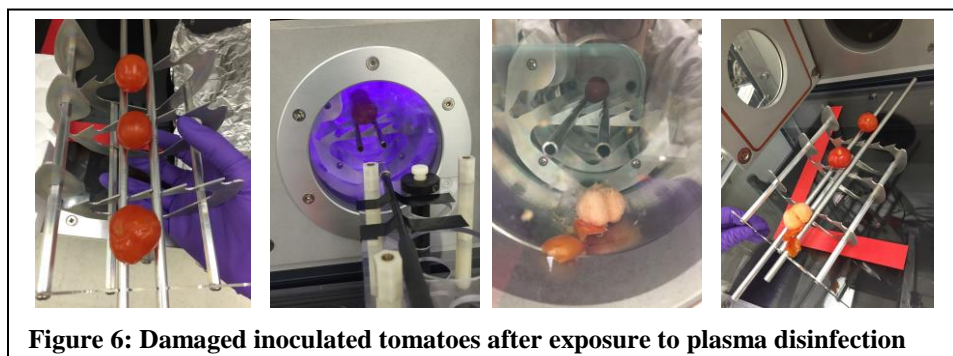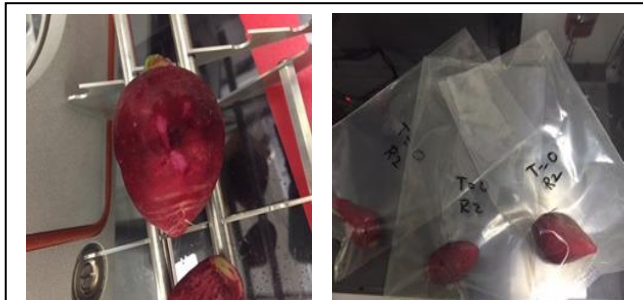
Figure 7: Inoculated radishes exposed to plasma and ready for log reduction test

The radishes were inoculated with *E. coli* as well and were tested within the same period of time to ensure microbes did not degrade. When radishes were tested, three were placed at a time on the rack for disinfection. Testing with radishes was simple as they did not present any damage that could lead into test failing. Different sets of radishes were exposed to plasma radiation at 5, 10, and 15 min, and one set was exposed for 15 min to vacuum without plasma power to create a blank for the test (Figure 7). After plasma exposure radishes did not present any physical changes; however, it was identified that the smallest ones were softer after a couple hours of contact.

C. Produce microbiological analysis after plasma treatment

Recovery of the bacterium was also lower than expected on all treatments and controls tested with a starting inoculum of approximately 2.2 x 10^5 cells per tomato. Therefore, results were inconclusive and tomato testing will be repeated using firmer fruit and a higher inoculum density.

Radishes were inoculated with a stationary phase culture of *E. coli* using the methods described above. The initial starting density was approximately 3 x 10^7 per radish. Plasma exposure times were 0, 5, 10 and 15 minutes. The zero time was under low pressure for 15 minutes. Treated radishes were compared to untreated inoculated controls. Each run (2 replicates of each time) included 3 radishes. Results (Figure 8) showed an average $\log_{10}$ reduction of 1.5, 1.73 and 1.76 for 5, 10 and 15 minute treatments respectively with no significant difference between the treatments although the reduction was higher than the low pressure control. The log reduction of *E. coli* on the surface of the radish was much less than that for *B. pumilus* on flat coupons (discussed below) suggesting that the rough topography of the radish surface may impact the microorganism's exposure to the plasma thus It is lethal effects.
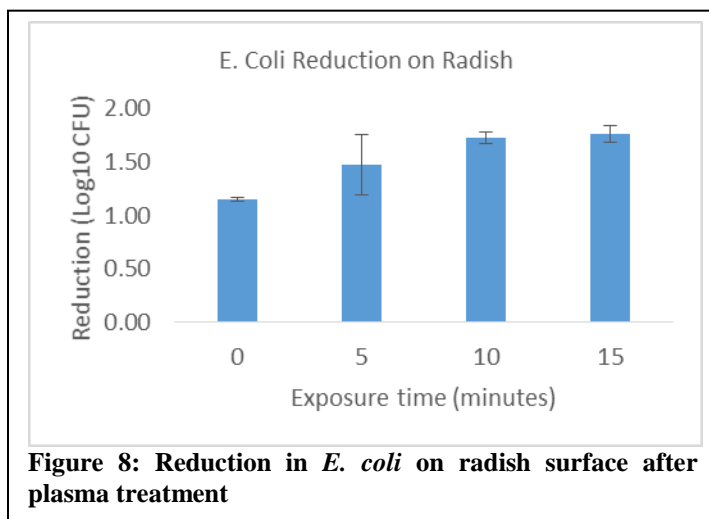
Figure 8: Reduction in *E. coli* on radish surface after plasma treatment

### D. Optimization parameter for coupon disinfection under plasma

The first step for disinfection of goods using low pressure air plasma, was to find the optimal conditions for application in produce and metallic components. Because of the diverse physical characteristics of the two materials to be introduced inside the plasma chamber under vacuum, different factors had to be considered for its use. The trials performed in this section allowed the selection of two different set of conditions: one set for all produce and another for metallic coupons.

The parameter's characterization for plasma disinfection in metallic objects was simple and straightforward. Previous precision cleaning studies used contaminated rectangular metallic coupons and generated a hanging rack that could be used for this testing. Those preliminary trials set conditions of 0.10 mbar pressure, up to 10 coupons at a time, and up to two hours of plasma cleaning. These same settings were used and successfully tested on pre-made rectangular 30 x 10 mm metallic coupons.

E. Plasma treatment of inoculated coupons with Bacillus Pumilus

Once parameters were optimized for the treatment of metallic coupons and a variety of produce, resistant fungi and bacteria that are usually present in this type of goods were chosen for its inoculation. Coupons were inoculated with *B. pumilus* spores and all produce was inoculated with *E. coli* bacteria to study disinfection capabilities of plasma at different exposure times. The analysis was done by SEM imaging and calculation of bacteria and fungi log reduction.

Since the coupons and produce will be exposed not only to plasma light discharge but also to vacuum pressures, a blank test with maximum exposure time of 60 min for coupons and 15 min for produce to vacuum without plasma power was performed. Then, each set of coupons and produce were exposed to plasma treatment for 5, 10, and 15 min. An additional test was done only for coupons for 30 and 60 min to determine greater reduction with longer exposure.
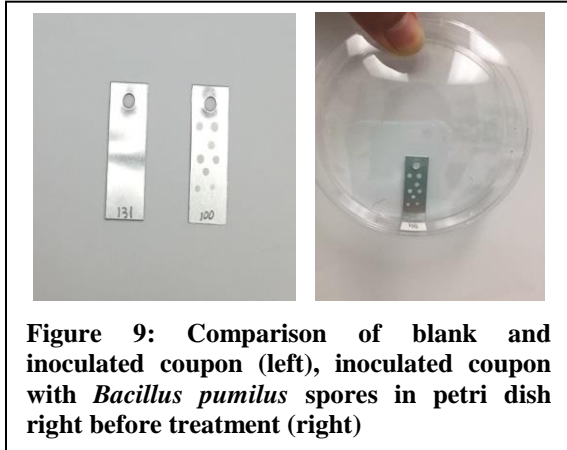
Figure 9: Comparison of blank and inoculated coupon (left), inoculated coupon with *Bacillus pumilus* spores in petri dish right before treatment (right)

Inoculated coupons were received for testing. Triplicate runs for each trial was implemented to ensure reproducibility of the treatment conditions. Figure 9 shows a picture of the coupons before and after inoculation. Each run had a total of 8 coupons that were later distributed into four coupons for SEM and four coupons for log reduction testing. Therefore, each trial had a total of 24 coupons for testing at different times. The first set called T = 0, were the coupons exposed for 15 min no power only vacuum by a subsequent 60 min test under the same conditions. The succeeding set of coupons called T = 5, were exposed to plasma for 5 min, followed by T = 10, and T = 15 exposed for 10 min and 15 min respectively. The coupons were placed hanging into the available rack hooks and coupon numbers were written for each run. Figure 10 illustrates a picture of the coupons being exposed to plasma at 0.10 mbar pressure. Several spectrograms of the substance fragments produced by the air plasma and the irradiated metallic coupons were also recorded during the radiation process at different time intervals and results will be discuss in the following section. Once the coupons were exposed to plasma disinfection, the items were inspected for any physical variation but no changes were observed. The only evident change was an increase in temperature which was measured in the next set of trials.
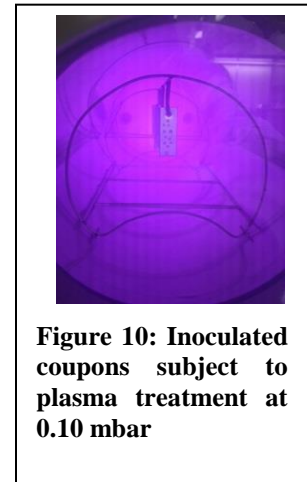
Figure 10: Inoculated coupons subject to plasma treatment at 0.10 mbar

SEM images showed a significant reduction and destruction of spores but not complete elimination was observed. However, it was detected that the higher the time of exposure, the greater the spore reduction. Because of this trend determination, a new trial was set with duplicate runs of 30 and 60 min exposure to plasma, T = 30 and T = 60 respectively. SEM and log reduction studies still have to be completed. Also, since an increase in temperature was observed in the initial studies, temperature measurements were performed with an initial T = 19.0 ˚C and an average final T = 37.3 ˚C ± 0.4. This increase in temperature might have to be studied in the future to see if it affects the destruction of the spores.
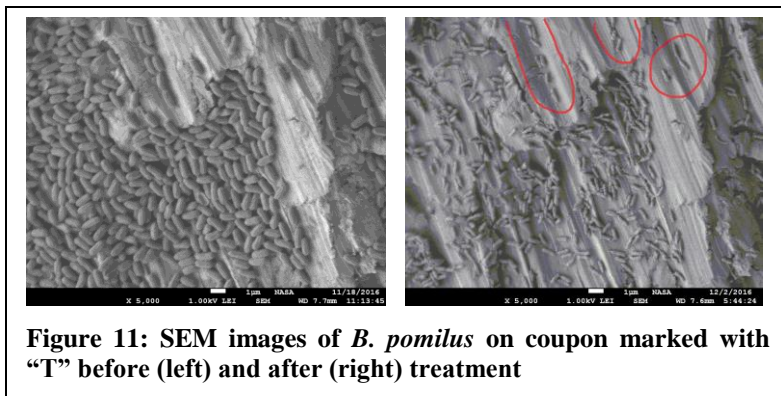
Figure 11: SEM images of *B. pomilus* on coupon marked with "T" before (left) and after (right) treatment

F. Plasma treatment of inoculated coupons with Bacillus pumilus over marked coupons

Based on the results stated in the previous section, where a significant reduction and destruction of the spores was observed, an additional test to complement these outcomes was planned. The main purpose was to determine if the reduction observed was based on the size of the spores (physical appearance) or an actual destruction reducing the amount of them (per count). In order to determine this, each coupon was marked with a "T" and a single spot containing the spores was deposited on top. This would allowed to focus the SEM image at the exact same place before and after treatment, followed by a count in the number of them. A significant reduction was observed after 30 min exposure; a quick assessment seems to show no significant reduction in the number of spores, but that the obvious visual difference is mostly due to the reduction in size. Figure 11 shows a picture of the SEM images for the coupon before and after treatment at 30 minutes.

G. Plasma treatment of inoculated coupons with Aspergillus niger over marked coupons

This new set of spore testing was also performed on marked coupons with a "T" in order to take SEM images in the same exact spot before and after treatment. The coupons containing *A. niger* were received for testing but in this case each coupon had only one spot of the inoculated spores instead of the multiple spots as it was observed before with the *B. pomilus* inoculated coupons. One of the important characteristics to consider in this set, is the pigment of the spores; a dark spot was observed in every coupon before treatment. Figure 12 shows a picture of the coupon before treatment where the pigmentation can be clearly observed. The same triplicate runs for each trial were implemented to ensure reproducibility of the treatment conditions. Each run had a total of 4 coupons, one of the coupons was previously set apart for SEM image before treatment; then, the four coupons were treated at the same time and the same coupon previously selected was once again submitted for SEM imaging; the other

Figure 12: Inoculated coupon with Aspergillus niger over marked "T" before treatment

three coupons were later distributed for log reduction testing. Therefore, each trial had a total of 12 coupons for testing at different times. The first set called T = 0, were the coupons exposed for 30 min no power only vacuum by a subsequent 30 min test under the same conditions. The succeeding set of coupons called T = 5, were exposed to plasma for 5 min, followed by T = 10, and T = 15 exposed for 10 min and 15 min respectively. The coupons were placed hanging into the available rack hooks and coupon numbers were written for each run. It is important to note that the coupons exposed to only vacuum (T = 0) did not show any discoloration of the pigments. On the other hand, all the coupons exposed to any amount of plasma showed discoloration. Figure 13 illustrates a picture of the coupons being exposed to only vacuum and to 15 min plasma. As it can be observed the coupon exposed to vacuum only kept the pigmentation intact as opposed to the coupon that was exposed to plasma treatment. After analyzing the SEM images and observing the significant reduction and destruction of spores (Figure 14), two conclusions could be drawn: one is that the plasma is making the spores lose its pigmentation or the plasma is killing such a great amount of spores that it losses the pigment having only a few left in the coupon. The second conclusion could be more accurate as the SEM pictures really show not only a reduction in the size of the spores but also a reduction of its amount.
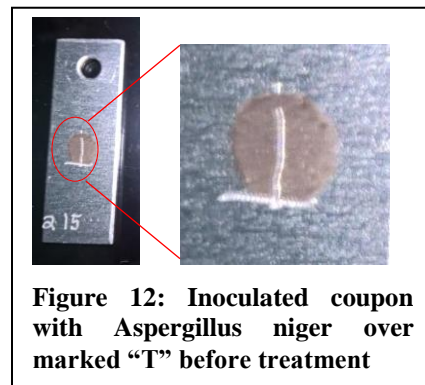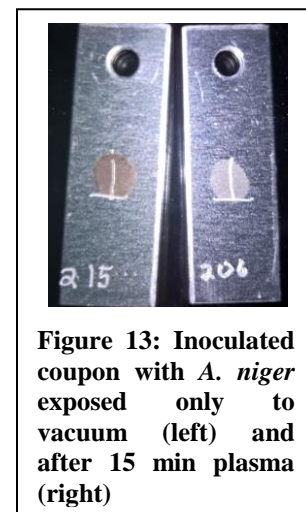

Figure 13: Inoculated coupon with *A. niger* exposed only to vacuum (left) and after 15 min plasma (right)
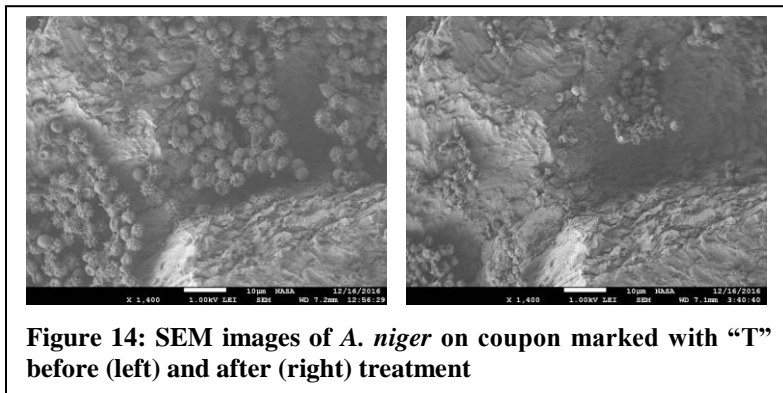

Figure 14: SEM images of *A. niger* on coupon marked with "T" before (left) and after (right) treatment

H. Coupon microbiological analysis after plasma treatment

A total of four coupons were tested in each triplicate run for each exposure time. As with the radishes, plasma durations were 0, 5, 10, and 15 minutes at 0.10 bar pressure. To determine the number of viable endospores present after treatment, the most probable number method was used. Coupons were placed into 10 mL of sterile pure water in glass test tubes. The tubes were vortexed for 2 minutes followed by sonication in a bath sonicator for 15 minutes to remove spores. It was observed that the inoculum spots on the 10 and 15 min treated coupons readily dissolved in the water before vortexing and sonication unlike the 0, 5 min and untreated control coupons. Spore suspensions were serially diluted in 9 mL of sterile water and 20 uL of each dilution was pipetted in triplicate into two columns (16 wells) of a 96 well plate containing 180 µL MPN medium. The plates were incubated at 35°C and read after 40

hours. The number of wells with growth out of 16 for each dilution were recorded and a Most Probable Number table was used to calculate the number of viable bacteria per coupon. Dilutions were also plated onto nutrient agar to confirm counts and detect any contaminants. 15 and 10 minute exposures to the plasma resulted in a > 5 $\log_{10}$ reduction (Figure 15). We plan to lower the detection limit in the next series of tests so that we can detect a 6 $\log_{10}$ reduction. Based on the theoretical D value (treatment time required for a 1 decimal reduction in viable cells) calculated as 2.17 minutes based on the 5 minute treatment, a 15 minute exposure should decrease the number of viable cells on the coupon by 7 $\log_{10}$. This would result in killing approximately 10^7 cells on the surface of the coupon in 15 minutes. We plan to test 30 and 60 minute exposure times.

IV. Conclusions

Low pressure cold plasma was used in order to observe its effects in the sterilization and/or inactivation of microorganisms, using different strains spores and fungi. CP was successful with respect to inactivation of microbes in produce and metallic coupons. However, produce quality and physical properties were lost making this process not viable for space applications. Nonetheless, the coupons used as a simulation of medical instrumentation showed promising results with a reduction greater than 5 $\log_{10}$ after 10 and 15 minutes of plasma exposure.
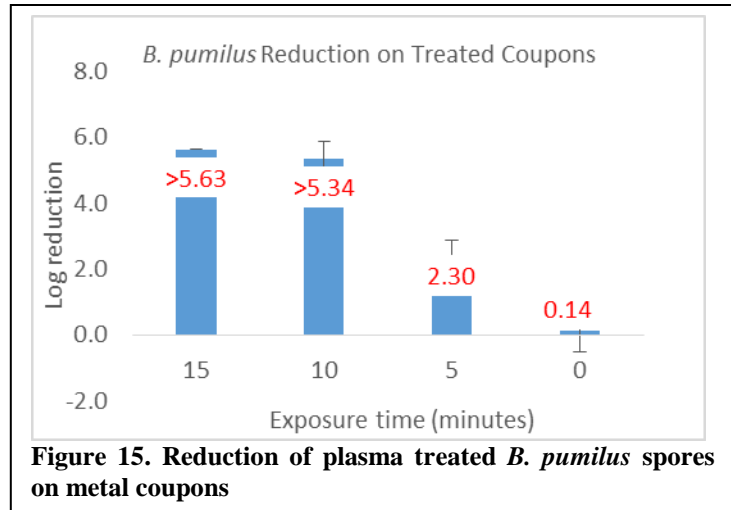
Figure 15. Reduction of plasma treated *B. pumilus* spores on metal coupons

V. Acknowledgements

References

A Lacombe, B. A. (2015). Atmospheric cold plasma inactivation of aerobic microorganisms on blueberries and effects on quality attributes. *Food Microbiology*, 479-484.

D Bermudez-Aguirre, E. W.-C.-P. (2013). Effect of atmospheric pressure cold plasma (APCP) on the innactivation of Escherichia coli in fresh produce. *Food Control*, 149-157.

N N Misra, K. M. (2014). In-package atmospheric cold pressure cold plasma treatment of cherry tomatoes. *Journal of Bioscience and Bioengineering*, 177-182.

N Philip, B. S. (2002). The respective roles of UV photons and oxygen atoms in plasma sterilization at reduced gas pressure: the case of N2-O2 mixtures. *IEEE Transactions on Plasma Science*, 1429-1436.

T B Klampfl, G. I. (2012). Cold atmospheric air plasma sterilization against spores and other microorganisms of clinical interest. *Applied and Environmental Microbiology* , 5077-5082.