International Online Plasma Seminar (IOPS)

Atmospheric Cold Plasma Treatment impact on Distillers Wet Grains

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BIOLOGICAL & AGRICULTURAL ENGINEERING
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Post Harvest and Engineering EDucation Research

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Outline

- ACP in package treatment
- Feed and Feedstock Storage Issues
- Results and Discussion
  - Ionization and Modified Atmosphere Combination Treatment
  - Critical Parameters for ACP treatment of Biological Materials
- Conclusion

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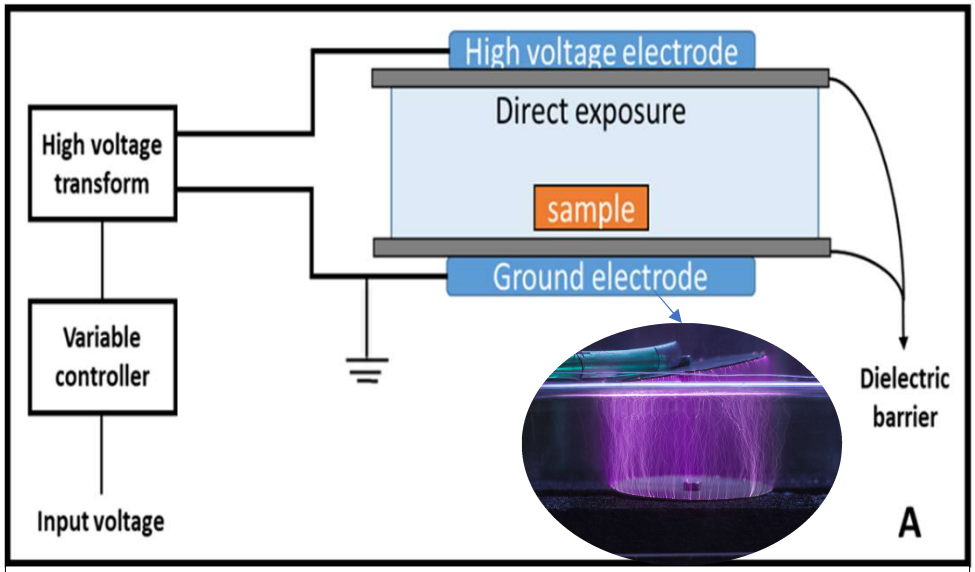
High Voltage Atmospheric Cold Plasma

- Inactivate bacteria, molds, yeasts, and other pathogens on agricultural products including fruits & vegetables, herbs & spices, food grains & nuts and meat & meat products.
- Dielectric barrier discharge
- Reactive Gas Species and Reactive Oxygen Species



Atmospheric Cold Plasma system with power input, high voltage, and dielectric barriers. Plasma image with system at 70kV, visible RGS creation.

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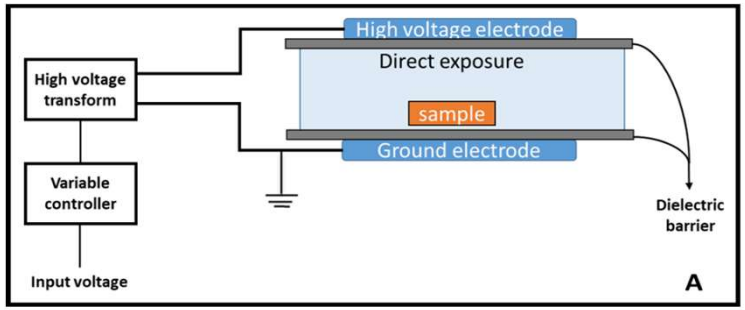
Operation of ACP Technology



- For air, ozone, nitrogen oxides, peroxides and other RGS are produced inside of the sealed container
- Works with any gas (air, oxygen, nitrogen, helium, argon, carbon dioxide)
- Multiple types of packaging materials – plastics, glass
- Proven bactericidal effects
- RGS decay back to their original state
- Operate between 30-120kV at 60Hz using less than 200 W power

Air Plasma Chemistry

Table 1. Ground neutrals considered.

Ground neutrals
N, N_2, O, O_2, O_3
NO, NO_2, NO_3, N_2O, N_2O_5
H_2O, H, OH, H_2, HO_2
H_2O_2, HNO_x ($x = 1, 2, 3$)
CO, CO_2, HCO, Ar

Table 2. Excited neutrals considered.

Excited neutrals
$N_2(A\,^3\Sigma_u^+, B\,^3\Pi_g, C^3\,\Pi_u, a\,^1\Pi_g, a'\,^1\Sigma_u^-)$
$O_2(a\,^1\Delta_g, b\,^1\Sigma_g^+)$, $O(^1D, ^1S)$
$NO(A\,^2\Sigma^+)$, $N(^2D, ^2P)$, $Ar(^3P)$
$CO_2(0\,0\,1, 0\,1\,0, 1\,0\,0)$
$N_2(X\,^1\Sigma_g^+\,(v = 1, \ldots, v = 8))$

Table 3. Electrons and negative ions considered.

Negative ions
e, O^-, O_2^-, O_3^-
NO^-, NO_2^-, NO_3^-
H^-, OH^-, $H_2O_2^-$, $H_2O_3^-$, $H_2O_4^-$, $H_3O_2^-$
CO_3^-, CO_4^-

Table 4. Positive ions considered.

Positive ions
N_2^+, $N_2^+(B\,^2\Sigma_u^+)$, N_4^+
O^+, O_2^+, O_3^+, O_4^+
NO^+, NO_2^+, N_2O^+, $N_2O_2^+$
H_2O^+, H_3O^+, OH^+
Ar^+

J. Phys. D: Appl. Phys. **41** (2008) 234016 (33pp)

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Trends in Biotechnology

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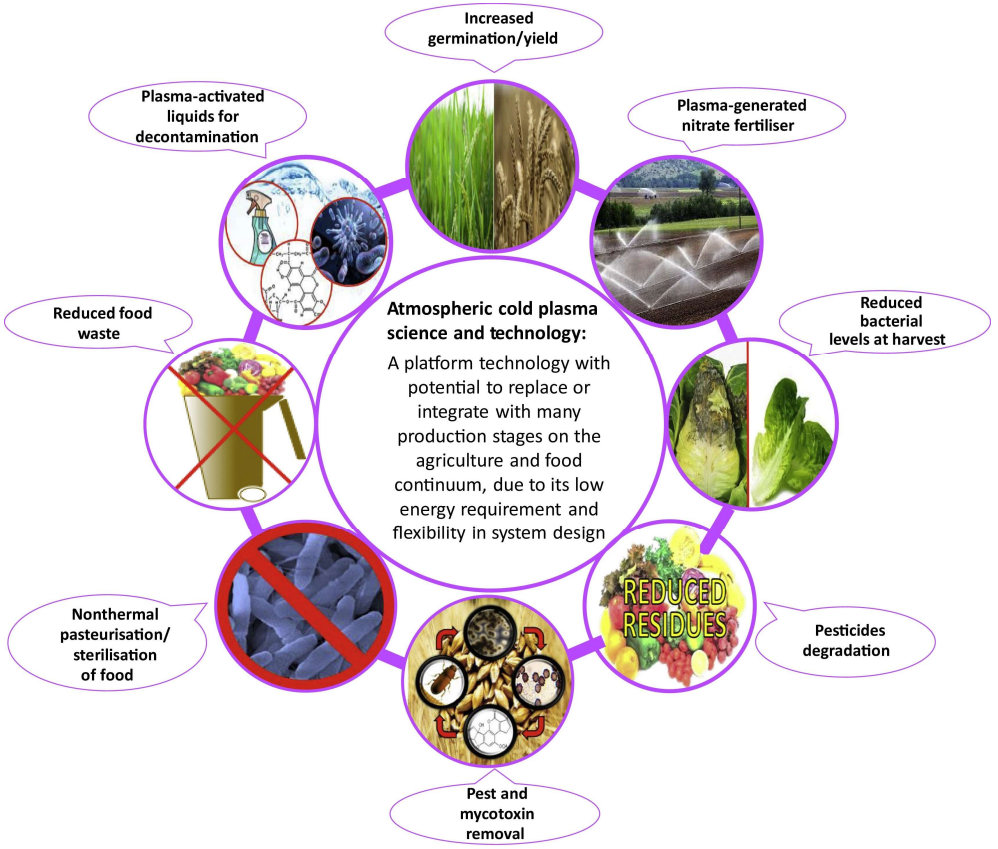
The Potential of Cold Plasma for Safe and Sustainable Food Production

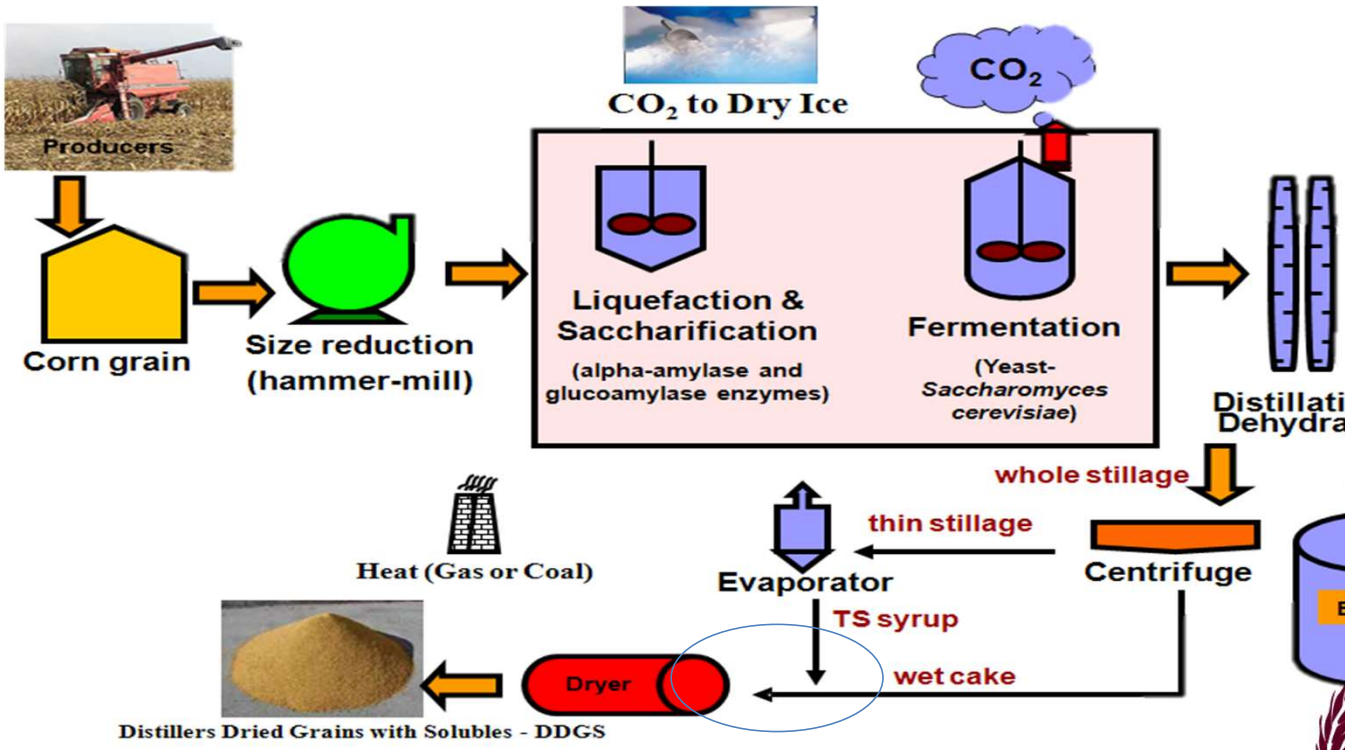
Paula Bourke • Dana Ziuzina • Daniela Boehm • Patrick J. Cullen • Kevin Keener

Published: January 09, 2018 • DOI: https://doi.org/10.1016/j.tibtech.2017.11.001 • Check for updates



DWG and Solubles Production

1 bushel of corn (25.4kg)
11.8 liters of ethanol
7.7 kg of DDGS



Schematic diagram of dry grind process of corn ethanol production (Ileleji, 2009)

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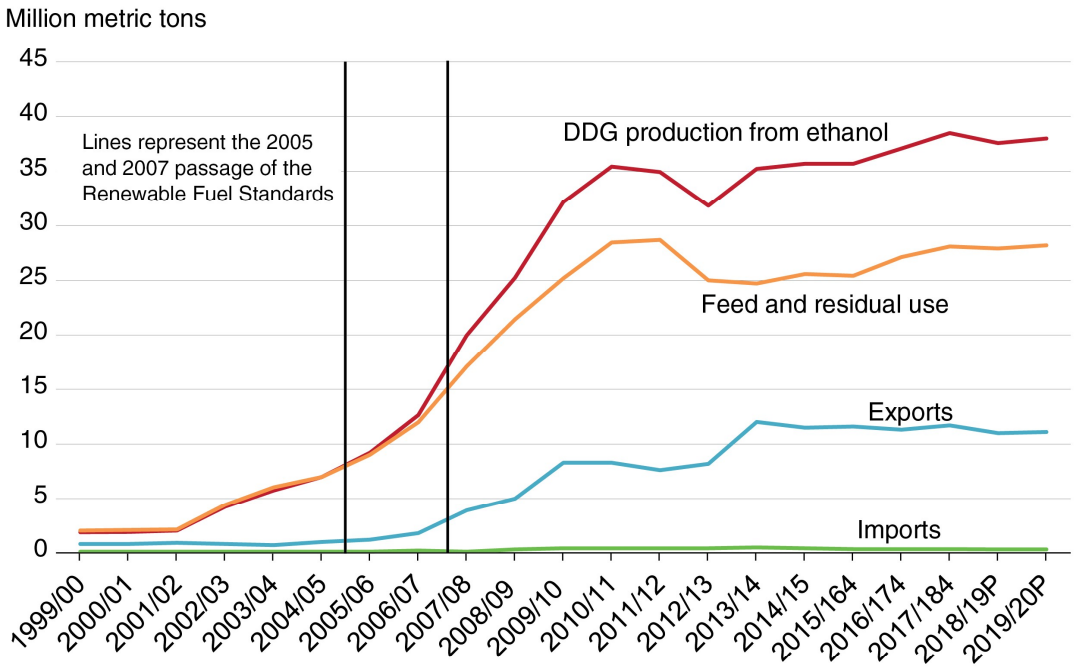
Distillers Grains as Animal Feed

Dried distillers' grains (DDGs) supply and use has risen in concert with ethanol fuel production



Note: P = projection. 2018/19 and 2019/20 data are projections. DDG = Dried distillers' grains.
Source: USDA, Economic Research Service Bioenergy Statistics data.

- High in Protein & Energy
  - 120 -135% nutrition of corn
- pH is low due to sulfuric acid during fermentation
- Wet Distillers Grains (DWG) – 30 % DM
  - Use within 7-10 days
- Dried Distillers Grains (DDG) – 90% DM
  - Easy to handle and transport

Current Storage Methods

DWG (without solubles) stored straight in a bunker lined with plastic and round bales. The pile was treated on the surface with 1 lb. of stock salt per square foot then covered in plastic

Visible mold growth

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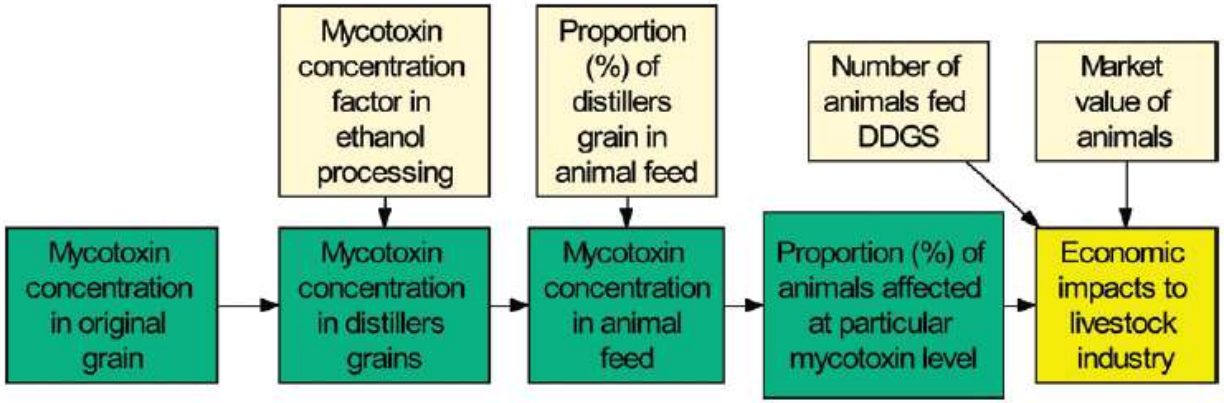
Higher mycotoxin level in Distillers Grains



Results in mycotoxin level ~3 times greater than level in original grain

(Wu et al., 2008)

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Table 2. Past Research on Atmospheric Cold Plasma Treatment of Various Stored Grains and Feed

Stored Product	Voltage	Freq	Gas Comp	Organism	Log Reduction	Treatment time	Author
Distillers Wet Grains	70 kV	60 Hz	$O_2/CO_2/N_2$; package DBD	Total mesophilic	2.23	6 min	McClurkin Moore et al., 2017
Wheat grain	80 kV	50 Hz	Air; package DBD	Yeast	2.5	20 min	Los et al., 2018
Barley grain	80 kV	50 Hz	Air; package DBD	Total mesophilic	2.4	20 min	Los et al., 2018
Maize	10 kV	25 kHz	Air; plasma jet	A. flavus and A. parasiticus	5.48 and 5.20	1-5 min	Dasan et al., 2016
Maize	90 kV	50 Hz	$O_2/CO_2/N_2$; package DBD	Aflatoxin	89-90% reduction	30 min	Shi et al., 2017
Brown rice	10 kV		Argon; plasma jet	A. flavus	20-day shelf life extension	20 min	Suhem et al., 2013b

Major Goal

- To increase the shelf-life of wet distillers grains; to triple the shelf-life of DWG from its current
  - 3-4 days in warm summer conditions and
  - 5-7 days in cold winter conditions

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Determine the effect of feedstock chemistry on the shelf-life of DWGS stored under warm temperatures (20 – 30°C) and cold temperatures (0 – 15°C)

Hypothesis: DWGS samples will deteriorate less under cold temperatures than warm temperatures.

McClurkin, J., Ileleji, K., 2015. The effect of temperature and condensed distillers solubles on the shelf-life of distillers wet grains stored aerobically. Journal of Stored Product Research, 62, 58-64.

Part one

Methods

CO_2 Kit

Digital Color Reader

<u>Also Tested</u>

MC

N.F.T.A. 2.2.2.5 - 2 g
105°C for 3 hours

CFU

Dilution plating on MSA

Mycotoxin

AgraQuant ELISA

pH

pH solids electrode

Water Activity Measurement

Free Fatty Acid Determination

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RESULTS

Sample analysis for DWG at 3 different blends stored for 7 days at 3 different temperatures

Storage Temp	CDS (%)	MC (d.b.) Initial	MC after 7 day storage	A_w Initial	A_w after 7 day storage	pH Initial	pH after 7 day storage	FA Initial (mgKOH/100g)	FA after 7 day storage
10	0	74.14(0.95)[a]	62.42(3.16)[b]	0.98(0.00)[a]	0.98(0.00)[b]	3.81(0.03)[a]	3.79(0.02)[a]	1.64E+02(1.50E+01)[a]	4.95E+02(6.05E+01)[b]
10	20	73.00(1.59)[a]	65.22(2.40)[b]	0.97(0.00)[a]	0.98(0.00)[b]	3.78(0.02)[a]	4.92(0.47)[b]	2.01E+02(1.30E+01)[a]	5.57E+02(3.90E+01)[b]
10	30	70.39(0.17)[a]	55.24(0.19)[b]	0.97(0.00)[a]	0.97(0.00)[a]	3.77(0.01)[a]	3.94(0.01)[b]	2.57E+02(7.10E+00)[a]	2.45E+02(2.01E+01)[a]
20	0	72.58(7.27)[a]	34.56(7.61)[b]	0.98(0.00)[a]	0.98(0.01)[a]	4.03(0.04)[a]	5.62(0.29)[b]	1.64E+02(1.50E+01)[a]	3.22E+02(6.45E+01)[b]
20	20	68.89(5.80)[a]	32.32(4.54)[b]	0.98(0.00)[a]	0.97(0.02)[a]	4.02(0.02)[a]	5.37(0.13)[b]	2.01E+02 (1.30E+01)[a]	3.34E+02(7.36E+01)[a]
20	30	71.06(0.32)[a]	32.89(1.71)[b]	0.97(0.00)[a]	0.96(0.01)[a]	4.01(0.13)[a]	5.44(0.09)[b]	2.57E+02(7.10E+00)[a]	4.65E+02(5.14E+01)[b]
25	0	74.14(0.95)[a]	39.67(10.03)[b]	0.98(0.00)[a]	0.93(0.00)[b]	3.81(0.03)[a]	5.88(0.22)[b]	1.64E+02(1.50E+01)[a]	3.47E+02(7.07E+00)[b]
25	20	73.00(1.59)[a]	35.94(1.68)[b]	0.97(0.00)[a]	0.95(0.00)[b]	3.78(0.02)[a]	5.76(0.32)[b]	2.01E+02(1.30E+01)[a]	4.19E+02(5.31E+01)[b]
25	30	70.39(0.17)[a]	30.90(2.23)[b]	0.97(0.00)[a]	0.96(0.00)[b]	3.77(0.01)[a]	5.69(0.36)[b]	2.57E+02(7.10E+00)[a]	4.81E+02(8.49E+01)[b]

*standard deviation in parentheses

[a] Within temperature and CDS level, the means for indicators (MC, aw, pH and FA) before storage and after 7 days of storage followed by different letters are significantly different (P<0.05; t-test).

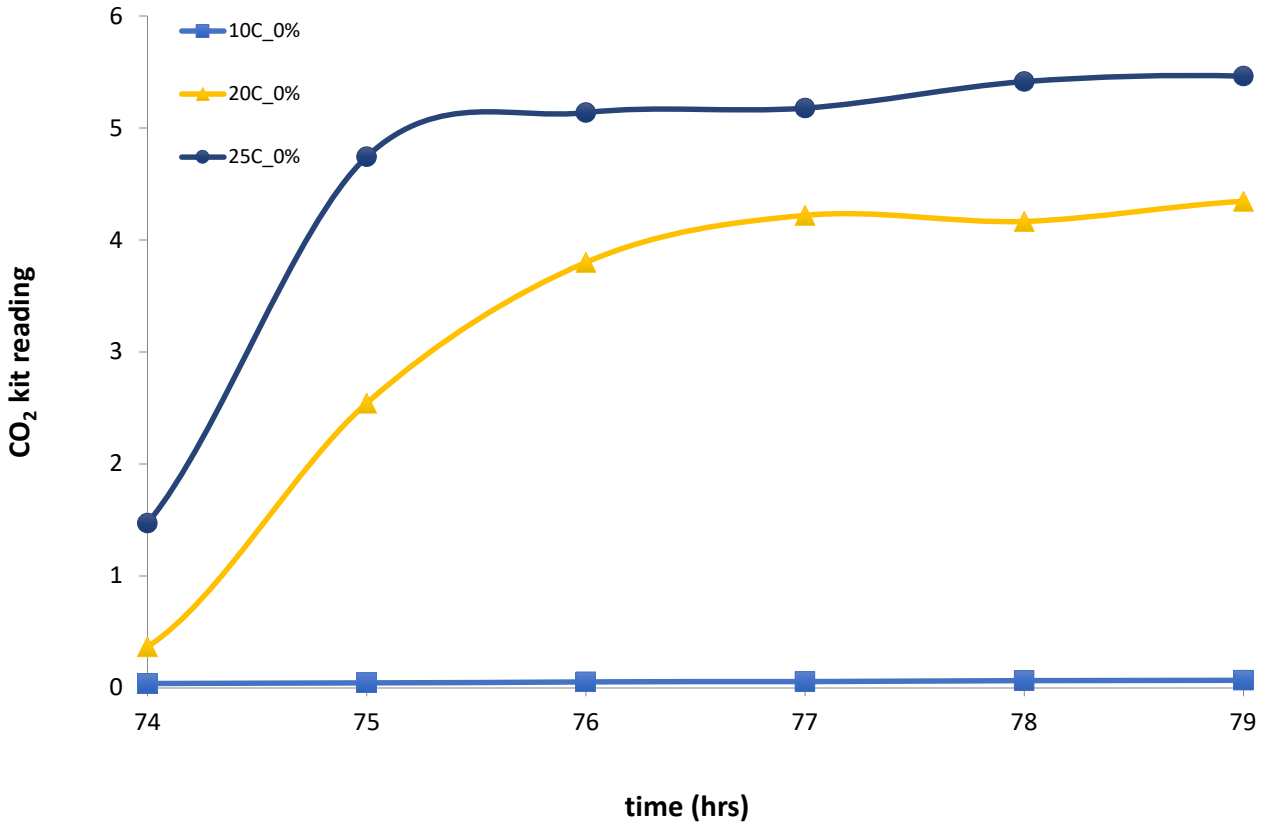
Microbial Analysis for DWG at 3 different blends stored for 7 days at 3 different temperatures

Storage	CDS (%)	CFU/g Initial	CFU after 7 days storage	AFLA (ppb) Initial	AFLA (ppb) after 7 day storage	FUM (ppm) Initial	FUM (ppm) after 7 day storage	ZEA (ppm) Initial	ZEA (ppm) after 7 day storage
10	0	2.00E+03^{a}	8.87E+04(2.92E+06)^{bA}	1.47^{a}	1.63(0.04)^{bA}	1.35^{a}	1.27(0.29)^{aA}	0.19^{a}	-0.03(0.00)^{bA}
10	20	3.78E+03^{a}	1.78E+05(1.80E+05)^{aA}	1.54^{a}	1.55(0.07)^{aA}	0.63^{a}	1.23(0.06)^{aA}	0.33^{a}	0.13(0.02)^{bA}
10	30	4.67E+03^{a}	2.07E+05(2.00E+04)^{bA}	1.94^{a}	1.41(0.02)^{bA}	0.59^{a}	0.87(0.76)^{aA}	0.55^{a}	0.19(0.02)^{aA}
20	0	2.00E+03^{a}	3.70E+04(9.65E+05)^{aB}	1.47^{a}	1.55(0.13)^{aA}	1.35^{a}	2.12(0.38)^{bB}	0.19^{a}	0.05(0.03)^{bA}
20	20	3.78E+03^{a}	2.00E+05(3.61E+05)^{aB}	1.54^{a}	1.42(0.08)^{aA}	0.63^{a}	1.84(0.40)^{aB}	0.33^{a}	0.09(0.04)^{bB}
20	30	4.67E+03^{a}	1.56E+05(1.02E+05)^{aB}	1.94^{a}	1.32(0.01)^{bA}	0.59^{a}	0.29(1.12)^{bB}	0.55^{a}	0.05(0.04)^{bB}
25	0	2.00E+03^{a}	3.06E+05(4.65E+06)^{aC}	1.47^{a}	1.24(0.18)^{aA}	1.35^{a}	3.16(1.03)^{bB}	0.19^{a}	-0.05(0.04)^{bB}
25	20	3.78E+03^{a}	3.04E+05(1.89E+05)^{aC}	1.54^{a}	1.42(0.08)^{aA}	0.63^{a}	2.42(0.96)^{aB}	0.33^{a}	-0.03(0.05)^{bB}
25	30	4.67E+03^{a}	3.63E+05(2.33E+05)^{aC}	1.94^{a}	0.96(0.54)^{bA}	0.59^{a}	3.20(0.79)^{bB}	0.55^{a}	0.00(0.02)^{bB}

*FDA Guidance Levels: Aflatoxin can be no more than 20ppb in lactating dairy feeds and up to 300ppb for finishing (feedlot) beef cattle, 100ppb for swine. Fumonisin levels must not exceed 60ppm for ruminants less than 3 months old being raised for slaughter and 30ppm for breeding ruminants, 20ppm for swine. Zearalenone no FDA guidance levels are available.

b.d. – indicates readings were below detection level of AgraQuant Testing Kit

Comparison of changes in CO_2 for 0% CDS inclusion at varying temperatures



CONCLUSIONS

Conclusions

- Temperature had the greater effect on the parameters measured than CDS content.
- Of the parameters, which correlate to shelf-life, FA, pH and CFU values were sufficient in expressing spoilage.
- Mycotoxin levels did not exceed FDA threshold.
- The study indicated that DWGS deteriorated less at 10°C (cool weather) than at 20 or 25°C (warm weather).

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Implications

- It is necessary to find a process that can address the microbial degradation that occurs in the product over time. The next objective will work to find a treatment method that can increase the shelf life 3-4 fold.

Determine the effect of ionization and modified atmosphere with CO_2 and their combination on shelf-life of DWG.

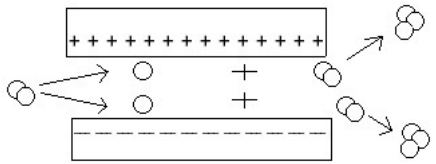
Hypothesis: The combination treatment of CO2 and ionization will increase shelf-life of DWG 3-4 fold.

McClurkin-Moore, J.D., Ileleji, K.E., & Keener, K.M, 2017. The effect of high-voltage atmospheric cold plasma treatment on the shelf-life of distillers wet grains. Food and Bioprocess Technology 10, 1431.

Part One

Increase Storage Time (Shelf-Life)

- Elimination of microbial growth through sterilization via HVACP treatment



- Suppression of microbial growth through CO_2

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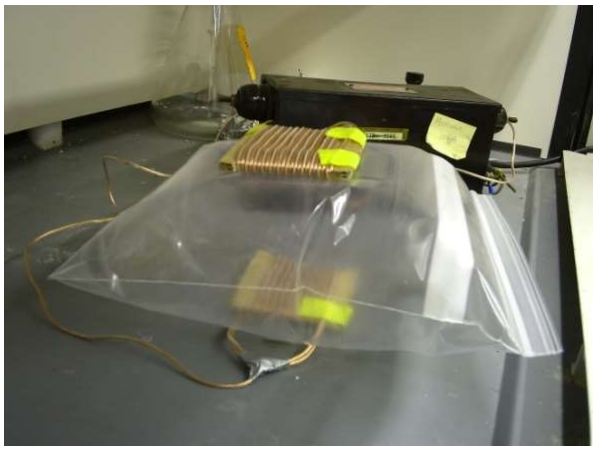
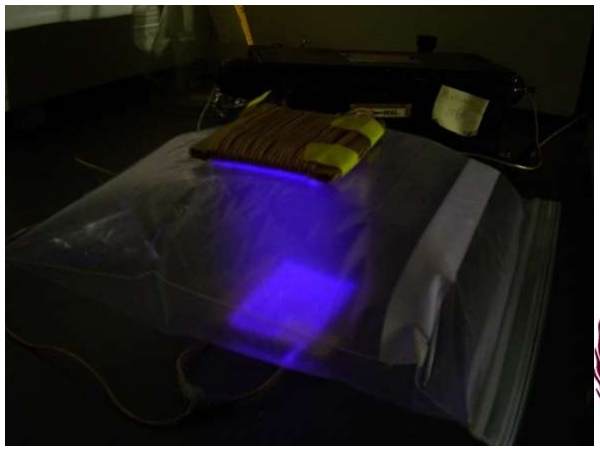
High Voltage Atmospheric Cold Plasma

- HVACP
  - In oxygen, currently generate 3,000 ppm ozone with 5 min treatment. Potential to increase generation rates with system modifications.
  - Ozone designated as “GRAS” (Generally Recognized As Safe) by FDA.





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METHODS

- Treatment
  - non-thermal, atmospheric plasma that creates ionization species which will be used to sterilize already packaged DWGS (Klockow & Keener, 2009)
- Analysis
  - Moisture Content - N.F.T.A. 2.2.2.5 - 2 g of sample, 105°C for 3 hours
  - pH - Mettler-Toledo pH Electrode LE407, Solids Probe
  - Microbial Load (CFU) on PCA – Dilution plating with 0.01% Peptone Water

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28 day storage test set-up

- Design Matrix for 25g of Sample

	Control	Elimination	Suppression	Combination
Time (s)	0	360	0	360
Temperature	10°C, 25°C	10°C, 25°C	10°C, 25°C	10°C, 25°C
Ionization level	0kV	70kV	0kV	70kV
Fill Gas	MAP	MAP	CO_2- Dry Ice	MAP/CO_2

MAP is modified atmosphere gas combination - 65% O_2 /30% CO_2 / 5% N_2

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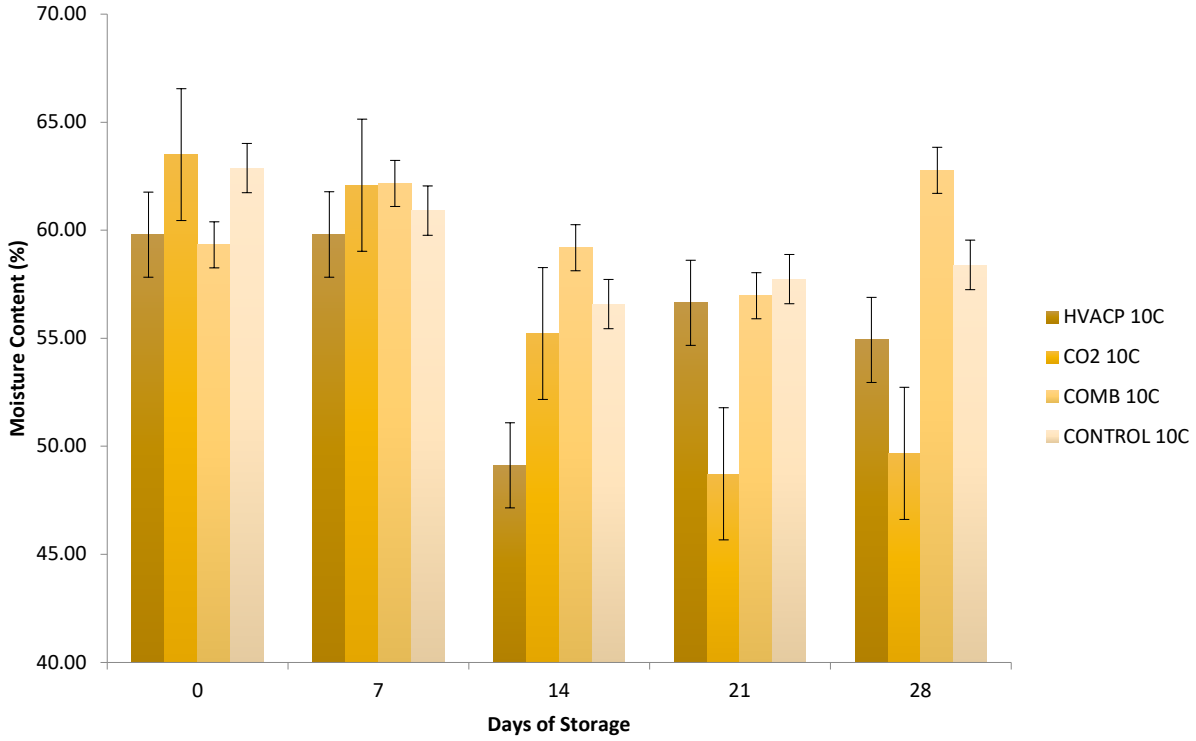
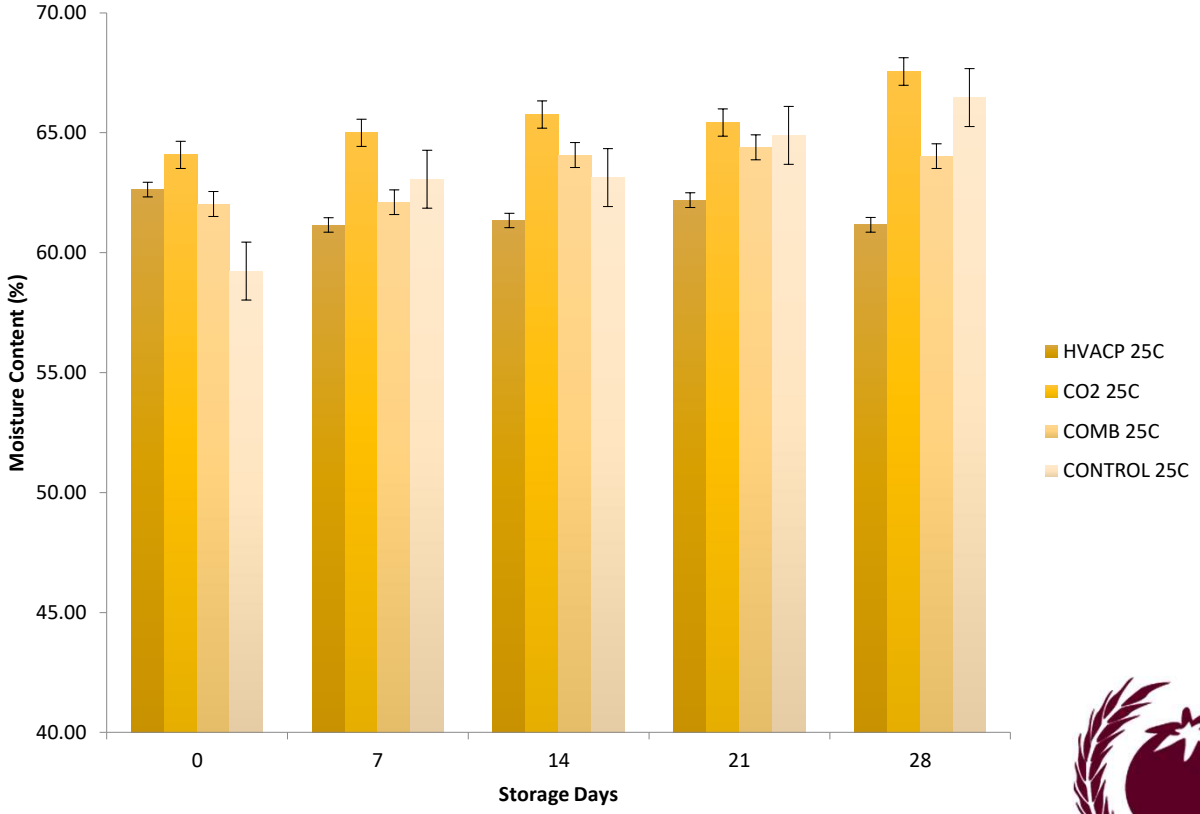
Results

Moisture content values for treated samples and control stored at 10°C over 28 day period



Moisture content values for treated samples and control stored at 25°C over 28 day period



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H_2O_2 for Treated Samples stored at 10°C & 25°C

		H_2O_2 Strip (100 mg/l H_2O_2)				
Storage Days		0	7	14	21	28
10°C	HVACP	100	65	100	30	20
25°C	HVACP	100	100	30	55	5.5
10°C	Comb	100	65	10	6.5	2
25°C	Comb	100	100	65	30	1

*Values are the average of results from two test strips, each strip measures 0, 1, 3, 10, 30, or 100 mg/l (H_2O_2)

Although peroxide values at day 14 for HVACP samples appear to be out of place they show that peroxides are still present.
We know that samples on these days had high microbial loads, and therefore must assume that there is an anomaly on this day for these samples.

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pH values for treated samples and the control stored at 10°C and 25°C over 28 day period

- There are microorganisms that are acidifiers (i.e. Aspergillus spp., Lactobacillus spp. and Penicillium spp.) present in the sample after 28 days. Likewise, at 25°C, the increase in pH could be attributed to the waste products from these bacteria. Therefore, we think that temperature induced mold growth influenced the difference for the changes in pH.

Temp	Treatment					
Storage Days		0	7	14	21	28
10	HVACP	$3.72\pm0.01^{a\,2}$	$3.85\pm0.06^{a\,2}$	$4.13\pm0.13^{b\,2}$	$3.60\pm0.01^{b\,4}$	$3.81\pm0.34^{b\,5}$
25	HVACP	$3.62\pm0.00^{a\,2}$	$3.75\pm0.13^{a\,2}$	$5.05\pm0.27^{b\,2}$	$5.83\pm0.19^{b\,2}$	$5.45\pm0.15^{b\,3\,4}$
10	CO_2	$4.31\pm0.07^{a\,1}$	$4.62\pm0.03^{a\,1\,2}$	$4.86\pm0.02^{b\,2}$	$5.30\pm0.08^{b\,2\,3}$	$4.73\pm0.18^{b\,4\,5}$
25	CO_2	$3.75\pm0.00^{b\,2}$	$4.26\pm0.13^{b\,2}$	$4.49\pm0.23^{b\,2}$	$7.84\pm0.60^{a\,1}$	$7.21\pm0.49^{a\,1\,2}$
10	Comb	$4.32\pm0.12^{a\,1}$	$4.62\pm0.16^{a\,1\,2}$	$4.81\pm0.13^{b\,2}$	$5.49\pm0.20^{b\,2\,3}$	$5.88\pm0.53^{b\,2\,3\,4}$
25	Comb	$3.70\pm0.03^{b\,2}$	$4.38\pm0.81^{b\,2}$	$4.89\pm0.00^{b\,2}$	$5.26\pm0.10^{a\,2\,3}$	$5.33\pm0.28^{a\,3\,4}$
10	Control	$3.76\pm0.00^{a\,1}$	$4.56\pm0.17^{b\,1\,2}$	$5.91\pm0.34^{b\,1\,2}$	$6.31\pm1.05^{b\,2\,3}$	$7.29\pm0.07^{b\,2\,3}$
25	Control	$3.76\pm0.00^{a\,2}$	$4.91\pm0.06^{b\,1}$	$4.96\pm0.06^{b\,1}$	$6.04\pm0.11^{c\,3}$	$6.40\pm0.14^{d\,1}$

[a-d] Within temperature and treatment, the means for indicators (storage days) followed by different letters are significantly different ($P<0.05$; Tukey).
[1-5] Within the storage day, the means for indicator (treatments) followed by different numbers are significantly different ($P<0.05$; Tukey).
n = 3, three replicates per storage period per treatment.

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pH

- Difficult to show the effect of plasma because different conditions can have different effects and interactions on microbes.
- Research has shown varying gas fill can have greater affect on gram negative compared to gram positive bacteria.
- For CO_2 we are displacing O_2 so the organisms that grow would be anaerobic.
- Lactobacilli are able to grow well in low pH high temperature environments

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Log Reduction of Microbial Load

Temp	Treatment	Log Reduction of microbial load (log_{10}CFU/g)				
Storage Days -		0	7	14	21	28
10	HVACP	2.23	2.86	-1.33	0.86	0.34
25	HVACP	2.00	3.23	-1.59	0.87	0.59
10	CO_2	-0.26	0.36	0.54	0.92	1.55
25	CO_2	0.99	0.45	0.20	-0.55	0.77
10	Comb	1.16	1.21	1.30	1.64	2.36
25	Comb	1.55	2.78	0.17	1.41	1.20

$$Log\ Reduction = log_{10}\left(\frac{A_c}{B_T}\right)$$

- For samples treated with HVACP and stored at 10°C the log reduction was 2.23 log (99.4% reduction) after initial ionization on day 0 changing to 0.34 log (54.3% reduction) at day 28.
- For the samples stored at 25°C there is a 2.00 log (99.0% reduction) reduction after initial ionization on day 0 reaching a 0.59 log (74.3%) reduction at day 28.

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- CO_2 limits the availability of oxygen where microbiological species are growing.
- An oxygen-depleted environment, with high levels of CO_2 can result in reduced growth of these common stored grain molds.
- CO_2 concentration above 14% is detrimental to mold growth (Nofsinger et al., 1983), however for some mold species, growth can occur at an oxygen concentration of about 0.2%.

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- The samples with the combination treatment stored at both 10°C and 25°C had the lowest microbial load and was able to maintain a consistent log reduction over time when compared to the other treatments.

Post-Harvest Engineering and Education

Implications

- This work showed that Microbial Load and Mycotoxin levels correlate to shelf life of DWG and that pH and FA values can be indicators of shelf life

Post-Harvest Engineering and Education

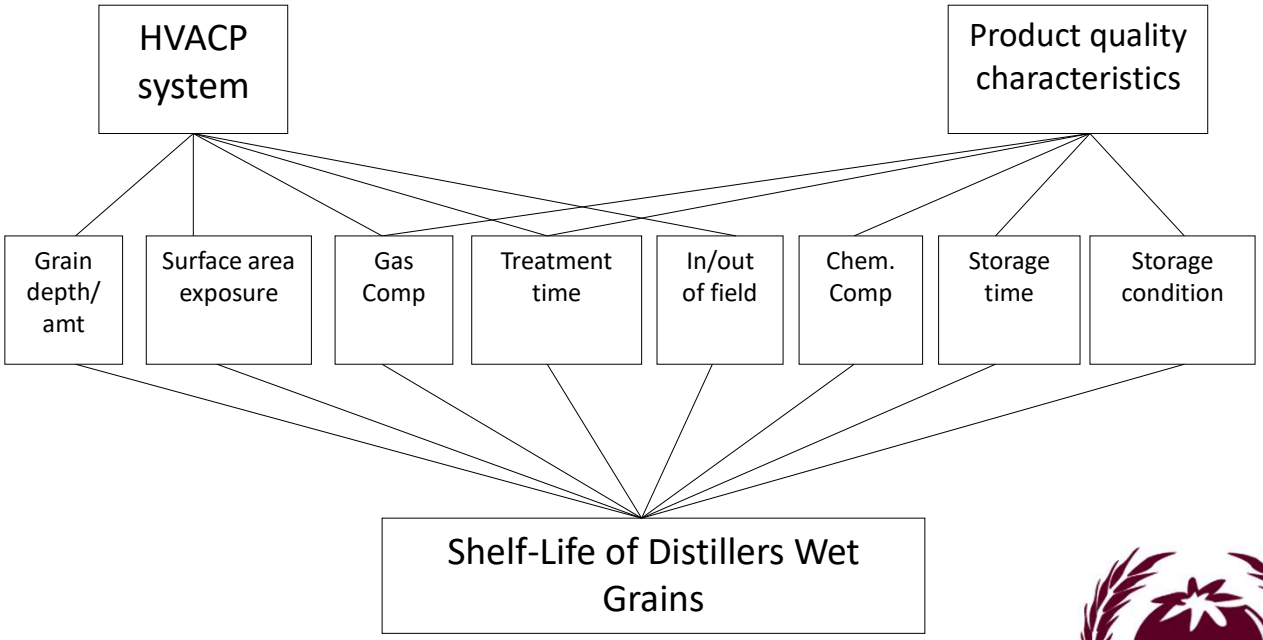
Identify and evaluate critical parameters for increase in shelf-life of DWG based on previous results.

Hypothesis: There are critical parameter that can be characterized to better model sterilization of DWG through ionization

> Moore, J.M., Ileleji, K.E., Keener, K., 2020. Factors that affect high voltage atmospheric cold plasma treatment efficacy on wet distillers' grains: Shelf-life and nutrient composition. Journal of Cereal Science, 103034. https://doi.org/10.1016/j.jcs.2020.103034

Part Two

Engineering a perfected process



Post-Harvest Engineering and Education

Model for Sterilization

- Characterize each parameter to determine levels of uncertainty in affecting sterilization
- Output for sterilization will be microbial log reduction
- Desire is to precisely control parameters to achieve the required sterilization conditions

Post-Harvest Engineering and Education

Package Gas Compositions

- Modified atmosphere gas blend of
  - 65% O_2 + 30% CO_2 + 5% N_2
- Air gas sample
  - 78% N_2 + 21% O_2 + 1% CO_2
- The increase in O_2 and CO_2 for the MA create increased opportunities for the production of ROS.

Post-Harvest Engineering and Education

Reactive Gas and Oxygen Species

- Formed during HVACP treatment
- Including O_3, CO, H_2O_2
- $O^{\cdot}$, $^{\cdot}OH$, $N^{\cdot}$, $HO_2^{\cdot}$, N_2^*, N^*, OH^-, O_2^-, O^-, O_2^+, N_2^+, N^+, NO, and NO_2

Post-Harvest Engineering and Education

METHODS

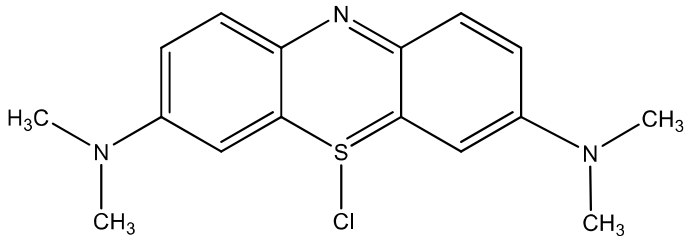
Methods

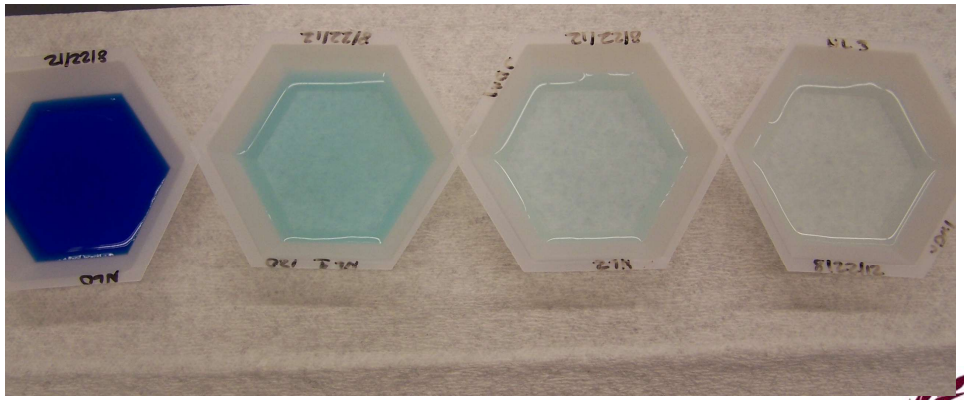
- Treatment
  - In-Field vs Out-of-Field
  - Sample amount (g) and grain depth – evaluate penetration of species through the grain mass
- Analysis
  - Methylene Blue Discoloration – evaluates efficacy of different gas concentrations (includes treatment time) and combinations
  - Microbial Analysis – evaluates efficacy of different gas concentrations (includes treatment time), as well as changes between samples treated in vs out of the field, and variations in bag size
  - Amino Acid Profiles (AOAC 982.3) and
  - ANOVA and stepwise regression polynomial model given by

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_n X_n + \varepsilon$$

Post-Harvest Engineering and Education

MB Discolourisation





Post-Harvest Engineering and Education

Test Grain Depth Penetration

Post-Harvest Engineering and Education

RESULTS

Analysis of aerobic plate count results compared with the change in color from no load MB test in "air" for samples "in" the field.

Sample	Microbial Count (Log_{10}CFU/g)	Log Reduction	% discoloration of MB (%)
Control	5.75(0.55)	0	3.95
Treatment 1 (120 sec)	4.78(0.73)	0.97	87.25
Treatment 2 (240 sec)	4.75(0.59)	1.00	97.13
Treatment 3 (360 sec)	4.98(0.95)	0.77	98.12

Analysis of aerobic plate count results compared with the change in color from no load MB test in "MA" for samples "in" of the field.

Sample	Microbial Count (Log_{10}CFU/g)	Log Reduction	% discoloration of MB (%)
Control	4.56(0.73)	0	0.30
Treatment 1 (120 sec)	3.28(0.37)	1.28	99.12
Treatment 2 (240 sec)	3.47(0.22)	1.09	99.63
Treatment 3 (360 sec)	3.29(0.26)	1.27	99.90

Post-Harvest Engineering and Education

Analysis of aerobic plate count results compared with the change in color from no load MB test in "air" for samples "out" the field.

Sample	Microbial Count (Log_{10}CFU/g)	Log Reduction	% discoloration of MB (%)
Control	4.97(0.58)	0	3.95
Treatment 1 (120 sec)	4.46(1.06)	0.51	87.25
Treatment 2 (240 sec)	4.80(1.26)	0.17	97.13
Treatment 3 (360 sec)	4.57(1.46)	0.40	98.12

Analysis of aerobic plate count results compared with the change in color from no load MB test in "MA" for samples "out" of the field.

Sample	Microbial Count (Log_{10}CFU/g)	Log Reduction	% discoloration of MB (%)
Control	3.40(0.00)	0	0.30
Treatment 1 (120 sec)	2.70(0.00)	0.70	99.12
Treatment 2 (240 sec)	3.20(0.28)	0.20	99.63
Treatment 3 (360 sec)	2.90(0.35)	0.50	99.90

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Reactive Oxygen Species

- MA has more O_2 so produces more O_3, and ROS
- Therefore, these species are most likely the main ones contributing to discoloration
- Fill gas and treatment time are the critical parameters (and significant parameters) of the HVACP system for optimal treatment and thus extending the shelf-life.
- The type of fill gas used determines which reactive gas species are produced in the package when placed in the HVACP system.

Post-Harvest Engineering and Education

Log reduction of microbial load after HVACP treatments of 3 different times and two different bag sizes.

			Full Bag	Half Bag
Amount (g)	Delay time (h)	Treatment time (s)	Log reduction	Log reduction
25	0	120	1.046[b]	0.845[b]
25	0	240	2.057[a]	0.982[a]
25	0	360	2.949[a]	1.766[a]
25	24	120	0.141[b]	1.681[b]
25	24	240	1.579[a]	2.438[a]
25	24	360	2.141[a]	2.681[a]
12.5	0	120	0.530[b]	1.000[b]
12.5	0	240	3.602[a]	2.073[a]
12.5	0	360	3.643[a]	2.750[a]
12.5	24	120	1.155[b]	0.176[b]
12.5	24	240	2.620[a]	0.834[a]
12.5	24	360	2.854[a]	2.477[a]

[a-b] Within the amount of sample and storage time the means for indicators (treatment time) followed by different letters are significantly different (P<0.05; Tukey).

- Gap space is fixed so changing the bag size meant changing the volume.
- Volume to Mass Ratio is reduced by half for “half” bag size
- Volume/Mass Ratio
  - 3.89L / 25g
  - 1.945L / 25g
  - 3.89L / 12.5g
  - 1.945L / 12.5g

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Volume to Mass Interaction

- Larger mass – half bag needed 24 hours to interact with and penetrate through the grain mass (species have less volume to move around package and can be concentrated on the product)
- Half-life of the gas species produced will be depleted before 24 hours after ionization with HVACP
- Smaller mass – 0 hours was sufficient for smaller amount of sample.

Post-Harvest Engineering and Education

Volume/Mass/Delay Time

- A given amount of gas volume for HVACP will create certain RGS
  - The more you have for a given mass the higher log reduction on microbial load. If you reduce gas volume you have less RGS.
- Which will require additional time before testing to allow for interaction with the product

Post-Harvest Engineering and Education

Amino Acid and Chemical Composition

	Treated Sample Analysis Day			Untreated Samples Analysis Day		
Item	0	14	28	0	14	28
Lysine	1.13(0.02)	1.12(0.03)	1.11(0.02)	1.12(0.05)	1.13(0.03)	1.12(0.04)
Methionine	0.56(0.01)	0.56(0.04)	0.58(0.01)	0.55(0.03)	0.59(0.02)	0.59(0.01)
Threonine	1.21(0.01)	1.20(0.06)	1.22(0.01)	1.21(0.06)	1.22(0.02)	1.23(0.02)
Tryptophan	0.23(0.01)	0.23(0.01)	0.22(0.00)	0.23(0.01)	0.24(0.01)	0.23(0.00)
Total	30.02(0.26)[a]	30.00(1.42)[a]	30.71(0.32)[a]	30.00(1.06)[a]	30.42(0.62)[a]	30.83(0.48)[a]

	Treated Sample Analysis Day			Untreated Samples Analysis Day		
Item	0	14	28	0	14	28
Crude Protein*	35.37(1.4)[a]	34.82(1.80)[a]	37.10(0.09)[a]	34.24(0.81)[a]	36.43(0.36)[a]	36.99(0.28)[a]
Moisture	3.57(0.2)[a]	3.61(0.19)[a]	3.62(0.36)[a]	3.68(0.04)[a]	3.58(0.17)[a]	3.54(0.15)[a]
Crude Fat	9.59(0.40)[a]	9.37(0.20)[a]	9.10(0.17)[a]	9.60(0.05)[a]	8.97(0.29)[a]	9.05(0.05)[a]
Crude Fiber	11.16(0.11)[a]	11.04(0.34)[a]	11.22(0.40)[a]	11.10(0.39)[a]	11.25(0.13)[a]	11.19(0.27)[a]
Ash	2.99(0.10)[a]	3.10(0.11)[a]	3.13(0.07)[a]	3.01(0.06)[a]	3.11(0.02)[a]	3.17(0.05)[a]

[a-c] Within the day and treatment the means for indicators (total and chemical composition) followed by different lower-case letters are significantly different (P<0.05; Tukey).
Values are the average of three replications (n = 3) and standard deviation is represented in parenthesis.

Total includes the following amino acids: Taurine, Hydroxyproline, Aspartic Acid, Threonine, Serine, Glutamic Acid, Proline, Lanthionine, Glycine, Alanine, Cysteine, Valine, Methionine, Isoleucine, Leucine, Tyrosine, Phenylalanine, Hydroxylysine, Ornithine, Lysine, Histidine, Arginine, Tryptophan

Post-Harvest Engineering and Education

Amino Acid and Chemical Composition

	Treated Sample Analysis Day			Untreated Samples Analysis Day		
Item	0	14	28	0	14	28
Taurine	0.06	0.06	0.05	0.06	0.06	0.06
Hydroxyproline	0.06	0.11	0.09	0.14	0.09	0.09
Aspartic Acid	2.07	2.05	2.12	2.03	2.12	2.13
Threonine	1.21	1.20	1.22	1.21	1.22	1.23
Serine	1.46	1.45	1.43	1.46	1.44	1.43
Glutamic Acid	4.50	4.64	4.94	4.31	4.81	4.95
Proline	2.72	2.71	2.84	2.73	2.76	2.83
Lanthionine	0.00	0.00	0.00	0.00	0.00	0.00
Glycine	1.30	1.25	1.23	1.36	1.20	1.23
Alanine	2.36	2.35	2.45	2.36	2.38	2.45
Cysteine	0.59	0.58	0.60	0.58	0.59	0.59
Valine	1.34	1.32	1.37	1.33	1.38	1.40
Methionine	0.56	0.56	0.58	0.55	0.59	0.59
Isoleucine	1.08	1.07	1.07	1.11	1.06	1.09
Leucine	3.95	3.98	4.09	3.96	4.06	4.10
Tyrosine	1.48	1.44	1.47	1.51	1.45	1.45
Phenylalanine	1.68	1.69	1.71	1.68	1.71	1.71
Hydroxylysine	0.09	0.08	0.10	0.11	0.05	0.10
Ornithine	0.06	0.04	0.03	0.06	0.03	0.03
Lysine	1.13	1.12	1.11	1.12	1.13	1.12
Histidine	0.72	0.72	0.66	0.75	0.69	0.69
Arginine	1.36	1.35	1.33	1.37	1.35	1.33
Tryptophan	0.23	0.23	0.22	0.23	0.24	0.23
Total	30.02	30.00	30.71	30.00	30.42	30.83
Crude Protein	35.37	34.82	37.10	34.24	36.43	36.99
Moisture	3.57	3.61	3.62	3.68	3.58	3.54
Crude Fat	9.59	9.37	9.10	9.60	8.97	9.05
Crude Fiber	11.16	11.04	11.22	11.10	11.25	11.19
Ash	2.99	3.10	3.13	3.01	3.11	3.17

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Moisture Content, pH and Aflatoxin levels for untreated and HVACP treated samples stored for 0, 14 and 28 days with three different depth levels of product tested.

	Depth	M.C.		pH		Microbial Load (CFU/g)		Aflatoxin (ppb)	
Day	(cm)	No Treatment	HVACP	No Treatment	HVACP	No Treatment	HVACP	No Treatment	HVACP
0	1	63.86(1.80)^{b2}	64.19(0.30)^{b1}	4.40(0.01)^{a2}	4.45(0.04)^{a2}	1.08E+08(2.58E+06)^{b2}	3.83E+07(3.06E+06)^{b1}		
0	2.5	64.65(0.20)^{a}	64.59(0.27)^{a}	4.40(0.03)^{a}	4.35(0.18)^{a}	1.22E+08(5.96E+06)^{b}	6.83E+07(4.34E+06)^{b}		
0	4	63.48(2.12)^{a}	63.97(1.68)a	4.44(0.09)^{a}	4.40(0.03)^{a}	1.21E+08(8.06E+06)^{b}	7.05E+07(3.05E+06)^{b}	10.00^{a}	0.00^{a}
14	1	64.28(0.28)^{b1}	65.28(0.62)^{b1}	4.29(0.11)^{a3}	4.26(0.06)^{a3}	3.21E+08(1.08E+07)^{a1}	1.08E+08(7.09E+06)^{a1}		
14	2.5	65.31(0.19)^{a}	58.76(0.91)^{a}	4.30(0.07)^{a}	4.44(0.16)^{a}	3.56E+08(1.13E+07)^{a}	1.12E+08(2.09E+06)^{a}		
14	4	65.77(0.14)^{a}	65.29(0.46)^{1}	4.33(0.12)^{a}	4.40(0.08)^{a}	4.43E+08(2.83E+07)^{a}	1.01E+08(4.26E+06)^{a}	NT	NT
28	1	63.08(0.76)^{b12}	62.84(0.44)^{b1}	4.33(0.06)^{a1}	4.32(0.05)^{a1}	3.20E+08(2.96E+07)^{ab1}	1.80E+08(7.51E+06)^{ab1}		
28	2.5	65.40(0.29)^{a}	64.96(0.42)^{a}	4.30(0.05)^{a}	4.31(0.02)^{a}	3.70E+08(5.42E+07)^{ab}	1.60E+08(2.41E+06)^{ab}		
28	4	66.29(0.28)^{a}	66.17(0.33)^{a}	4.29(0.06)^{a}	4.27(0.04)^{a}	4.00E+08(4.00E+08)^{ab}	1.70E+08(5.83E+06)^{ab}	11.00^{a}	0.00^{a}

NT – sample was not tested.

$^{a-c}$ Within the day and treatment the means for indicators (depth) followed by different lower-case letters are significantly different (P<0.05; Tukey).

$^{1-2}$ Within the treatment, the means for indicator (day) followed by different number are significantly different (P<0.05; Tukey).

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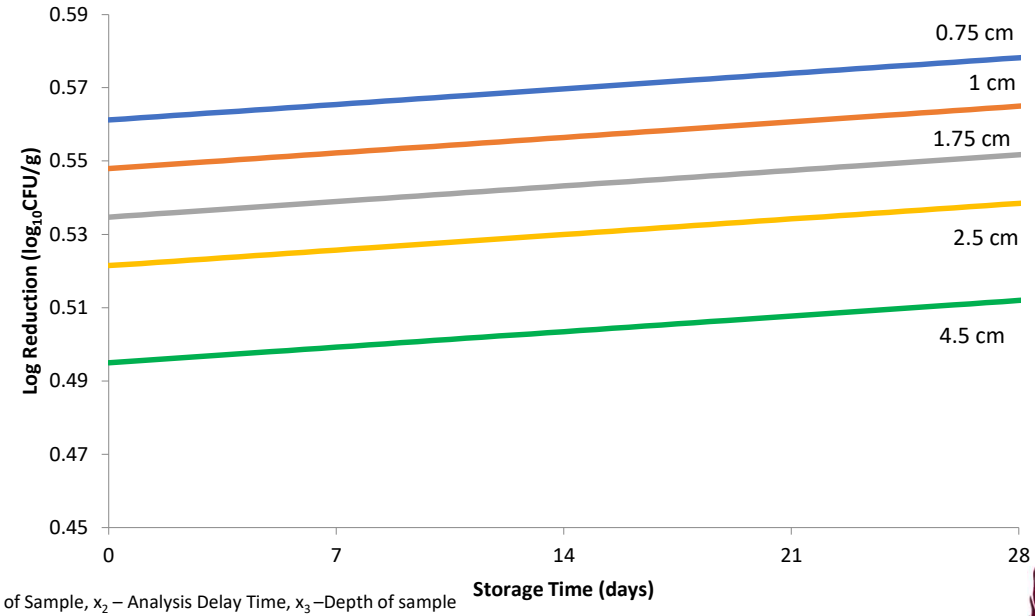
Log reduction as a function of DWG depth (inches) over time (days)

Stepwise regression polynomial model

$y=0.3226 + .0012*x_1 + 0.0006*x_2 – 0.05299*x_3$



x_1 – Amount of Sample, x_2 – Analysis Delay Time, x_3 –Depth of sample

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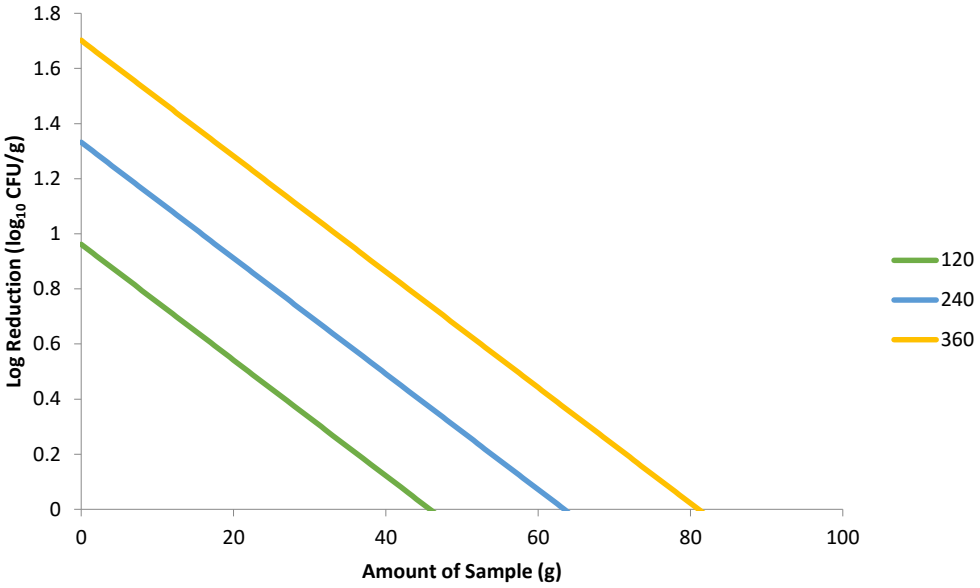
Microbial log reduction as a function of treatment time and amount of sample

$y = 0.124 - 0.021^* x_1 + 0.006 ^* x_2$



x_1 – Amount of Sample, x_2 –Treatment time

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Grain Depth

- The model obtained log reductions less than 1 log at the treatment parameters tested, 70kV, 360s. It is likely that with an increase in treatment time higher log reductions will be obtained for these larger DWG samples.
- Results presented are still promising as they show that with an increase in depth there is a decrease in log reduction.

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CONCLUSIONS

Conclusions

- Changes to DWG, Amino Acid Profile and Chemical Analysis, after HVACP treatment and storage are minimal
- Volume to Mass interaction HVACP reactive gas species penetrate through the depth of the grain mass, however reactions initially occur on exposed surfaces.
- Microbial reduction of stored grain fungi on HVACP treated DWG can be modeled as a function of the amount of sample, treatment time, package size and depth of sample

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- RGS produced from higher O_2 fill gas had higher microbial loads. ROS and H_2O_2 may be contributing to the higher log reductions
- The volume/mass/delay time interaction, showed that giving RGS time to return back to their original fill gas composition yielded a higher log reduction for smaller volume to mass ratios.

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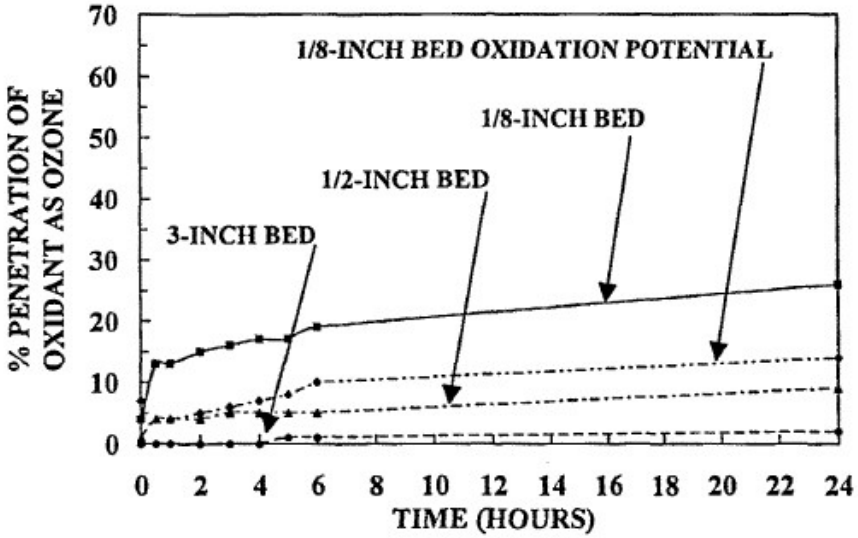
Ozone penetration through granular activated carbon



Cannon, F.S., Dusenbury, J.S., Paulsen, P.D., Singh, J., Mazyck, D. & Maurer, D.J., 2010. Advanced oxidant regeneration of granular activated carbon for controlling air-phase VOCs. Ozone: Science & Engineering: The Journal of the International Ozone Association, 18:5, 417-441, DOI: 10.1080/01919512.1996.10382853

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Applications

- What sterilization processes can we replace with ionization (i.e. aseptic process)
- Directly to target
  - High energy stream, in the direct path of ionization
- Degradation of lignocellulosic biomass

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QUESTIONS

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