Ozone treatment effects on microbial count on maize

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Abstract

The ultimate goal of this research was to develop a semi-continuous flow grain treatment system and predictive model that will reduce microorganisms on grain kernel surfaces with ozone. The focus of this research was to determine the concentration-time product (CTP) of ozone required to eliminate various levels of microbial growth on grain kernels. To examine the effect of ozone on surface microbes, samples of freshly-harvested and stored maize were treated with ozone for 1 and 3 hours at average ozone concentrations of 1752 ppm, 915 ppm and 37 ppm. Microorganisms were significantly decreased by 28 to 57% after maize samples were ozonated for 1 h at 37 to 1752 ppm and 45 to 80% for 3 h at 37 to 1752 ppm. Linear regression analysis of the CTP data indicated that percent mold reduction increased at a rate of 0.0088 times the CTP. The modified Gompertz equation applied to the microbial inactivation data indicated that a 0.5 to ~1 log mold reduction on maize kernels was attained for ozone concentrations between 37 and 1752 ppm. When compared to preliminary field data from a semi-continuous flow grain treatment system, the laboratory data and the model-predicted values were reasonably close with respect to the microbial load reduction observed on maize samples taken from the system.

Keywords: Ozone, Microorganisms, Treatment, Sterilization, Ozone concentration.

1. Introduction

There is great potential for the use of ozone to replace insecticides and fungicides used to treat stored grain and grain-based food and feed products. Stored grain products, such as maize, can harbor multiple fungi such as *Aspergillus* species that produce mycotoxins harmful to both human and animal health (Sharma, 2005). Ozone is an environmentally friendly gas that is produced by the excitation of electrons in air. Ozone has been shown to effectively sterilize grain and is thus a viable option for deterring grain loss. However, in order to effectively remove mold from grain, it is necessary to determine the best treatment approach. These approaches involve adjusting the ozone concentration and treatment times to yield an optimum concentration-time product (CTP). CTP is a measurement that relates gas concentration with treatment time. Once various CTPs are evaluated the optimum concentration and treatment time to reduce the microbial load on grain can be determined. Analyzing the microbial load on grain before and after ozone treatment will yield the percent in mold reduction for various CTPs. As a result, ozone treatment effects on grain can be quantified and modeled.

In order to control the efficacy of ozone on mold growth on maize in a scaled up treatment system, a model needs to be developed. The model equation needs to describe the inactivation of mold as a function of reduction in colony forming units (CFU) on maize at varying ozone concentrations. A modified Gompertz model equation and a linear model equation will be considered to express fungal inactivation of maize due to ozone treatment. The objective of this research was to quantify the treatment effects on grain exposed to high ozone concentrations with respect to microbial load reduction and to develop a model to quantify microbial load reduction as a function of ozone concentration and time.

In a study by Fan et al. (2007) ozone treatment with a concentration of 100 nanoliters per liter (nL L^{-1}) for 2 hours reduced the microbial population by 2-3 log CFU per milliliter (mL). The average time to obtain a 2 log CFU mL^{-1} reduction was 1.3 hours at 20°C and 2.5 hours at 5°C. This was determined by fitting the Gompertz model to an ANOVA statistical program. The model was used to determine the time required for a 2 log CFU mL^{-1} reduction. White et al. (2010) treated high moisture maize with ozone to determine its ability to control deterioration of high moisture maize. White et al. (2010) was able to decrease dry matter loss with ozone treatments of 2.4 and 4.8 mg min^{-1} for 24 hr.

According to Strait et al. (1998) grain not previously treated with ozone has inherent sites on its surface that react with ozone during an initial treatment (i.e., Phase 1). After the sites have reacted with the ozone (Phase 2), the rate of ozone degradation decreases (Kells et al., 2001). The reactions in Phase 1 will continue to occur until all possible ozone-surface reactions have completed. Therefore, when treating with ozone, it may be necessary to sterilize the inside of a container with ozone for a period of time before running experiments.

2. Materials and methods

2.1. System set-up

An ozone treatment and intermittent grain sampling system was designed and fabricated for this project. Five identical chambers were connected to make the complete system and allow grain to be placed in the individual chambers. Each chamber was equipped with a drawer that allowed removal of the bottom layer of grain. The bottom of each drawer consisted of a perforated screen to hold grain and allow ozonated air to pass through. The design held approximately 1 kg of grain kernels per chamber, which was about 130 g of maize per drawer pull.

2.2. Ozone generator and ozone analyzer

The ozone generator was manufactured by O_3Co Inc. (Idaho Falls, Idaho) and utilized the corona discharge to produce ozone. The unit contains four electrodes where the oxygen-to-ozone transformation takes place. The generator produces about 2.75 gh^{-1} of ozone at 115 V with dry air as the feed gas. The input voltage was varied from about 50 V to produce 0.5 g/h to 140 V to produce 3.5 $g.h^{-1}$.

The ozone analyzer used to monitor the ozone concentration from the generator was an IN2000LC unit from IN USA, Incorporated (Norwood, Massachusetts). According to the analyzer's manual, the unit has a measuring range from 0 to 2000 ppm, and is calibrated according to US NIST traceable standards (+/- 1%). The ozonated air mixture is pumped into the analyzer at 1.0 $l.min^{-1}$. When multiple chambers were filled with grain and ozonated concurrently, an automatically operated valving system was used to switch ozone readings from chamber to chamber.

2.3. Microbial load analysis

The percentage of mold growth is a variable that needed to be determined in each sample by microbial load count which was attained through dilution plating followed by counting colony forming units (CFU). To measure the effect of ozone on fungal populations samples were serially diluted onto malt salt agar medium. After 3 days of incubation, colonies were counted giving CFU values. Maize kernels were also plated and number of CFUs were counted prior to and after ozone treatment. There was an ozone concentration and treatment time (CTP) combination associated with each percent difference value. The CTP versus the percent difference was graphed to compare the percent reduction in surface mold to the CTP.

2.4. Models for mold growth analysis

The modified Gompertz equation was used to fit CFU counts at various ozone concentrations. By comparing the CTP and percent mold reduction values, an inactivation curve was developed. The graph of the curve is a log (N) versus time graph. The N value is the number of microorganisms at time t and N_0 is the number of microorganisms at time t = 0. The log of N is dependent upon the lower N value, the inactivation rate of the microbial load and the phase of disappearance. This model was useful in determining the reduction in microbial load over time.

A linear correlation of CTP and percent mold reduction was also explored. This correlation was used to directly compare treatment CTP to the percent mold reduction. This will be useful for treatment system scale up in order to determine the expected percent mold reduction for the CTP used to perform sterilization. After the optimal CTP is determined, it can be used in the modified Gompertz curve to model the inactivation of the surface microbes over time.

Fan et al. (2007) used a modified Gompertz model developed based on the statistical analysis performed in their research. The experimental factors of concentration, exposure time, half-life time as a function of airflow, temperature and relative humidity made up the factorial combinations. These combinations were used for the experimental series. In this current research, the modified Gompertz model was used to compare the inactivation response data to the ozone treatment parameters over time.

3. Results

3.1. Microbial load reduction

During the treatment, 30 g of maize was removed after one hour and the remaining 100 g were treated for two additional hours. The controls for these tests were the results from the samples which did not receive treatment. Three replications were performed. Samples from replications two and three were taken from a different bag of grain. The results show that there was a reduction in CFUs after ozone treatment for one hour, and a further reduction after three hours. These results showed reduction in CFU by up to 100% after 3 hours of treatment. Previous literature (Fan et al., 2007) also showed reduction in microbial load after high ozone treatment.

In Figure 1 the average percentages of mold reduction during the 1752 ppm, 915 ppm and 37 ppm treatments for 1 and 3 hours are graphically illustrated. The standard error bars represent a 95% confidence interval. The 3-hr results indicate increased mold reduction as ozone concentration increased from 37 to 1752 ppm as would be expected. Unfortunately, the 1-hr results for the 1752 ppm concentration did not display the same trend. These results were not consistent, considering the fact that this was the highest ozone concentration tested.

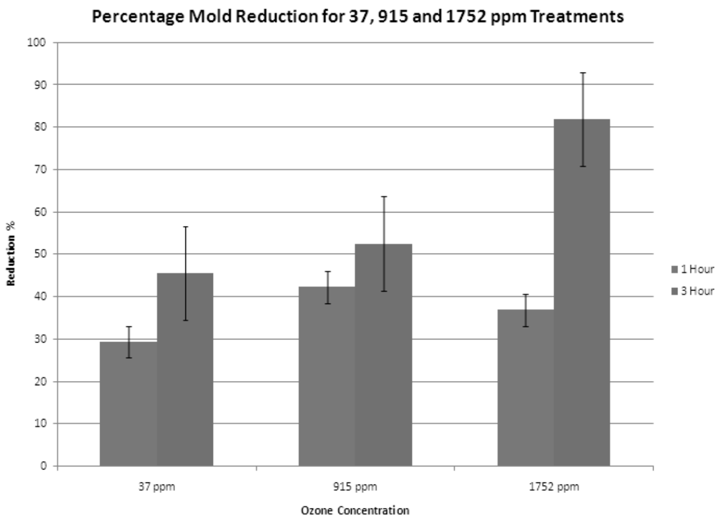

Figure 1 Average of three replications of percent mold reduction for ozone treatments of maize at 1752 ppm, 915 ppm and 37 ppm and treatment periods of 1 and 3 hours.

Because of the variation in mold reduction for the 1-hr results, a trend in percent reduction could not be identified. The 37 ppm results show that the percent reduction went from 31% for 1 hour to 50% reduction after 3 hours. At an ozone concentration of 915 ppm the percent reduction went from 47% for 1 hour to 57% for 3 hours. The percent reduction at 1752 ppm after 1 hour was 38% and went to 80% for 3 hours. An additional replication was run and the four percentage reduction values for 1752 ppm after 1 hour were 24.3%, 45.3%, 43% and 8.8%, which caused the average percent reduction to remain lower than for the 915 ppm treatment effect. Based on the results from replications 2 and 3 for the 1-hr treatment at 1752 ppm, the average percent mold reduction was 44.2%, which would be similar to the effect achieved at 915 ppm. That would be a more reasonable result than the observed reduction. Therefore, the 3-h treatment effect appears to be more predictable and reliable with respect to the percent mold reduction than a 1-hr treatment as the ozone concentration increased. During one replication (i.e., 1752 ppm and 3 h) a complete (100%) sterilization of surface molds was observed.

3.2. Linear model

Figure 2 depicts the x-y relationship for mold reduction and CTP for the ozone treatments. The linear correlation for the CTPs initially showed a low R^2 value of 0.38 indicating that the fit was not very strong. There were several outliers in the data which caused the linear fit line not to be as accurate. These outliers were identified and removed from the data set analyzed statistically. The data analysis was then performed again and a higher R^2 value (0.65) was obtained, indicating a better fit. The final equation obtained through Microsoft Excel (2007) by fitting a linear regression to the plot of scattered points (Eqn. 1) was:

$$y = 0.0088 * x + 44.097 \quad (1)$$

where x represents the CTP and y gives the percent mold reduction. From Equation 1 a CTP of 392 ppm-hr predicts a 47.5% reduction and for 340 ppm-hr a 47% reduction. This comparison of data from grain bins shows that the values are reasonably close, and thus the linear equation appears useful in predicting the percent mold reduction. Additional tests will need to be performed to determine how effective Equation 1 is in predicting field data.

For field applications it would be expected that the lower range of Figure 2 would be most commonly used, as ozone generators currently used on grain bins do not have sufficient capacity to produce sufficient ozone concentrations for the higher range.

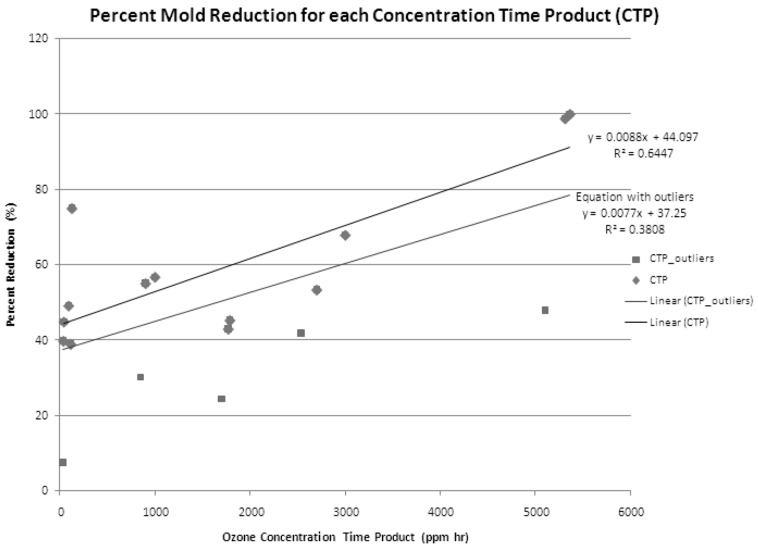

Figure 2 Linear graph of percent reduction versus ozone concentration time product for tested values with and without outliers.

3.3. Modified gompertz model

The modified Gompertz model was applied to each ozone concentration and the microbial reduction previously determined. Values for N and N_0 were obtained by taking the number of CFU present on the grain at time t and time t = 0. These values were then used to determine A, (log (N/N_0)), μ (min^{-1}), and λ (min). The model application gave the inactivation curve in Figure 3 over time and is comparable to the Gompertz inactivation curve (Erkman, 2001). The most identifiable value is the inactivation rate (μ), which is the slope of the line (see Figure 3). The slope for the line which represents 1752 ppm concentration of ozone was μ = -0.008, for 915 ppm the slope was μ = -0.004, and for 37 ppm the slope was μ = -0.0035. The lag phase for each concentration began around 120 minutes. This is the period when microbial reduction has been completed and no further reduction is expected. There is a large difference between the 915 ppm and 1752 ppm curves. As the concentration increases, the inactivation rate increases substantially. A log reduction near 1 log is seen at the 1752 ppm concentration but only a log reduction of < 0.5 log is observed for the 37 ppm and 915 ppm concentrations. This is not comparable to Fan et al. (2007) who showed a log reduction of 2 in microbial load. However, with a larger initial mold concentration, a log reduction of 2 could be attained.

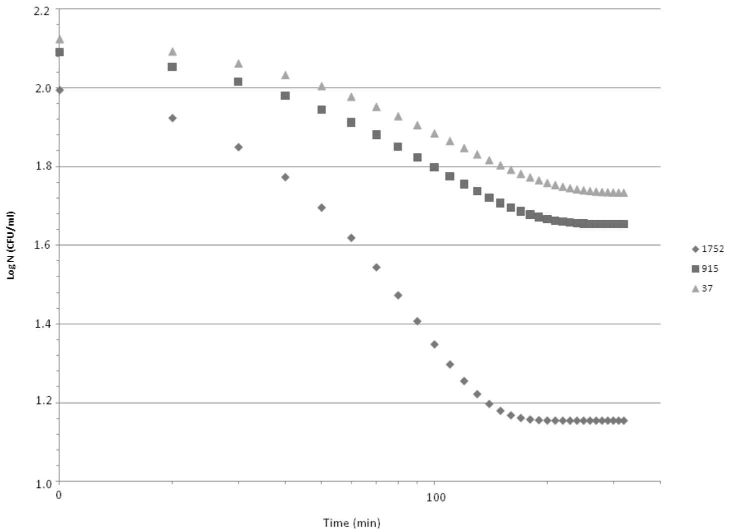

Figure 3 Graph of Log_{10} CFU ml^{-1} for three ozone concentrations representing the inactivation curves.

4. Discussion

As a result of the experiments conducted in the laboratory ozone treatment and grain sampling system, the following conclusions can be drawn in terms of quantifying the treatment effects on grain exposed to high ozone concentrations with respect to microbial load reduction and developing an equation to quantify microbial load reduction as a function of ozone concentration and time. First, the experimental results showed a reduction in mold CFUs on maize of 47.4% and 56.6% at 915 ppm ozone treatment after one and three hours, respectively, versus 37.5% and 80.2% at 1752 ppm after one and three hours, respectively. The reduction in mold CFUs after one and three hour treatments at ozone concentrations of 37 ppm was only 28% and 45%, respectively. The treatment effect after 1 hour was lower for 1700 ppm than for 800 ppm and no reasonable explanation for that behavior could be found. Secondly, linear regression analysis of the CTP data indicated that percent mold reduction increased linearly at a rate of 0.0088 times the CTP. This showed that higher ozone concentrations and/or longer treatment periods can decrease the amount of CFUs found on maize samples. Lastly, the modified Gompertz equation was successfully applied to the microbial inactivation data. From the results it was concluded that a 0.5 to ~1 log mold reduction on maize kernels was attained for ozone concentrations between 37 and 1752 ppm.

References

Erkman, O., 2001. Mathematical modeling of *Escherichia coli* inactivation under high-pressure carbon dioxide. Journal of Bioscience and Bioengineeirng 92, 1, 39-43.

Fan, L., Song, J., McRae, K.B., Walker, B.A., Sharpe, D., 2007. Gaseous ozone treatment inactivates *Listeria innocua* in vitro. Journal of Applied Microbiology 103, 2657-2663.

Kells, S., Mason, L.J., Maier, D.E., Woloshuk, C.P., 2001. Efficacy and fumigation characteristics of ozone in stored maize. Journal of Stored Products Research 37, 371-382.

Sharma, P.D., 2005. Environmental Microbiology. Alpha Science International, Ltd. India.

Strait, C.A., Maier, D.E., Woloshuk, C.P., 1998. Controlling stored grain insects with ozone fumigation. ASAE Annual International Meeting.

White, S.D., Murphy, P.T., Bern, C.J., van Leeuwen, J. 2010 Controlling deterioration of high moisture maize by ozone treatment. Journal of Stored Products Research 46, 7-12.