GMO CORN STALK DECOMPOSITON TRIAL / ASSESSMENT

Submitted to

Biodyne Midwest

Submitted by:

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INTRODUCTION

The purpose of this study was to determine if inoculation with **Environoc 501** can increase the decomposition rate of genetically modified organism (GMO) corn stalks.

This would be shown as an increased loss in overall dried corn stalk mass when compared with a control over a 28 day test period.

Environoc 501 is a blend of beneficial microorganisms that provides enhanced degradation of many polymers such as cellulose, chitin, and lignin. It also increases the efficiency for composting and vegetation degradation applications.

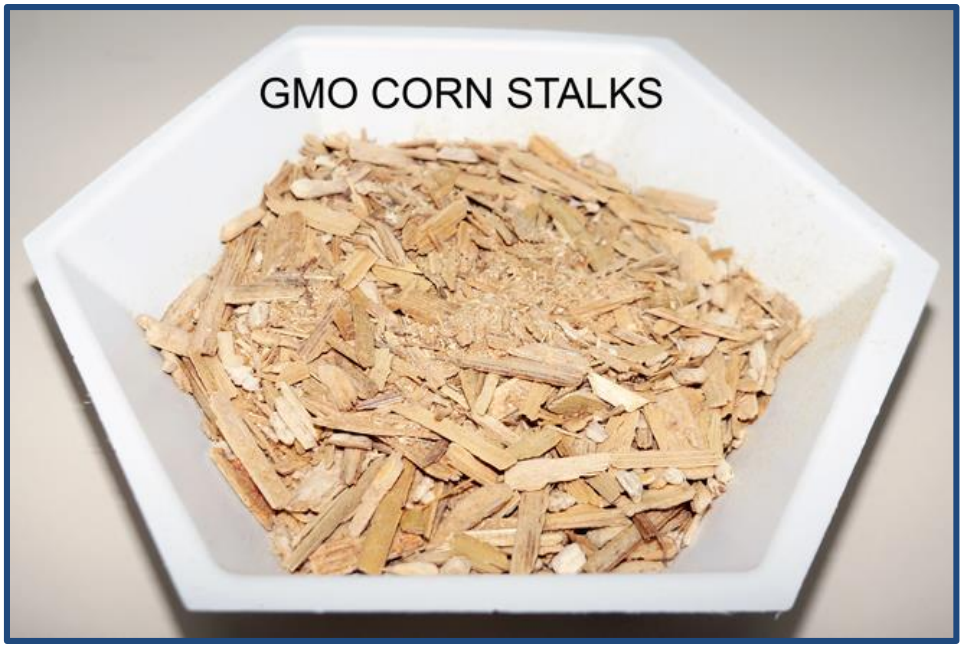
One partially dried (GMO) corn stalk was received from a GMO Corn Crop, on August 20, 2013 for testing purposes.

MATERIALS AND METHODS

Sample Preparation

The corn stalk was cut into 5 cm segments and macerated in a food processor for 10 minutes. It was then placed into a metal pan and dried at 105°C for 24 hours to reduce the initial moisture content of 27% until there was no change in mass.

The dried material was then ground in a food processor for 5 minutes. The final processed material was reduced to 1-2cm chips of dried corn stalk with powder like fines.

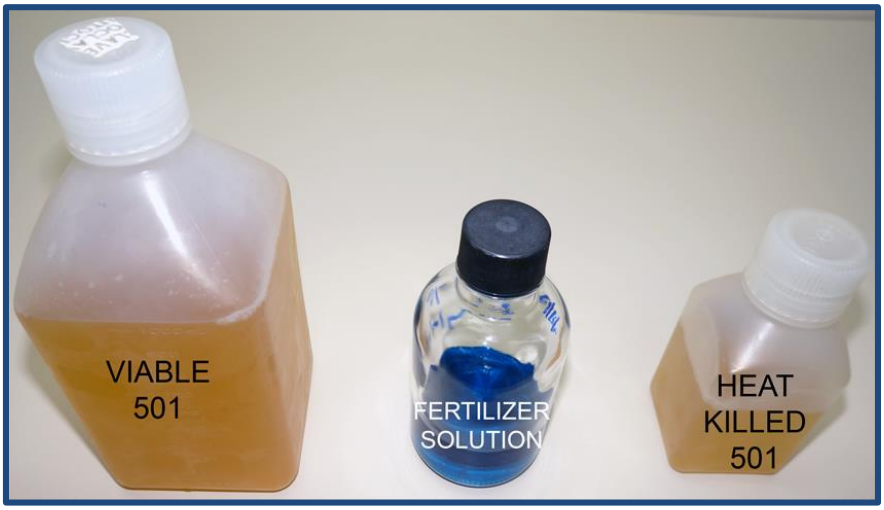


Using a precision balance, the mass of six empty, clean, and dry porcelain crucibles was determined to within 1 milligram. The mass of each was recorded and referenced as ($M_{p)}$. The crucibles were tared individually to 0 on the same balance. Approximately 1 gram (+/- 2 milligrams) of the final dried corn stalk was placed into each one. The corn stalks masses were recorded and referenced as ($M_{c)}$

Inoculum Preparation and Application

To achieve a 100:5:1 (Carbon: Nitrogen: Phosphorus) ratio for the 1 gram samples of dried corn stalk, approximately 1ml of a 20% solution of 25-5-15 soluble greenhouse grade fertilizer needed to be added to each of the samples for optimum biodegradation.

Three crucibles were inoculated with **Environoc 501** at a rate of 4.6 billion cfu/gram (5ml) and fertilizer solution. The three control samples were inoculated with heat killed **Environoc 501** (5ml) and fertilizer solution.



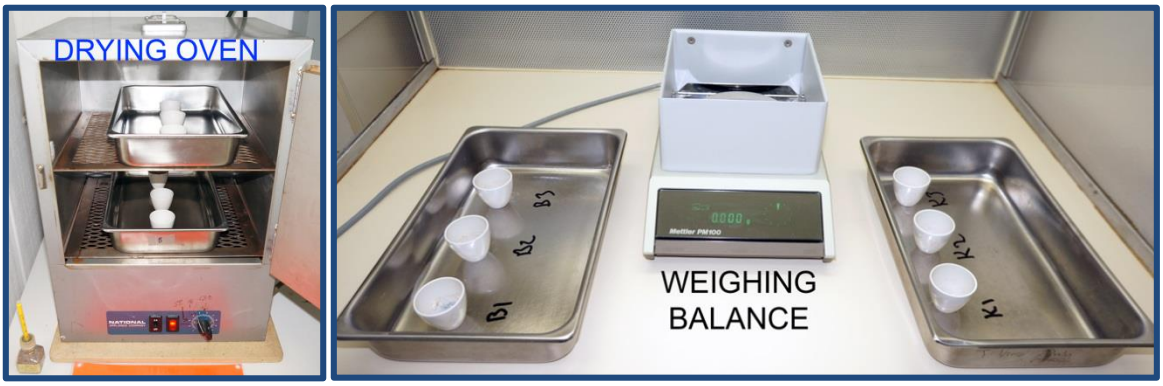
Heat killed **Environoc 501** was used so that the control samples received the same amount of nutrient carryover present in the viable inoculum. The nutrient carryover could influence the overall mass comparison. In addition, the nutrient carryover would need to be equal for all samples because it could serve as a nutrient source for the native microorganisms present on the corn stalks. The heat killed **Environoc 501** was autoclaved at 121**°**C at 15 psi for 30 minutes. This was repeated 4 times over multiple days to render the microbes non-viable.

Following the inoculation, the crucibles were placed into a 27**°**C incubator. The inoculations were repeated weekly for 4 weeks, however the volume of inoculum was reduced at 7 days to 2ml **Environoc 501**/ Heat Killed **Environoc 501** and no additional fertilizer was added.

The moisture content of the samples was maintained in-between inoculations with 1ml of water (approx. 1 ml/wk).

Post Treatment Measurements

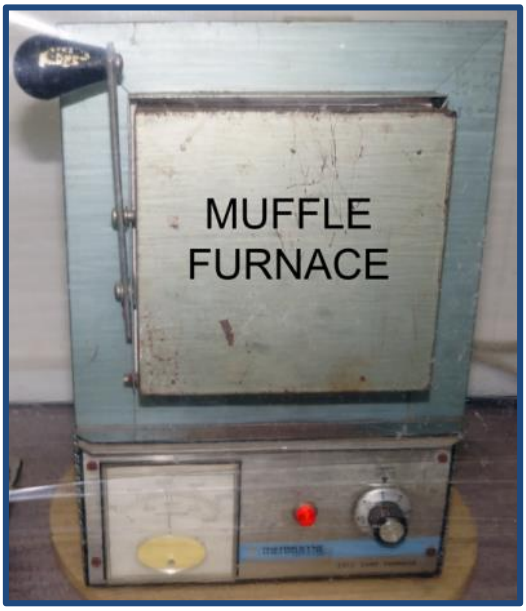
After 28 days the samples were dried at 105°C for 24 hours. The individual crucibles with the corn stalks were placed on a precision balance and the final mass of the corn stalks determined to within 1 milligram. The mass was recorded and referenced as ($M_{d)}$. The final dry mass of corn stalks (M_d) was compared with its respective starting dry mass (M_c). Using the formula (M_d-M_c)/ M_c x 100, the % reduction in corn mass was determined. An average reduction in mass was calculated for both of the two treatments and compared.



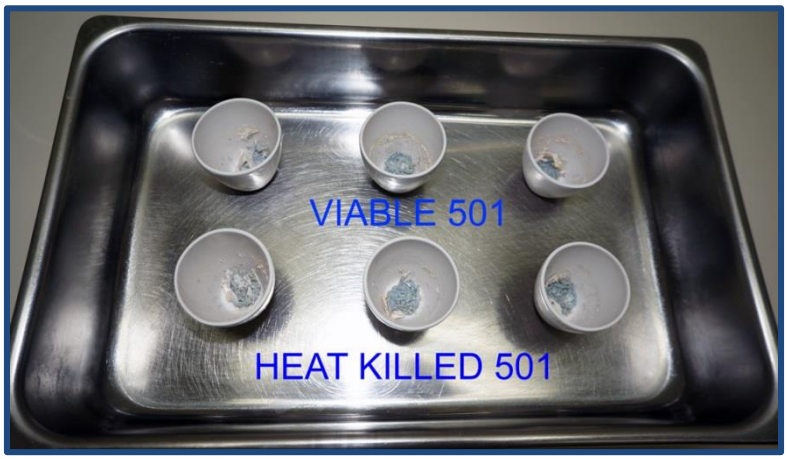
ASTM D 2974 (% Organic Matter Content)

The samples were then processed using a modified version of ASTM D 2974 (Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Organic Soils) to determine if they had similar % organic matter contents. Even though there may be a difference in the mass loss over time the remaining mass should be composed of the roughly the same % organic matter content for all samples. This testing was done as a check to make sure the samples were all consistently the same material and had been handled similarly.

The samples were loaded into a high temperature muffle furnace and heated to up to 630°C over the course of 8 hours.



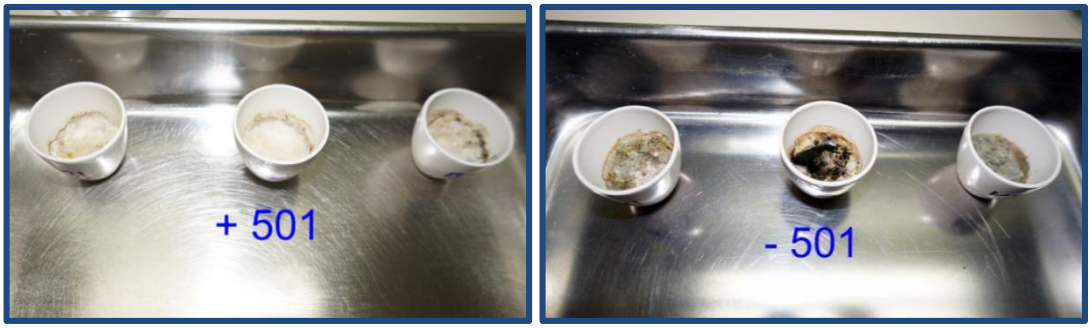
They were then left to cool in the muffle furnace overnight. The individual crucibles were placed on a precision balance and the mass of the remaining ash determined.



By subtracting the mass of the ash ($M_{a)}$ from the mass of the final dry corn stalks ($M_{d)}$ the mass of the organic matter ($M_{o)}$ from the samples was calculated.. The percent organic matter content of the samples was calculated using the formula M_o/M_d x 100.

RESULTS

Over the 28 day period the three samples treated with **Environoc 501** developed a homogenous mat of white fluffy mycelium. The controls had variability in the overall appearance of their fungi ranging from black to powdery green. It can be assumed that all samples had native fungi present, but that fungi present in the **Environoc 501** inoculum tended to out compete the native fungi, leaving only a white cottony appearance rather than the colorful variability of the heat killed samples.



		Environoc 501				Control		
TO (Dry)		B1	B2	B3		K1	K2	K3
Crucible Mass (grams)	M_p	17.697	20.371	22.143		20.234	18.487	17.069
Corn Mass (grams)	M_c	1.002	1.001	1.002		1.000	1.000	1.001
Crucible+Corn Mass (grams)		18.699	21.372	23.145		21.234	19.487	18.070
T28 (Dry)								
Post Oven								
Crucible+Corn Mass (grams)		18.532	21.228	23.031		21.126	19.397	18.016
Corn Mass (grams)	M_d	0.834	0.857	0.887		0.892	0.910	0.946
Loss in corn mass over 28 days (grams)		0.168	0.145	0.115		0.108	0.090	0.055
Corn Mass Loss over 28 Days (%)		-16.72%	-14.44%	-11.46%		-10.75%	-9.00%	-5.44%
% Average Corn Mass Loss over 28 days				**-14.20%**				**-8.40%**
Crucible+Ash Mass (grams)		17.774	20.443	22.217		20.310	18.564	17.145
Ash Mass (grams)	M_a	0.077	0.072	0.074		0.076	0.078	0.076
Mass of Organic Matter (grams)	M_o	0.758	0.784	0.814		0.816	0.833	0.871
% Organic Matter		90.77%	91.59%	91.70%		91.43%	91.48%	91.97%
Average % Organic Matter				**91.35%**				**91.63%**

During the 28 day test, samples treated with **Environoc 501** displayed more rapid decomposition when compared with the controls. This was evidenced by a 69.20% greater loss of dry corn mass in the viably inoculated samples than in the heat killed samples.

The results of ASTM D 2974 (% Organic Matter Content) indicated that all samples were consistently composed of the same % organic matter content (91-92%) and had been handled similarly throughout the trial.

DISCUSSION

Overall there was 69.20% more mass loss in the cornstalks treated with **Environoc 501** than with heat killed inoculum. Since the only difference between the two sets of samples was the addition of viable **Environoc 501** microbes, the greater reduction in organic matter must be attributable to the microbes. This means the microbes in **Environoc 5**01 metabolized organic components in the corn for growth and in the process reduced the carbon mass through the production of carbon dioxide (CO_2) and water (H_2O). The CO_2 would leave the corn mass immediately as a gas and the H_2O would be driven off during the drying cycle, leaving less weight in the corn treated with the **Environoc 501**. While there was a loss of dry weight in the samples that had only the indigenous and heat killed **Environoc 501**, the reduction was significantly greater in the **Environoc 501** viable samples.

While both samples contained the same indigenous population, the homogeneity of the mycelium present on the samples treated with viable inoculum versus the control indicates that the microbes present in **Environoc 501** colonized the corn material more quickly thereby reducing the surface available to the indigenous microbes. A faster elaboration of the **Environoc 501** over the surface of the corn coupled with their enhanced enzymatic abilities would inevitably lead to an increased decomposition rate in those samples. This is borne out by the 69.20% greater reduction in the organic dry mass of the **Environoc 501** treated corn.

CONCLUSIONS

According to the literature, besides inherent hybrid vigor, GMO corn has traits that allow it to experience less loss from invasive insects and less competition from weeds due to their resistance to certain weed control chemicals. However these improvements can come with some unexpected challenges like increased lignin in the stalks. The increased lignin may assist in repelling some boring insects, but it has a tendency to make the stalks more resistant to decomposition, thereby increasing stubble longevity in the field.

Environoc 501 contains proven cellulose and lignin degraders. Their added presence on the GMO corn used in this study produced an increased removal of organic dry weight over indigenous strains present on the corn. It is likely that similar success would take place in the field thereby leading to a reduction of some of the problems associated with the longevity and structural rigidity of stubble currently being experienced during the growth of GMO corn.