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## BIOLOGICAL DEGRADATION OF COTTON BALES DUE TO EXCESS MOISTURE

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### Abstract

The practice of adding water to ginned cotton to reduce bale packaging forces and increase the bale weight was studied in regard to potential biological degradation of the cotton due to excess moisture. This study showed that added moisture did indeed increase bale weight, but at the same time, caused significant increases in yellowness and grayness of lint. The excessive moisture levels stimulated microbial activity; especially troubling was the large increase in mold activity, which may be considered as an unnecessary health risk. Microbial activity was not uniformly dispersed through the treated bales and this spotty behavior may add to difficulties during lay down at the mill.

### Introduction

Producers and ginners have long restored some moisture to ginned lint to make up for excessively dry cottons and reduce excessive bale packaging forces (Anthony, 2001), but have avoided excess moisture in cotton bales since common sense and research have shown this to be a prudent practice. However, any improvement that can increase profitability is quickly adopted, especially in this recent downturn in the cotton industry. Water typically is added just before baling the ginned cotton to make up and add to the moisture lost during the production and ginning processes. The advantage of this practice is obvious, since water is relatively inexpensive and cotton is sold by the pound; and hence, an immediate additional value is added to such a treated bale. This practice is neither done nor expected to provide the beneficial effects of moisture on fiber quality and processing as put forth by McAlister (1997). On the contrary, current practice and research development attempts to avoid excessive moisture in baled cotton (Anthony, 2001). This practice of intentionally adding water raises serious concerns that degradation may occur in the fiber by this additional moisture during storage (Lalor, 1994). The purpose of this study is to determine if degradation occurs during storage of cotton bales that have been augmented with water after ginning and before baling.

### Materials and Methods

#### Cotton and Moisture Treatment

Cotton was from the 2000 harvest year. Sufficient Stoneville 4892BR seed cotton for five bales was harvested October 11, 2000. On May 25, 2001, the cotton was ginned in the full-scale gin at the Cotton Ginning and Research Laboratory, Stoneville, MS. Added moisture was applied after ginning but before baling. The moisture treatments consisted of a non-treated control and four levels of water applications to the cotton. Approximately 1400 pounds of seed cotton were ginned for each approximately 500-pound bale treatment and each treatment bale was placed in a six-mil plastic bag with two additional layers of plastic bags placed over the first bag even though polyethylene bags with small diameter holes, woven polypropylene, or burlap is typically used industry wide, in increasing permeability, respectively. The bales were then stored near the bale press in Building 21 at the Research Laboratory.

The ginning sequence consisted of a cylinder cleaner, stick machine, cylinder cleaner, extractor feeder/gin stand, and one saw-type lint cleaner. For the non-treated control, Treatment 1, no moisture overspray was applied. For the water treatments 2, 3, 4 and 5, three conventional spray nozzles applied water as an overspray to the surface of the cotton as the lint came down the lint slide. The water treatments consisted of sufficient water oversprays for four levels of water: approximately 10, 20, 40 and 50 pounds per 500-pound bale of cotton, treatment 2, 3, 4 and 5, respectively. The amount of water overspray was regulated by using three nozzle tips with either 0.001, 0.001, 0.002 or 0.003 inch hole diameters, for treatments 2, 3, 4 and 5, respectively. These nozzle tips were on a pipe connected to a standard residential water hose. Since the pressure and water volume were from a municipal source, the output of the tips at various valve settings was calibrated by capturing water from the tips over time.

Just before each of the water applications, five samples were taken as the cotton came up the battery condenser for HVI evaluation and nine samples were taken for moisture evaluation. After moisture was added, nine samples were taken for lint moisture evaluation from each of the treated cottons. Each of the cottons was pressed into a bale for a total of 5 bales, each

consisting of one of the 4 water applications and the non-treated control. The bales were pressed to a platen separation of about 19 inches. The bales were then weighed and placed in storage.

On September 18, 2001, after the storage period, the bales were laid on the floor in the full-scale gin and marked with 10 intermediate locations (layers) from one side to the next before the bale ties were removed so that samples could be taken. Sub-samples were taken at each layer: 10 samples for moisture content, 5 samples for AFIS (Advanced Fiber Information System), 3 samples for HVI (High Volume Instrument) classification and 10 for biological degradation evaluation. The cotton was separated at each layer and samples were taken. The moisture analyses were done at the Cotton Ginning Research Laboratory, and the AFIS and HVI analysis was done at the AMS Classing Office at Dumas, AR. The 100+ gram samples taken for biological degradation study were stored in two Ziploc polyethylene bags in order to reduce drastic changes in their moisture composition during transport. The samples were transported directly by government vehicle directly to the Cotton Quality Research Station (CQRS) at Clemson, South Carolina.

### **Biological Degradation Evaluation**

The extent of biological degradation in each moisture treatment, aside from changes to the physical properties of cotton measured by HVI and AFIS analysis, consisted of measuring the microbial populations (total bacteria, Gram-negative bacteria and fungus populations), cotton dust potential, endotoxin content of the dust and potential airborne endotoxin load. Endotoxin content is measured as endotoxin units per mg of dust (EU/mg dust). Cotton dust potential was measured with the Microdust Trash Monitor (MTM) and the potential airborne endotoxin load is the product of the dust endotoxin content and the cotton dust potential. The cotton dust potential and endotoxin assay were not completed when this paper was written so only the microbial population determinations will be reported here.

The samples were sorted, randomized and sub-sampled immediately on arrival at CQRS, for microbial and cotton dust potential assays. Cotton dust potential assay will be performed by the Testing Laboratory at CQRS using the MTM (Chun and Perkins, 1996). Dust laden filters will be randomly chosen for endotoxin analysis and to calculate potential airborne endotoxin load using the methods described by Chun and Perkins (1996). Microbial assay was made from 1-gram of lint from each sample for total bacteria and total Gram-negative bacterial populations using the method described by Chun and Perkins (1996); for fungal populations the method described by Chun and McDonald (1987) was used. However, because of the size of the study, incubation was made at room temperature,  $20^{\circ}\pm 2^{\circ}$  C, and ranged from 4 to 11 days for the total bacterial and Gram-negative bacterial assays, and from 10 to 17 days for the fungal population assay.

### **Statistical Analysis**

For the microbial and dust assays, the 100 samples from each treatment bale were separated into four zones and from each zone, 15 samples were randomly selected for assay. These samples were then randomly assigned a number from 1 to 300 and the samples assayed sequentially for a completely randomized split block design. A  $\log_{10}(\text{cfu}+1)$ , where cfu = microbial population as colony forming unit per gram lint (corrected for dry weight), transformation was used for the analysis.

Data were analyzed using release 8.00 or earlier releases of SAS (SAS, Statistical Analysis System; SAS system for Windows version 4.90.3000, SAS Institute Inc., Cary, NC, USA) for making mean comparisons. Otherwise, additional testing and data manipulation was done with Microsoft EXCEL 2000 or earlier releases of EXCEL (Microsoft Corporation, USA).

## **Results and Discussion**

The actual amount of moistures applied to the bales differed slightly from the target values of 0, 10, 20, 40 and 50 lbs per 500-pound bale. The actual moisture applied were 0, 12.8, 19.5, 47.9 and 55.3 pounds per bale, respectively (Table 1) for starting moisture contents of 5.97%, 7.26%, 8.87%, 13.87% and 15.41%. After storage from May 25 to September 18, 2001, some loss of moisture was observed with most of the treatments even though the bales were triple sealed in polyethylene bags. Upon opening the bales, unusually strong earthy and musty odors escaped from the polyethylene bags covering the water-augmented bales. Water damage was observed on the surface of the heavily water augmented bales (Figure 1). The final overall moisture contents were 6.06%, 7.92%, 8.21%, 11.59% and 12.92 percent, respectively for treatments 1, 2, 3, 4 and 5. The two bales with no overspray or just a small amount of water added actually increased in water content slightly from 5.97% to 6.06 and 7.26% to 7.92%, respectively for treatment 1 and 2 (Tables 1 and 2) and did not exhibit water damage or unusual odors.

Sample fiber classification qualities are shown in Tables 3 and 4. HVI color index averaged 98.5, 98.2, 96.0, 91.1 and 85.6, respectively for treatments 1, 2, 3, 4 and 5. Likewise, Rd averaged 75.7, 74.7, 73.6, 70.6 and 69.3, while +b averaged 8.5, 9.9, 9.3, 10.1 and 10.6, respectively for treatments 1, 2, 3, 4 and 5. One can only deduce then that the bales became darker and more yellow as more moisture was added which is supported by previous research (Brushwood and Chun, 1998; Chun

and Brushwood, 1998); even adding small amounts (12.8 lbs) of water per bale substantially increased yellowness and grayness.

Microbial populations were influenced by the added moisture but not in a clear consistent manner (Table 5). The total bacterial populations did not follow a consistent trend as the level of water was increased. While the average population varied significantly from one treatment to another, the values could also be representative of the variation inherent in cotton. On the other hand, the Gram-negative bacterial populations show an obvious decrease in numbers with increased added moisture. This is inversely correlated to the observation of the yellowness and grayness trend with increased moisture (Table 4). In addition, this appears to contradict an observation that Fischer et al. (1980) made concerning raw cotton: the yellowness of raw cottons was significantly and positively correlated with Gram-negative bacterial populations. To explain this inconsistency, one must remember that bacterial population determinations are measurements of the viable bacteria present in the cotton. What we speculate to be happening is that the added water stimulated growth and broke the dormancy survival stage of many of the Gram-negative bacteria which then began to die off because conditions were not suitable to sustain growth. This trend seems to be an acceleration of the normal bacterial survival under conventional cotton storage (Chun and Perkins, 1996). Nevertheless, while the Gram-negative bacterial populations tended to die off, their remains were left behind intact on the cotton. Chun and Perkins (1996) showed that under long-term storage, Gram-negative bacterial populations declined during storage, but endotoxin, which is a component of the cell wall of Gram-negative bacteria and an indicator of the presence of Gram-negative bacteria, remained consistent. A likely occurrence here is that more Gram-negative bacteria were stimulated to short-term activity with the increased added moisture and because conditions were not suitable for sustained growth, the die-off was greater with added moisture.

The fungal populations showed a strong tendency to increase with added moisture (Table 5). This could easily account for the increased grayness as added water was increased (Figure 1). Not only does the added moisture potentially lower the color grade of the cotton, but also it may raise a serious health concern. The increased presence of fungal components may greatly aggravate the allergic response of susceptible workers and add an unnecessary health risk. This is supported by earlier work by Bargerion et al. (1986) that indicated that bales containing the most moisture, produced dust with the greatest microbiological activities. The extent of this risk will be better determined when the cotton dust potential and endotoxin content portion of the study is completed.

When the dispersal of the various microbial populations was examined (Table 6), the localization of the microbial populations showed greater variability through the zones when water was added. This was with the exception of the Gram-negative bacteria, which probably was exhibiting greater die-off with added moisture resulting in simplification of the number of surviving species and hence the lower variation. The greater variability in the zones with added moisture will probably add to the inherent variability of cotton and this increased spottiness in a bale could possibly make lay downs more difficult at the mill.

In conclusion, adding water after ginning, by overspray positively increases bale weight but at the potential of causing increased yellowness and grayness, thus lowering the color grade. At the same time, microbial activity will also be stimulated. While the dust potential data has not been gathered yet, there is reason to believe that the cotton dust potential of the water treated bales will be greater than the untreated bales since increased bacterial and especially fungal activity were observed. In addition, the greater fungal mass on the water treated bales adds an unnecessary potential health risk to the workers who will be handling the cotton in the mills. While adding water does inflate the bale weight, the added weight might be offset by penalties or a lower classification of the cotton due to increased yellowness and grayness. The change in color grade occurs after official classification; and it may or may not occur before mill consumption depending upon the storage time. But if the users of cotton adversely affected by excess moisture were to delve into the history of suspected bales, very likely the reputation of the gins and producers of such practices may suffer in reputation and possibly indirectly tarnish the reputation of the entire cotton industry. Additionally, the bales studied herein, were packaged in relatively impermeable polyethylene rather than the more conventional bagging. Further studies should examine the impact of permeable bagging.

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Table 1. Initial level of moisture added to bales (500-pound bales) during ginning.

Treatment No.	Target Moisture, lbs	Actual Moisture, lbs	Average Moisture, %	Std. Dev.,%	Minimum,%	Maximum,%
1	0.0	0.0	5.97	0.079	5.80	6.05
2	10.0	12.8	7.26	1.109	5.85	8.85
3	20.0	19.5	8.87	1.310	6.55	10.75
4	40.0	47.9	13.87	3.784	10.10	21.40
5	50.0	55.3	15.41	4.204	9.50	20.75

Table 2. Final level of moisture in bales (500-pound bales) after storage, from May 25 to September 18, 2001.

Treatment No.	Average Moisture, %	Std. Dev.,%	Minimum,%	Maximum,%
1	6.06	0.165	5.70	6.05
2	7.92	0.272	7.40	8.85
3	8.21	0.272	7.80	9.10
4	11.59	0.884	10.45	14.20
5	12.92	1.215	11.05	16.20

Table 3. Advanced Fiber Information System<sup>1</sup> of water treated bales.

Treatment No	Lw	Uqlw, in.	Sfcw, %.	Ifc, %	Matratio %
1	0.9616	1.1542	8.520	3.652	0.8950
2	0.9468	1.1444	9.042	3.682	0.8870
3	0.9440	1.1370	8.746	3.564	0.8888
4	0.9456	1.1422	9.094	3.466	0.8962
5	0.9402	1.1382	9.392	3.470	0.8912

  

Treatment No	Neps	SCN per per gm	Dust, gm	Trash, gm	VFM, gm
1	188.78	11.76	504.32	109.44	1.9980
2	203.72	10.10	389.40	91.40	1.6426
3	201.76	10.06	389.50	96.04	1.6990
4	208.66	10.56	418.76	104.44	1.9032
5	212.40	10.54	411.18	100.84	1.8386

<sup>1</sup>Lw=mean length by width; Uqlw=upper quartile by weight in inches; Sfcw=short fiber content by weight; Ifc=percent immature fiber content; Matratio=percent maturity ratio; SCN=seed coat neps count per gram; Dust, gm=dust count per gram; VFM=visible foreign matter.

Table 4. Average HVI data after bale storage.

Treatment No.	Moisture, %	Reflectance, Rd (%)	Yellowness, +B	Color Index	Mike	Uniformity	Strength g/tex	Percent area	Length, in.
1	6.1	75.7	8.5	98.5	4.46	83.2	29.18	0.49	1.086
2	7.9	74.7	8.9	98.2	4.50	83.2	29.38	0.42	1.084
3	8.2	73.6	9.3	96.0	4.50	83.0	29.15	0.48	1.082
4	11.6	70.6	10.1	91.1	4.50	83.2	29.04	0.50	1.082
5	12.9	69.3	10.6	85.6	4.46	82.6	29.29	0.46	1.086

Table 5. Overall average microbial population in treated bales.

Treatment No.	Final Average Moisture, %	Total <sup>1</sup> Bacteria, Log <sub>10</sub> (cfu+1)	G(-) Bacteria <sup>1</sup> , Log <sub>10</sub> (cfu+1)	Fungi <sup>1</sup> , Log <sub>10</sub> (cfu+1)
1	6.06	6.834 <sup>A</sup>	6.220 <sup>A</sup>	3.556 <sup>C</sup>
2	7.92	6.262 <sup>B</sup>	5.481 <sup>B</sup>	2.957 <sup>D</sup>
3	8.21	5.900 <sup>C</sup>	4.984 <sup>B</sup>	3.762 <sup>C</sup>
4	11.59	5.661 <sup>C</sup>	1.488 <sup>C</sup>	4.844 <sup>A</sup>
5	12.92	6.786 <sup>A</sup>	1.660 <sup>C</sup>	4.118 <sup>B</sup>

<sup>1</sup>Mean separation within column by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

Table 6. Average microbial population in each zone of the treated bale.

		Treatment <sup>1</sup>				
	Zone	1	2	3	4	5
Total	1	6.826 <sup>A</sup>	6.145 <sup>B</sup>	6.119 <sup>A</sup>	6.056 <sup>A</sup>	6.107 <sup>B</sup>
Bacteria,	2	6.796 <sup>A</sup>	6.290 <sup>BA</sup>	5.870 <sup>A</sup>	5.930 <sup>AB</sup>	6.908 <sup>AB</sup>
Log <sub>10</sub> (cfu+1)	3	6.850 <sup>A</sup>	6.408 <sup>A</sup>	5.684 <sup>A</sup>	5.213 <sup>C</sup>	6.934 <sup>AB</sup>
	4	6.864 <sup>A</sup>	6.204 <sup>B</sup>	5.927 <sup>A</sup>	5.446 <sup>BC</sup>	7.194 <sup>A</sup>
	Zone	1	2	3	4	5
Gram-negative	1	6.083 <sup>B</sup>	5.373 <sup>B</sup>	4.974 <sup>A</sup>	2.004 <sup>A</sup>	2.416 <sup>A</sup>
Bacteria,	2	6.203 <sup>AB</sup>	5.509 <sup>AB</sup>	4.779 <sup>A</sup>	2.097 <sup>A</sup>	1.064 <sup>A</sup>
Log <sub>10</sub> (cfu+1)	3	6.255 <sup>AB</sup>	5.725 <sup>A</sup>	5.271 <sup>A</sup>	1.132 <sup>A</sup>	1.698 <sup>A</sup>
	4	6.338 <sup>A</sup>	5.317 <sup>B</sup>	4.911 <sup>A</sup>	0.719 <sup>A</sup>	1.462 <sup>A</sup>
	Zone	1	2	3	4	5
Fungi,	1	3.638 <sup>A</sup>	2.917 <sup>A</sup>	3.823 <sup>AB</sup>	5.413 <sup>A</sup>	3.424 <sup>B</sup>
Log <sub>10</sub> (cfu+1)	2	3.399 <sup>A</sup>	3.242 <sup>A</sup>	3.803 <sup>AB</sup>	4.681 <sup>AB</sup>	4.130 <sup>AB</sup>
	3	3.659 <sup>A</sup>	2.631 <sup>A</sup>	3.431 <sup>B</sup>	4.526 <sup>B</sup>	4.605 <sup>A</sup>
	4	3.529 <sup>A</sup>	3.038 <sup>A</sup>	3.992 <sup>A</sup>	4.744 <sup>AB</sup>	4.314 <sup>AB</sup>

<sup>1</sup>Mean separation within column by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.



Figure 1. Water damage observed when polyethylene bags were removed after storage.