Evaluation of Cleaning and Washing Processes for Cotton Fiber

Part VII. Microbiological Evaluation[1]

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ABSTRACT

Precleaning, washing, steaming plus washing, and steaming plus hot alkaline scour result in statistically significant decreases in the gram-negative organism counts and the endotoxin content of the cotton and of the airborne dusts in the model cardroom when the cotton is carded.

Introduction

Attempts to understand the pathogenesis of byssinosis have led to the concept that a water-soluble substance, present in the cotton or as a contaminant harvested with the cotton, is made airborne during the process of carding and is inhaled by workers in the cardroom of mills. Baled cotton contains significant numbers of weeds, grasses, and parts of the cotton plant [6, 7]. Frequently these are heavily contaminated with microorganisms [3]. These include fungi, gram-positive bacteria, and gram-negative rod bacteria. The latter decrease with time as they die off, but they leave an endotoxin—a lipopolysaccharide component of their cell walls—in the cotton. The structure of the endotoxin varies, depending on its source (*i.e.* the species of microorganisms), but it is a stable, highly-reactive substance [2] that causes recruitment of cells into pulmonary airways on inhalation; it can be detected in the blood of patients with severe infections; it causes vascular damage; and it triggers histamine release [4, 5].

Therefore, our microbiological studies were directed towards determining 1) the level of viable gram-negative microorganisms present during the cotton-carding process, and 2) the level of endotoxin activity in the cotton and in the airborne dusts during carding.

The procedure for processing and blending of the cotton, the source of the cotton, the facilities in the model cardroom at N. C. State, and the treatments listed in the charts are described in previous papers of this symposium. Studies were made on the cotton (after treatment and blending and prior to carding) and on the airborne dust when the cotton was carded in the model cardroom at N. C. State.

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Methods

The cotton was mixed with normal saline, sonicated briefly, and an aliquot of the extract plated out on appropriate dilutions of trypticase soy agar with and without added vancomycin to inhibit gram-positive microorganisms. The plates were counted and calculations made of the number of microorganisms (both gram-negative and gram-positive) per mgm of the cotton.

The filters were mixed with normal saline, sonicated briefly, and appropriate dilutions plated and counted.

The same types of extracts were made with endotoxin-free saline and in endotoxin-free glassware, and the endotoxin activity was determined by the Limulus Amoebocyte Lysate Test using a modification of a micromethod [5]. The actual range of endotoxin present was determined by testing several dilutions until an endpoint was reached.

Results

The basic microbiological data are shown in Table I (the means of the counts for each treatment modality). Endotoxin levels decrease progressively for both raw cotton and airborne dust as severity of treatment increases. The same is true for counts of gram-negative microorganisms in both the raw cotton and the airborne material.

A model was constructed by our statistician for the data on the airborne dust, and the various treatments were tested for significance with the following conclusions. Statistically significant ($P < 0.001$) treatments of the cotton are: 1) precleaning; 2) washing; and 3) steaming. However, the effect of steaming could not be separated from washing and hot alkaline scour, since no study was made of steaming as a single treatment. The observed and estimated (by the model) means are compared in Table II.

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TABLE I. Washed cotton. Means of raw data.

Treatment[a] No.	Dust, mg/m³	Cottons Bact.[b] cfu/mg	Cottons LPS,[c] ng/mg	Airborne Dusts Bact.[b] cfu/m³ × 10⁻²	No.	Airborne Dusts LPS,[c] ng/m³	No.
1-11	0.326	1456	8.3	16.2	24	202.1	10
1-1	0.356	939	33.3	25.7	12	162	6
2A	0.349	439	8.3	5.0	18	1500	8
2B	0.344	76	8.3	4.0		220.2	8
3	0.241	<0.1	0.32	<1.8	5	18.6	10
4	0.174	<0.8	0.08	<0.08	10	9.6	12
5	0.253	<0.03	0.06	<0.7	7	12.1	9
6	0.218	<0.03	0.008	<0.2	4	13.1	12
6A	0.151	<0.03	0.008	0.5	7	11.9	8
7	0.337	0.8	0.04	<0.3	7	12.6	8
8	0.223	<0.03	0.008	<0.3	9	15	10

[a] Treatments are as follows: #1 (control)-11 is bale held in warehouse and processed later; #1-(control)-1 is bale processed as control; #2A is precleaned once; #2B is precleaned twice; #3 is precleaned twice, washed and rinsed in hot water (60°C); #4 is precleaned twice, washed, and rinsed in hot water (82°C); #5 is precleaned twice, washed, and rinsed in hot water (60°C) and also steam treated; #6 is precleaned twice, washed in hot alkaline scour (71°C), and steam treated; #6 is rinsed at 82°C; #6A is rinsed at 32.2°C; #7 and #8 are preleaned twice, washed in hot alkaline scour (71°C), steam treated, rinsed at 82°C, finish added. In addition, #8 had bleach added. These treatments are outlined in Winch, A. R. [8].
[b] BACT. = gram-negative rod bacteria.
[c] LPS = Lipopolysaccharide.

TABLE II. Observed and estimated means of significant treatments for airborne gram-negative microorganisms.

	Treatments Preclean[a]	Wash[a]	Steam[b]	N	Mean cfu × 10² Observed	Estimated[c]
1 (1-1)	—	—	—	36	18.9	17.8
2A	+ (1×)	—	—	27	4.07	3.95
2B	+ (2×)	—	—	18	3.96	5.91
3	+	60°C	—	16	1.02	1.26
5	+	60°C	+	10	0.37	0.26
6, 6A, 7, 8	+	71°C	+	38	0.34	0.28
4	+	82°C	−	12	0.27	0.12

[a] $P = 0.0001$.
[b] $P = 0.0002$.
[c] = according to statistician's model.

Factors studied for which a significance could not be established are storage, run-speed, and elutriator number (see Table III).

For the endotoxin in the airborne dusts, the significant treatments were precleaning, washing, and storage. Significance was not established for steaming (plus alkaline scour).

TABLE III. Bales (pairwise comparisons) for airborne gram-negative microorganisms (Duncan's multiple range test $\alpha = 0.05$).

Significant comparisons Treatments	Significance not established Treatments
1–2A	3–5
2A–2B	4–5
2A–3	
2A–5	4–6
3–4	5–6
3–6	

Endotoxin (lipopolysaccharide or LPS) was measured by a modified Limulus Amoebocyte Lysate method. Previous studies have shown that in the air of cardrooms of mills [3], and in the air of the model cardroom at N. C. State, there are significant levels of endotoxin (see Table IV, which lists a few studies of microorganisms and endotoxin in these different areas).

TABLE IV.

	Airborne dusts Bacteria, cfu/m³	LPS, ng/m³
Model cardroom		
Bales—"Hi"	3 900	680
	8 600	250
Bales—"Lo"	700	110
	580	110
Research lab	14	—
Outside air	14	—
Mills	25 000	16–95

Table I shows a marked decrease in the levels of endotoxin in the airborne dusts as the severity of treatment of the cotton increases.

The correlation coefficient between the cotton and airborne dust levels of gram-negative rods is 0.68. A regression equation was arrived at after plotting the above data, and this equation can be used to calculate an estimated level of airborne dust microorganisms in cfu/m³ (cfu = colony-forming units) from a determined level of microorganisms in cfu/mgm of

a cotton. This equation applies only to the specific conditions in the model cardroom at N. C. State, because it was derived from data collected there.

$$y = 1.77 + 0.354x \quad ,$$

where y = log of airborne dust microorganisms in cfu/m³, and x = log of cotton microorganisms in cfu/mgm. Currently, we are studying the applicability of this and similar equations to other environments.

Conclusions

Precleaning of cotton significantly reduces the level of airborne microorganisms when that cotton is carded. Washing alone (#3) and washing and steaming (#5) do the same. Washing at 82°C is better than washing at 60°C (#4 *vs.* #3).

Alkaline scour at 71°C and steaming are better than only washing at 60°C (#6 *vs.* #3).

The counts are so low in treatments #6, 6A, 7, and 8 that differences between these treatments are not significant in this data set for either airborne microorganisms or raw cotton.

Precleaning drops the levels of gram-negative microorganisms in airborne dusts and in raw cotton about 1 log. Washing (60°C) drops the levels of gram-negative microorganisms in airborne dusts another log, and in raw cotton, another 2 logs. A further one-log drop is added by steaming and washing plus hot alkaline scour.

Endotoxin levels follow the same trend and drop about 3 logs with treatment of raw cotton but only about 1 to 1½ logs in the airborne dust. A significant amount of endotoxin in the airborne dusts may have a different source than the cotton being processed.

A regression equation ($y - 1.77 + 0.334\,x$) was determined from the data relating airborne dust levels to cotton levels of microorganisms.

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