Microbial Growth Inhibition by SO_2 or SO_2 Plus NH_3 Treatments During Slow Drying of Corn[1]

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ABSTRACT

Microbial growth in three bins containing corn with 26.5% initial moisture was monitored during ambient air drying. One bin was treated by the "trickle" procedure, with two equal applications of 0.066% SO_2 (weight of SO_2/weight of wet corn) at one and 28 days. The second bin was treated on the same days with 0.066% SO_2 followed immediately by 0.018% NH_3. The third bin was a control. Six kinds of fungi and bacteria grew rapidly in the control bin and reduced the corn to sample grade. The corn treated with SO_2 showed little microbial growth for the first 60 days of storage. Subsequently, however, *Penicillium* grew in the top third of the bin, but little visible deterioration occurred, and the SO_2- and SO_2-NH_3-treated corn was graded No. 2 or better. The sequential SO_2-NH_3 treatment was similar to the SO_2 treatment, with no indication that the combination treatment was superior. Neither treatment affected the market grade, milling yields, or protein and ash content of the corn, but both were mildly corrosive to galvanized steel.

During the past decade, there has been a trend away from on-farm high-temperature drying systems toward low-temperature in-bin drying of corn (*Zea mays* L.). Low-temperature systems conserve fossil fuels, but initial moisture content of the corn should be below 24% to prevent spoilage and mycotoxin formation during prolonged adverse drying conditions (Ross et al 1978, Tuite and Foster 1979). These spoilage problems might be circumvented by the application of fungicidal gases to control microbial growth. Nofsinger et al (1977, 1978) applied anhydrous ammonia (NH_3) via the air-distribution system of the bin (ie, the "trickle" process) to corn ranging from 24 to 26.5% moisture, wet basis (wb) in intermittent dosages of 0.009–0.09% by weight of dry corn. They reduced mold population by a factor of 100 during the tests while maintaining market grade. Eckhoff et al (1979) aerated 24% (wb) moisture corn treated with 0.3% SO_2 in two equal applications, at 0.56 m^3/min/t (metric ton) for 56 days and subsequently at 2.23 m^3/min/t for the next 116 days until dry. Mold and bacterial counts were better than 10^3 counts per gram lower in the SO_2-treated corn than in the control corn throughout the test, with the final grading being U.S. No. 1 for the SO_2-treated corn, and U.S. No. 5 for the untreated control. Kalchik et al (1979) compared drying cost, drying efficiency, and corn quality of five conventional drying procedures to the "trickle-NH_3" drying process. The "trickle" process had the lowest drying cost, the highest-quality corn, and a drying efficiency of 3.7 kJ per gram of water removed.

Vidal and Jayaraman (1979) sequentially applied SO_2 and NH_3 to corn in a 3:1 dosage ratio and air-dried it in the laboratory. The combination SO_2-NH_3 treatment showed a synergistic effect on the microbial efficacy, an increase in the drying rate, a decrease in corrosion on galvanized metal, and produced a product superior in color, flavor, and taste to corn treated with SO_2 or NH_3 separately. The treatment eliminated and inhibited microbial growth during the test. Based on these laboratory findings, a scale-up evaluation of the process was suggested. This article reports the findings of that scale-up and its comparison to the "trickle" SO_2 procedure.

MATERIALS AND METHODS

Three 6 t-capacity drying bins 3.1 m in height (2.2 m i.d.) and equipped with centrifugal blowers to deliver an airflow of 1.11 m^3/min/t, were filled with 26.5% wb moisture corn. One bin containing 5.43 t of corn was treated with 3.6 kg of SO_2 gas and the second containing 5.71 t of corn with an application of 3.6 kg SO_2 immediately followed by 1 kg of NH_3, using the "trickle" process as described by Nofsinger et al (1977, 1978). The third bin containing 5.39 t was a control. The gas-flow rate, monitored by a rotameter, was 20 g/min for the SO_2 and 16.5 g/min for the NH_3. A second treatment was applied after three weeks at the same dosage but at a flow rate of 60 g/min for the SO_2 and 25.1 g/min for the NH_3.

Initial samples were taken on 25 October 1979 from each bin before treatment and were analyzed for fungi, anaerobic bacteria, and moisture. Samples from three depths—top, middle, and bottom—were taken one, five, 12, 19, 26, 34, 41, 54, 89, 119, and 134 days after the initial treatment. Each sample was a composite of three probings—one from the center of the bin and two from the periphery. Final and initial samples from the three depths in each bin were dry-milled.

Corrosibility of the SO_2 and NH_3 treatments were determined by embedding 10-cm square pieces of galvanized sheet metal in the grain mass. Seven metal pieces were used in each bin; six were located at 30.4-cm intervals in the grain mass, starting 15.2 cm from the bin floor, and the seventh was located in the exhaust air stream. Corrosibility was measured visually and after wire-brushing by the loss in weight due to oxidation.

To determine fungal infection, kernels were plated on potato-dextrose agar containing 100 ppm Tergitol NPX and 30 ppm Chlortetracycline (PDTC) and malt salt (MS) agar containing 7.5% NaCl. Fifty kernels each were submerged in 5% NaOCl (Timesaver brand) for 1 min and rinsed twice in sterile water. The NaOCl had one to two drops of Tween 80/500 ml. Fungal infection was also determined on some samples after 59 days, using an alcohol pretreatment. Fifty kernels were submerged for 10–20 sec in 95% ethanol before treatment with NaOCl. These determinations were used for comparison of the procedures, and all data presented herein used the NaOCl treatment without alcohol pretreatment. Numbers of bacteria and fungi were determined by serial dilution. Twenty-five grams of corn were blended in a Waring blender in 0.1% water agar for 1 min and 1:10 dilutions in 0.1% water agar cultured in PDTC, MS, and beef peptone agar (BPA) containing 100 ppm cycloheximide. Visible mold damage was determined on four 50-seed replicates. Kernels were judged mold-damaged if spores or mycelia were observed on the surface or if kernels were sufficiently discolored from apparent internal mold infection. Seed germination was measured by plating NaOCl-treated kernels on 1.5% water agar at 22–24°C for five to seven days. Seeds were classified as germinated if they had both shoots and roots. Moisture content was determined by the whole-seed air-oven method at 103°C for 72 hr.

The corn was conditioned and dry-milled (roller-milled) into grits, meal, flour, germ, and hull fractions, following the procedure outlined by Kirleis et al (1982). Protein (N × 6.25) content was determined by the Kjeldahl method as modified by Noel (1979) and ash and moisture contents as described in AACC methods 08-01 and 44-40, respectively (1976). Color of grit, meal, and flour fractions, ground to pass through a U.S. Standard 50-mesh screen,

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were determined on a Hunter Lab color meter (model D25-2).

Airflow was measured by using a hot wire anemometer (Thermo-Systems, Inc., model 1650) and a 25:1 reducing funnel. Four locations were measured in each bin and averaged to give the air flow for the bin.

RESULTS AND DISCUSSION

The low airflow (1.11 m^3/min/t), high initial moisture content (26.5%), and the fall drying weather combined to stress the capabilities of the system and gave an adequate comparison of the chemical treatments. Under these conditions, five months of continuous drying were required to drop the average moisture content of the three bins below 15.5%. Most of the drying occurred during the first 45 days, with little drying during the winter. After 90 days, the average moisture in all three bins was below 18.0%. A second drying front, which passed through the corn in the spring, reduced moisture to less than 15.5%.

Figure 1 shows the progress of drying at three depths for each bin. Fan output varied between bins, causing drying to be slower in the control bin. When airflow differences were accounted for, no difference in drying rates were observed.

Both the SO_2-NH_3, and SO_2 treatments reduced bacteria counts by a factor of 1,000 for most of the test (Fig. 2). The 26-day sample (just before the second treatment) showed a marked increase in bacteria in the SO_2-NH_3 bin. Apart from this sample, the SO_2-NH_3 and the SO_2 treatment appeared to perform about the same. Figure 2 shows the average counts of the three sampling levels in each bin. Counts varied between levels in a bin by a factor less than 10.

The effect of the treatments on the predominant molds is shown in Figs. 3 and 4. Both treatments had fewer infected kernels and lower dilution counts than the control throughout the tests, although the treatments were not successful in completely controlling mold growth. The SO_2-NH_3 treatments had an increase in both infected kernels and dilution counts throughout the bin before the second treatment and in the top third of the bin after 45 days. SO_2 controlled growth throughout the first 45 days, but the top third showed fungal growth and sporulation thereafter.

Fungal dilution counts for the 12th and 19th days in the SO_2-NH_3-treated bin were high when compared to the percentage of infected kernels. Such high counts appeared to be caused by heavy sporulation in a few kernels rather than a widespread infection. Thus, high mold counts on a few kernels obscure the low counts of most of the other kernels. The counts on the 12th day in the top

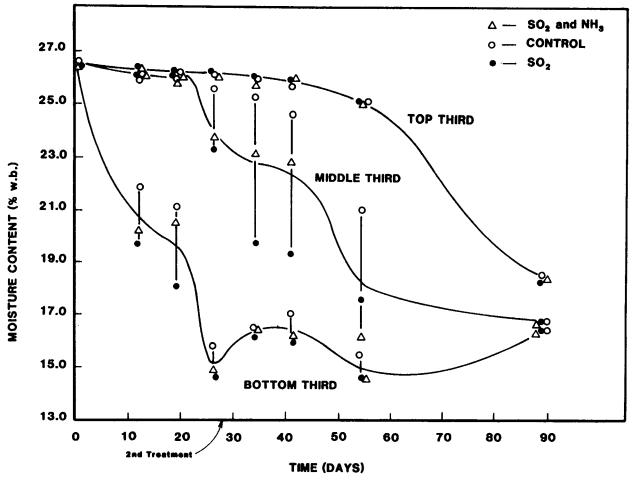

Fig. 1. Moisture content at three depths in all three bins.

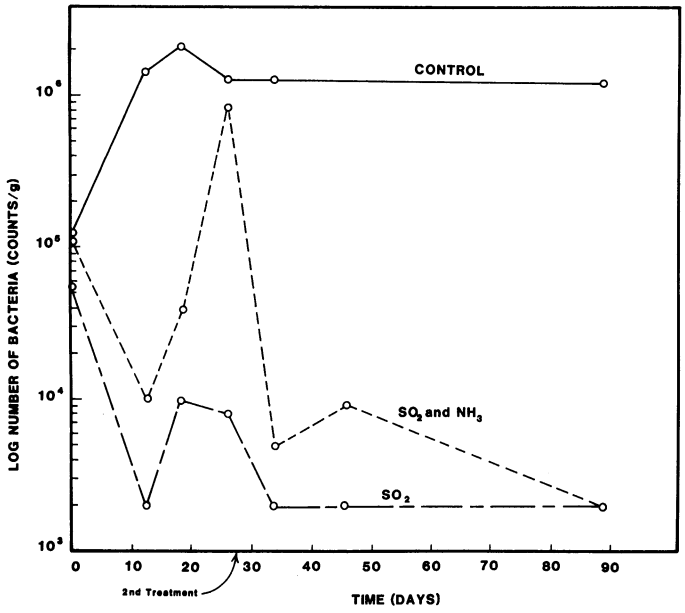

Fig. 2. Average number of bacteria per gram for untreated, SO_2-treated, and SO_2-NH_3-treated corn.

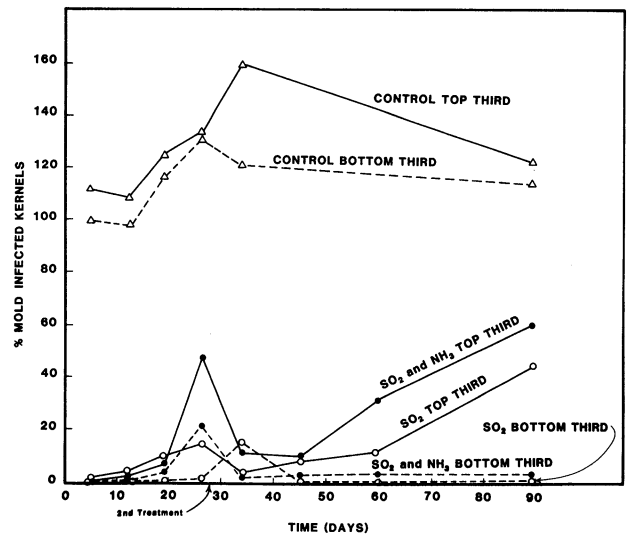

Fig. 3. Average total percent mold infection in top third and bottom two thirds of each bin.

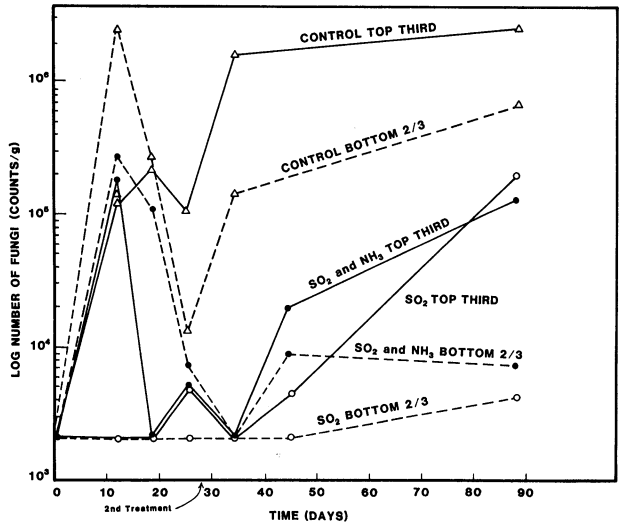

Fig. 4. Average numbers of fungi per gram in top third and bottom two thirds of each bin.

third of the bin were predominantly *Penicillium* spp., (primarily *P. cyclopium*), whereas on the 19th and 26th days no *Penicillium* was isolated. For the bottom two thirds, counts on the 12th day were due to *Cladosporium* in the middle third of the bin, on the 19th to *Penicillium* in the bottom third, and on the 26th only low levels of both organisms were observed. Such variations indicate that certain kernels either were inadequately treated (poor eradication) or localized growth was occurring (poor inhibition). Poor inhibition is probably caused by the in situ formation of ammonium sulfate when the NH_3 is added following the SO_2. Ammonium sulfate is not inhibitory and may actually promote growth because it can serve as a nitrogen source. In laboratory tests in which NH_3 was applied to corn followed by SO_2 (reverse of sequence used in this test), molds grew faster than in an untreated control.[5]

When repeated subsamples after 59 days storage from SO_2-NH_3 treatments (upper third of the bin) were plated after the usual disinfection treatment (5% NaOCl, two sterile water rinses), one or two out of five or six subsamples would have 60–98% kernels yielding *Penicillium* as compared to about 0–10% for the other three to four replicates. However, when the kernels were first treated with 95% ethanol[6] and then with 5% NaOCl, there were no subsamples with high *Penicillium* infection. Also, when the kernels were treated separately in a specially designed device, or when the sterile water rinse was omitted, the numbers of infected kernels remained low for all replicates. We believe this is evidence that spores of *Penicillium* were present in sufficient numbers on a few kernels in some subsamples that survived the NaOCl treatment without alcohol, and were spread to noninfected kernels by the water rinse. Replicates that had high numbers of kernels yielding *Penicillium* colonies were those that happened to include the kernel with the heavily sporulating kernels. The high mold counts in Fig. 4 compared with the lower percent mold infection kernels in Fig. 3 for SO_2-treated corn were caused by this phenomenon.

The control bin had a more diverse population of fungi dominated by *Alternaria*, *Cladosporium*, *Epicoccum*, *Gibberella zeae*, *Penicillium*, and yeasts, whereas *Penicillium* predominantly grew in the treated bins. This nearly pure culture growth is similar to that observed by Cleustrom et al (1981) when they treated hay with formic acid. The treatment initially suppresses the indigenous microflora, so that when the treatment begins to fail, the most resilient and opportunistic organism dominates. Such single-organism cultures appear to grow faster than mixed cultures because of a lack of competition, and depending upon the organism, may result in greater toxin production than in mixed cultures that usually occur on untreated corn.

Visible mold damage of corn at five sampling times is shown in Table I. The results indicate that low damage occurred in the SO_2-NH_3-treated corn and in the SO_2-treated corn, whereas significant damage occurred in the top two thirds of the control bin. Samples taken after 200 days from the start of the test were inspected and graded by a licensed grain inspector. The results of the inspection, along with a grade classification for each factor, are shown in Table II. Official grade is based on the lowest grade of any of the factors and on this basis, both the SO_2 and the SO_2-NH_3 were successful treatments. Some grade deterioration occurred in the top third of each of the treated bins, but the bin average was maintained above minimum standards for No. 2 corn.

Grade is a useful but not always adequate indicator of treatment success. For example, mycotoxin formation can occur even though little visible deterioration has occurred. To control mycotoxin production, a treatment should inhibit all fungal growth. Both treatments failed upon this criteria; growth of fungi was evident in the SO_2-NH_3 treatment after 20 and 50 days and was evident in the SO_2 treatment after 60 days. Growth in the bin may have been inhibited by using a third treatment at about 60 days, by using a higher SO_2–air ratio during treatment, by reversing the fan and pulling the SO_2 down through the bin, or by not aerating the bin until 24 hr after treatment. These options need to be explored. Complete inhibition of microbial growth should be possible by using adequate treatment procedures.

Milling tests were performed on a composite untreated sample of corn taken as the bins were loaded and on samples taken from the

[5]Personal communication with Dr. Rod Bothast, Northern Regional Research Center, Peoria, IL.
[6]Suggested by Dr. Dave Sauer, U.S. Grain Marketing Laboratory, Manhattan, KS.

TABLE I
Visible Mold Damage of Treated and Untreated Corn Sampled at Three Depths in the Bins at Five Sampling Times[a]

		Damaged Kernels (%) After				
Treatment	Location	26 Days	34 Days	55 Days	89 Days	124 Days
Control	Top	8.0	···	7.5	9.0	13.0
	Middle	13.0	···	13.0	10.0	9.5
	Bottom	6.0	···	8.5	2.0	3.5
SO_2-NH_3	Top	1.5	3.5	1.0	0.5	0.0
	Middle	2.0	0.0	1.5	0.0	0.0
	Bottom	0.5	0.0	1.5	0.0	0.0
SO_2	Top	0.5	1.0	2.0	3.0	0.0
	Middle	0.0	0.0	0.5	0.0	0.0
	Bottom	0.0	0.0	0.0	0.5	0.0

[a]Kernels determined damaged if visibly molded (obvious spores or mycelium present) or if discolored.

TABLE II
Official Grades[a] of Treated and Untreated Corn After 134 Days

		Control		SO_2-NH_3		SO_2	
Location	Grade Factor	Numerical Value	Grade	Numerical Value	Grade	Numerical Value	Grade
Top	Test weight	55.0 lb	2	55.5 lb	2	55.0 lb	2
	Moisture content	16.0%	3	11.9%	1	12.0%	1
	Total damaged kernels	26.1%	Sample	3.5%	2	4.5%	2
	BCFM	2.7%	2	2.1%	2	2.8%	2
Middle	Test weight	55.5 lb	2	56.0 lb	1	55.5 lb	2
	Moisture content	15.1%	2	11.0%	1	11.0%	1
	Total damaged kernels	22.9%	Sample	1.4%	1	1.8%	1
	BCFM	1.2%	1	1.8%	1	1.7%	1
Bottom	Test weight	56.5 lb	1	56.0 lb	1	56.0 lb	1
	Moisture content	12.8%	1	10.5%	1	10.5%	1
	Total damaged kernels	10.5%	5	1.4%	1	1.3%	1
	BCFM	0.9%	1	1.1%	1	1.6%	1

[a]Official grade is the lowest of the four grade factors.

TABLE III
Seed Germination at Three Levels in Each Bin for Four Sampling Dates

Treatment	Location	Seed Germination (%) After 1 Day	19 Days	119 Days	134 Days
Control	Top	92	78	59	56
	Middle	99	81	67	50
	Bottom	96	89	81	75
SO_2-NH_3	Top	96	93	49	28
	Middle	96	77	40	36
	Bottom	95	87	69	45
SO_2	Top	86	72	25	28
	Middle	68	77	22	8
	Bottom	24	60	43	24

treated bins 134 days after the start of the test. The bins were sampled at three depths—top, middle, and bottom. The samples from the control bin were not milled because a considerable amount of microbial deterioration had already occurred. There was no difference in the product yield, Hunter Lab color meter readings, and the protein and ash content of the various dry-milled fraction of the treated and initial untreated corn.

The germination of the corn determined on four dates (Table III) shows that the chemical treatments reduce the germination of the corn. The SO_2 treatment reduced the seed viability more than the SO_2-NH_3 treatment, although both treatments produce corn unacceptable for use in any process where germination is important.

In the corrosion test, the weights of the galvanized sheet metal samples were highly variable and showed no trends. Visual observation of the plates did show mild corrosion from the chemical treatments. The plates in the control bin showed little corrosion. Both the SO_2-NH_3 and the SO_2 treatments had visible corrosion, although the corrosion was not great enough to cause blistering or cavitation. The corrosion was most pronounced on the plates located in the lower parts of the bins where exposure to the chemicals was the highest. The corrosion on the plates between the SO_2-NH_3 and the SO_2 treatments were visually different. The corrosion on the plates from the SO_2-NH_3 bins was less uniform and exhibited a slightly whitish appearance compared to the SO_2 corrosion, apparently because of the formation of zinc oxide.

CONCLUSIONS

Both the SO_2 and the SO_2-NH_3 treatments inhibited microbial deterioration in 26.5% wb moisture corn to an acceptable level based on market grades in an ambient air-drying system. Both treatments were unsatisfactory in inhibiting microbial growth beyond 45 days in the upper third of the bin. A higher initial dose (>0.066%) or a third treatment may be necessary to provide satisfactory inhibition of growth. When growth occurred after 45 days in the treated bins it consisted of essentially a pure culture growth of *Penicillium* as compared to the mixed culture that grew on the untreated corn.

Treatment of wet corn with SO_2 or SO_2-NH_3 does not affect the market grade, milling yields, or protein and ash content of corn. The sequential SO_2-NH_3 treatment was not superior to the SO_2 treatment. Both SO_2 and SO_2-NH_3 caused mild corrosion to galvanized steel and reduced kernel germination.

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