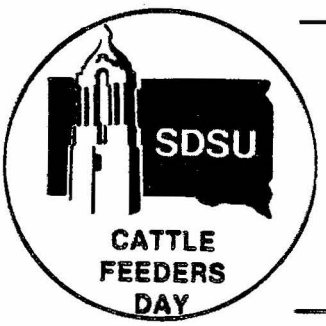
1984

Effect of Lactobacillus Plantarum on Microbial Populations, Silage Qualit, Utilization and Preservation

J. Nothnagel
South Dakota State University

R. M. Luther



EFFECT OF *LACTOBACILLUS PLANTARUM* ON MICROBIAL POPULATIONS, SILAGE QUALITY, UTILIZATION AND PRESERVATION

J. Nothnagel and R. M. Luther

Department of Animal and Range Sciences

CATTLE 84-6

Summary

Corn forage from the 1982 corn crop was harvested at approximately 61% moisture. One experimental concrete silo was filled with untreated forage and another with forage treated with a *Lactobacillus plantarum* inoculant. The Lactobacillus inoculant was added prior to ensiling by mixing 10 g of inoculant (10 billion CFU (Colony Forming Units) /g) per ton of forage.

Measurements taken were counts of microbiological groups, chemical characteristics and heat production during fermentation, dry matter perservation and nutrient digestibility and nitrogen retention by beef steers.

The treated silage appeared to have a more favorable microbial profile as evidenced by higher anaerobic populations and lower yeast and mold counts. Although chemical quality data are not complete, results show that the treated silage has a higher lactic acid content (4.80 vs 3.98) than untreated silage. The digestibilities of dry matter, crude protein and organic matter were essentially the same for both silages. However, more nitrogen was retained by steers fed the treated silage (41.59% vs 34.57%). Recovery of feedable silage from both silos was unusually low at about 69% for both of the silages.

Introduction

The making of quality corn silage is largely dependent upon a fermentation process that takes place in the forage after ensiling. Research has shown that microbial populations present in the forage are responsible for fermentation. These microbes may vary widely even within the same field of forage. Low microbial populations appear to slow the rate of (extent of) fermentation. Inoculation of forage with desirable bacteria offers a means of providing adequate types and numbers to initiate fermentation. The expected result would be to improve preservation and recovery of nutrients.

The objective of this study was to determine the effect of treating corn forage with a *Lactobacillus plantarum* silage inoculant on corn silage quality. Response to inoculation was

compared to untreated forage in terms of microbiological composition during the fermentation period, chemical characteristics and preservation of dry matter. The study also emphasized digestibility of nutrients and nitrogen utilization by beef steers.

Procedures

Corn forage from the 1982 corn crop was harvested at approximately 61% moisture with a conventional forage chopper. The chopped forage was weighed in a feed mixing wagon[a] equipped with a scale. Forage was allowed to mix for about 10 minutes and then packed into reinforced concrete culvert silos which were 6-foot high with a 5-foot diameter. One silo was filled with untreated forage and the other with forage inoculated with 10 g of product per ton (10 billion *Lactobacillus plantarum* colony forming units/g)[b]. Each silo was covered with plastic and a wooden lid placed on the top. Approximately 1200 lb of cement blocks were added for weight and another layer of plastic placed over the top. A thermistor probe was inserted into the center of the silage mass to permit recording of temperatures. Samples of forage were taken during filling and as the silage was removed for feeding. In addition, samples were removed through ports located in the wall of each silo during the first 21 days of the fermentation process. Each port was flushed with carbon dioxide after sampling.

Microbiological Analysis. A series of plating studies were made using 10-g sub samples of ensilage taken from a port. The sub sample was placed in individual, sterile Waring blenders containing 90 ml of sterile distilled water. Inoculants of 0.1 ml were distributed to incubation plates to give a 10^{-4}, 10^{-5} and 10^{-6} final plate dilution with fresh forage, whereas 10^{-8} and 10^{-9} and 10^{-10} final dilutions were used for ensilage. The media selected for enumeration of total aerobes and total anaerobes was tomato juice agar (Difco); for *Lactobacilli* spp. MRS (Difco) and for yeasts and molds potatoe dextrose agar with an antibiotic. Incubations were conducted in the dark at 30 C. Anaerobic conditions involved the use of a BBL Gas Pak anaerobic system. Three plates were used per dilution for the determination of colony-forming units. Samples for this phase of the study were taken of the standing corn crop; chopped forage at ensiling; and ensilage at 2, 4, 6, 9, 12, 15, 18 and 24 hr after ensiling on day 1, at 30, 36, 42 and 48 and 52 hr after ensiling on day 2 and at 52, 60, 66 and 72 hr on day 3. Thereafter samples were taken every 12 hr each day for the next 3 days and once a day for the next 10 days. Standard microbiological plate count techniques were used.

---

[a] Blair Manufacturing Company, Blair, NE.

[b] Biomax SI. Chr. Hansen's Laboratory, Milwaukee, WI.

Silage Quality Studies

Chemical silage quality determinations were completed in the laboratory on samples collected at storage, during ensiling and at feeding. The tests included moisture, pH, titratable acidity, total nitrogen, ammonia nitrogen, lactic acid and organic acids (acetic, propionic, butyric).

Digestion-Nitrogen Balance Trial

Utilization of nutrients from untreated and treated silage was determined in a digestion-nitrogen balance trial with beef steers. Twelve steers averaging 577 lb were placed in individual pens in the Animal Science Complex. The steers were fed corn silage (untreated) from another source for about 2 weeks. The steers were then weighed and allotted to the two silage treatments with 6 steers per treatment. The experimental silages were full-fed for an additional week and then the steers were placed in metabolism crates. The steers were allowed to adjust to the crates and a 5-day total collection digestion-nitrogen balance trial conducted. Corn silage was fed to appetite and a protein supplement was top-dressed to the silage at a rate of 1.14 lb per head daily. The supplement consisted of 58% soybean meal (44% protein), 28.7% ground corn, 2.0% ground limestone, 6.3% dicalcium phosphate, 5.0% trace mineral salt and vitamin A (10,000 IU/lb).

Urine and feces were collected once daily, measured or weighed, and a 10% aliquot of each saved for chemical analysis. The fecal material was frozen and the urine stored under refrigeration. Digestibility coefficients were calculated for dry matter, crude protein, organic matter. Nitrogen retention was reported as a percentage of that consumed.

Dry Matter Recovery Studies

Preservation of nutrients was determined on the basis of dry matter stored versus dry matter removed from the silo. Spoiled silage was separated from good silage, weighed and sampled as the silos were emptied. Dry matter and protein preservation were determined for each silage treatment.

Results

Microbiological Analyses

The microbial composition of the experimental silages during the first 15 days of storage are presented in figures 1 through 4. The colony forming unit counts were somewhat variable which may have been due to port-to-port variation. Also, each port was sampled several times. There were several general trends in the various microbiological profiles. The total aerobic bacteria counts shown in figure 1 did not appear to differ

greatly between the two silages. However, somewhat larger numbers were observed in the untreated silage. The total anaerobic counts presented in figure 2 were generally higher for the treated silage for most of the days of ensiling than for the untreated silage. The higher number of anaerobic bacteria did not appear to be due to Lactobacillus spp. Figure 3 shows that the number of Lactobacillus organisms was about the same for each silages. Number of yeasts and molds (figure 4) seemed to increase earlier and more rapidly in the treated silage but then dropped off faster than in the untreated silage.

While the data for the various microbe groups do not present a definite trend, the treated silage tended to provide a more favorable medium for microbial growth than the untreated silage. Further research is needed to substantiate these results.

Temperature Measurements and Silage Quality Studies

The results of temperatures taken during the fermentation period are shown in figure 5. Temperatures at storage were 25.5 C and 26.5 C. In the untreated ensilage peak temperatures of 30 C were reached at 4 days and in the treated silage a high of 29 C was observed at 3 days. The treated silage had generally lower temperatures throughout fermentation than the untreated silage.

Data from the chemical analyses of the samples of forage and silage are presented in table 1. The pH values were well below 4 which would indicate formation of quality silage. A titratable acidity value of 7.75 for the untreated silage versus 8.92 for the treated silage points toward higher acid formation in the treated silage. The higher level of acid was partially the result of a higher lactic acid level (4.80% of the dry matter) in the treated silage as compared to 3.98% the untreated silage. Ammonia nitrogen values were essentially the same for the untreated silage as for the treated silage. Volatile fatty acid analyses have not been completed.

Digestion-Nitrogen Balance Studies

Results of digestion and nitrogen balance studies with steers are presented in table 2. Digestibility data for dry matter, crude protein and organic matter show only small differences between the two experimental silages. The retention of nitrogen was 34.57% of that consumed for the steers fed the untreated silage as compared to 41.59% for those fed the trated silage. Nitrogen intake was about 10% greater for the steers fed the treated silage, but nitrogen retention by these steers greatly exceeded this value.

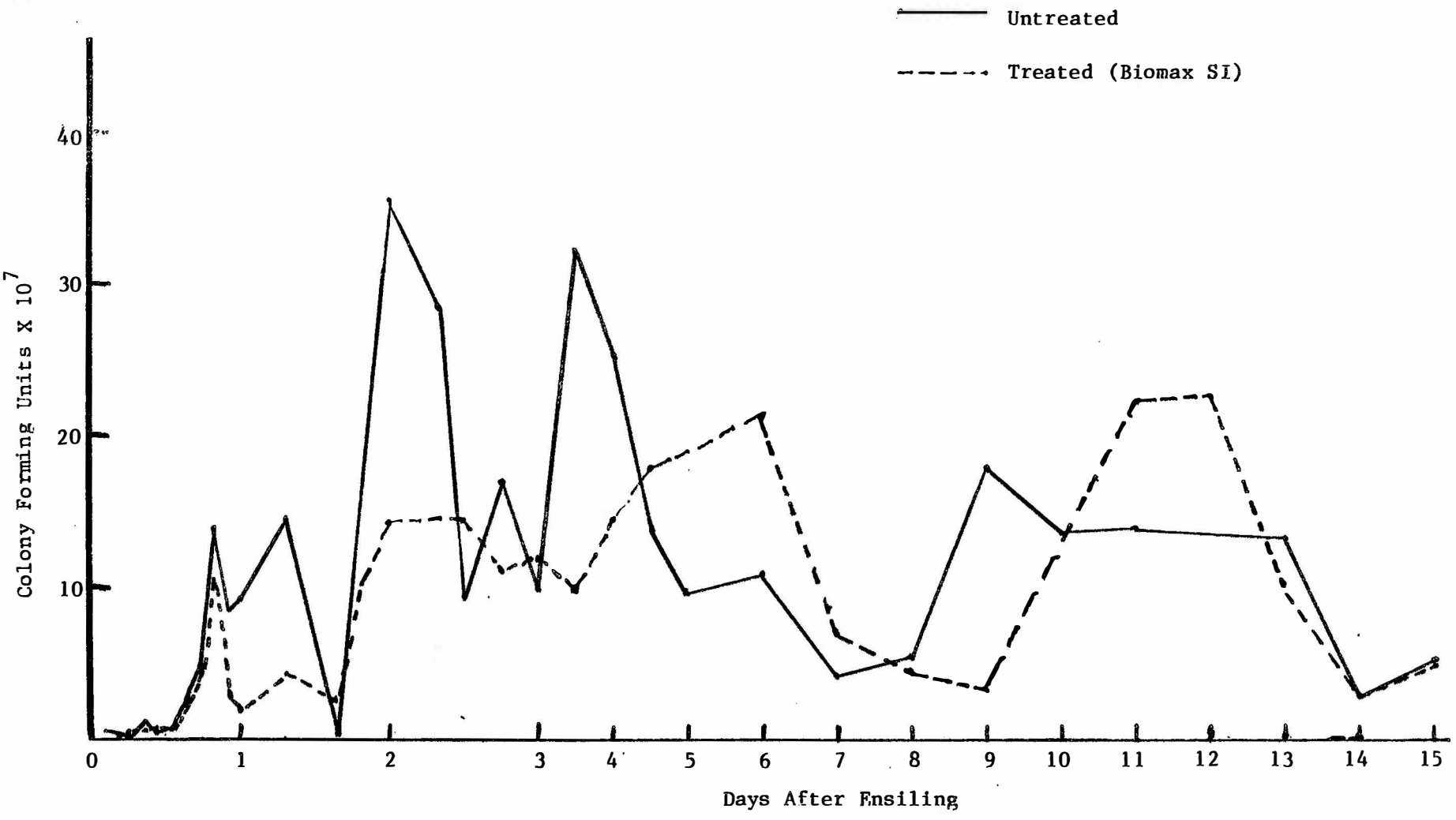


Figure 1. Total aerobic microbial colony counts from untreated and inoculated corn silage.

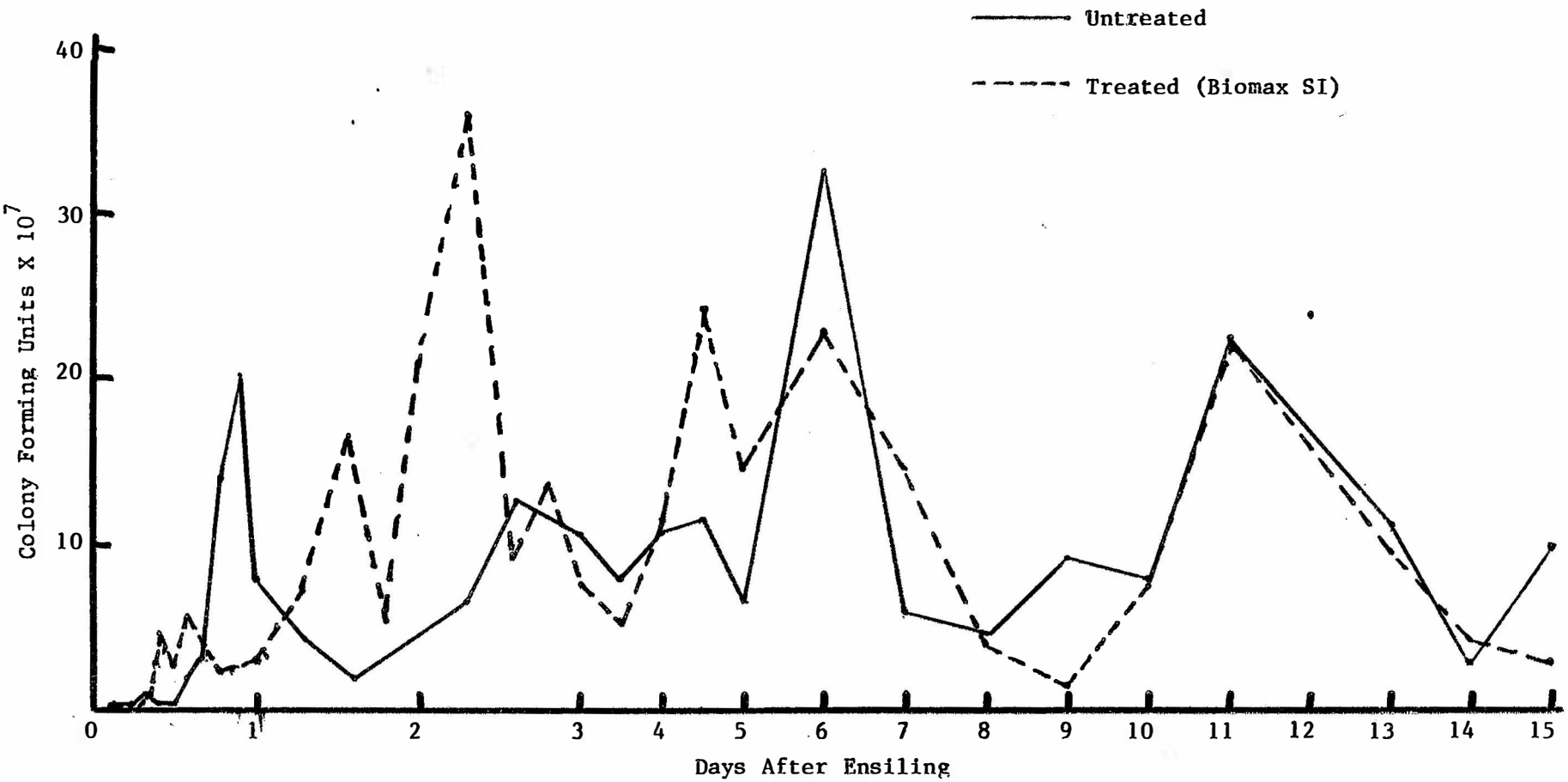


Figure 2. Total anaerobic microbial colony counts from untreated and inoculated corn silage.

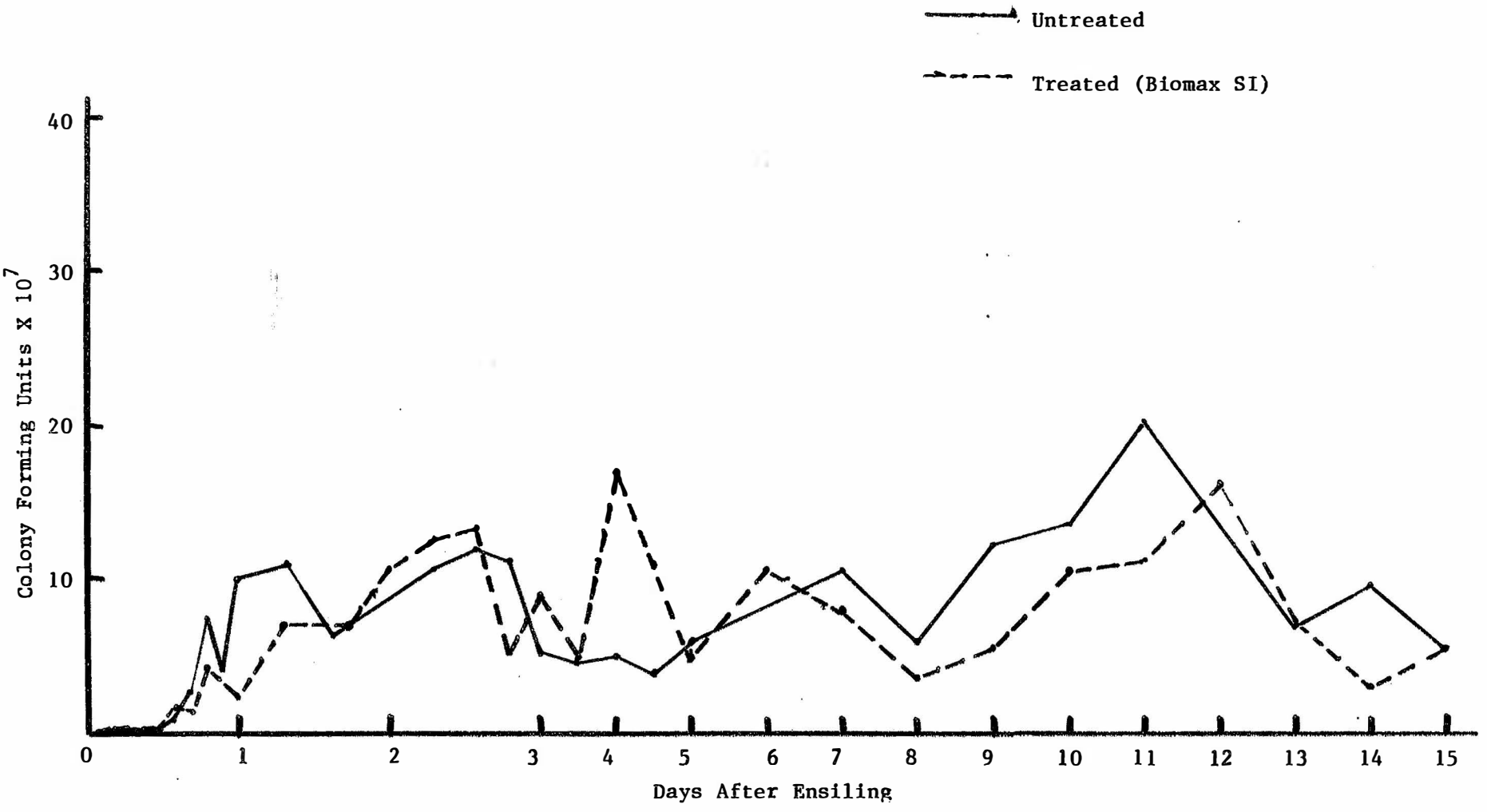


Figure 3. Lactobacillus spp. colony counts from untreated and inoculated corn silage.

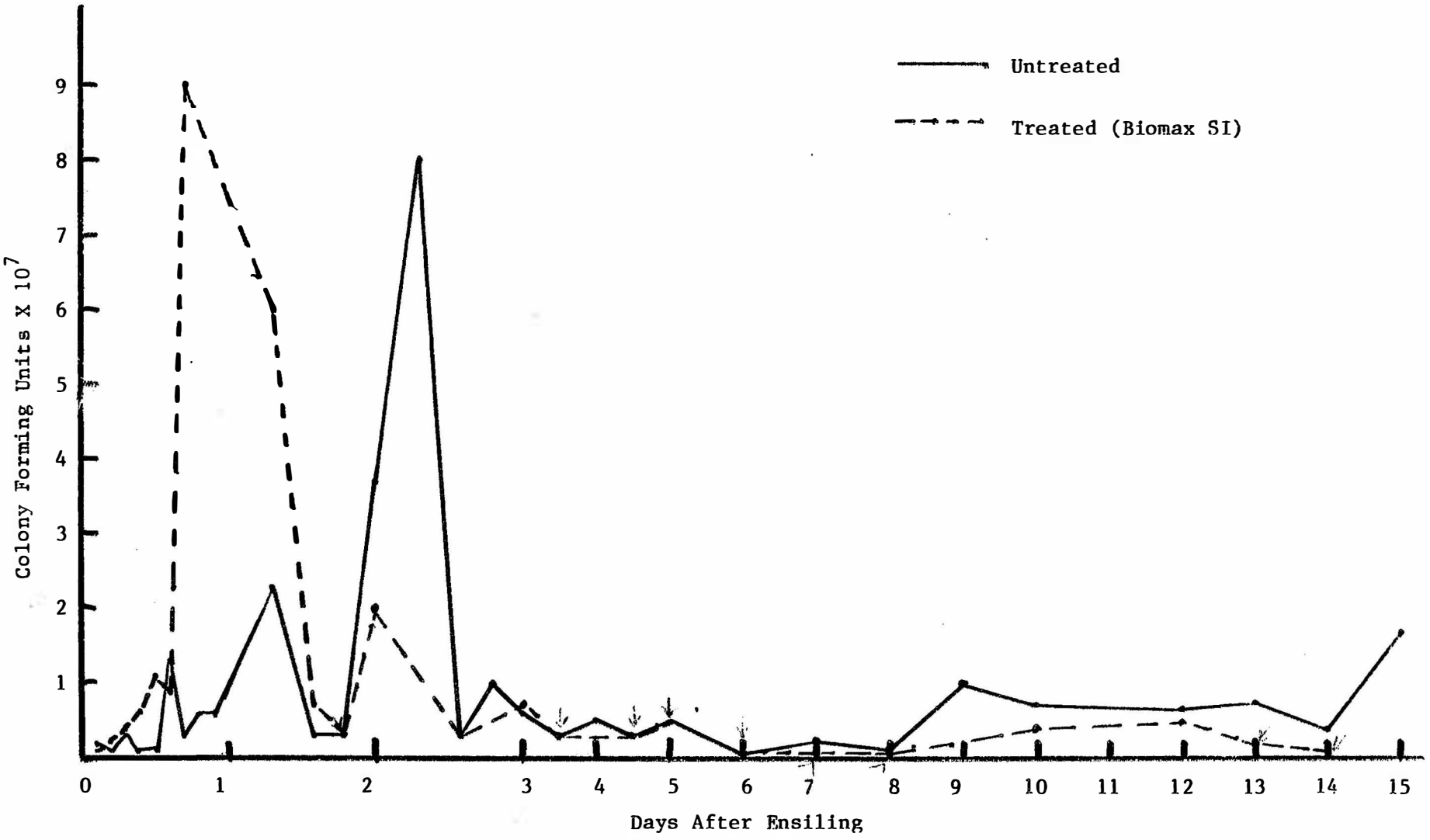


Figure 4. Yeast and mold colony counts from untreated and inoculated corn silage.

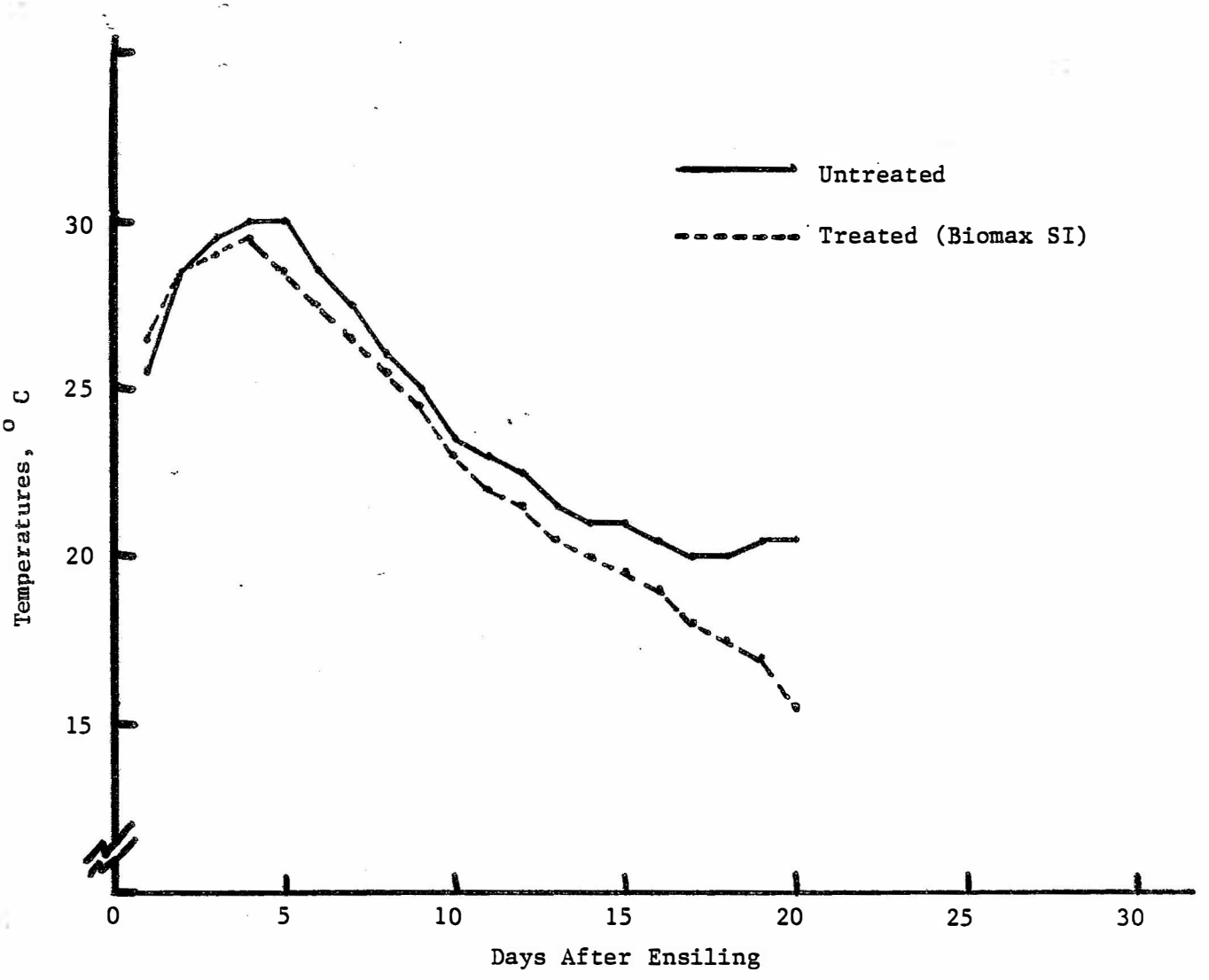


Figure 5. Fermentation temperatures of untreated and inoculated corn silage.

Preservation of Dry Matter

Dry matter recovery of the treated and untreated silage is presented in table 3. Feedable dry matter as a percentage of dry matter ensiled was low (about 69%) in this study. Spoilage losses were higher than previously observed in these silos. The reason for these losses may have been due to the exposure to air through the port openings with repeated sampling. While carbon dioxide was flushed into the ports, perhaps the quantity was not sufficient to maintain anaerobic conditions.

TABLE 1. CHEMICAL PROFILES OF CORN SILAGE FOR FEEDING AS AFFECTED BY BACTERIAL INOCULATION

	Silage for feeding	
	Untreated	Treated[a]
Dry matter, %[b]	37.41	37.15
pH	3.89	3.82
Titratable acidity[c]	7.75	8.92
Ammonia nitrogen[d]	0.164	0.154
Percent of dry matter		
Crude protein	8.37	8.45
Lactic acid	3.98	4.80

[a] Inoculated with *Lactobacillus plantarum* fermentation product at rate of 10 g per ton of forage.

[b] Toluene distillation with acid correction.

[c] Milliliters of .1N KOH to raise pH to 7.

[d] Percent of total nitrogen.

TABLE 2. DIGESTIBILITY AND NITROGEN BALANCE OF (UNTREATED AND MICROBIAL INOCULATED CORN SILAGE) FED TO BEEF STEERS

	Untreated	Treated[a]
No. of steers	6	6
Avg weight, lb	582	572
Avg daily dry matter intake, lb	12.34	13.37
Avg daily nitrogen intake, gm	89.36	98.32
Digestibility, %		
Dry matter	69.43	70.36
Crude protein	59.02	61.27
Organic matter	70.65	71.60
Nitrogen balance, g/day		
Fecal	36.64	38.02
Urinary	21.83	19.41
Retained	30.89	40.89
Percent retained of consumed	34.57	41.59

[a] *Lactobacillus plantarum*, Bio Max SI, Chr. Hansen's Laboratory, applied at 10 g per ton of forage.

TABLE 3. DRY MATTER RECOVERY OF CORN SILAGE DRY MATTER AS AFFECTED BY A MICROBIAL SILAGE INOCULANT

	Untreated	Microbial inoculated[a]
Dry matter of corn forage, %	38.85	38.65
Total dry matter stored, lb	1557.97	1592.46
Total dry matter of fed silage, lb		
As percent of dry matter	1087.37	1069.03
Stored, %	69.79	68.81
Dry matter losses		
Spoilage, lb		
As percent of dry matter	345.64	317.23
Stored, %	22.19	19.92
Nonrecovered, lb		
As percent of dry matter	124.96	206.20
Stored, %	8.02	12.95

[a] *Lactobacillus plantarum*, Bio Max SI, Chr. Hansen's Laboratory, applied at 10 g per ton of forage.