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Effects of Corn Silage Inoculants on Aerobic Stability

Richard E. Muck, Agricultural Engineer

USDA, ARS, US Dairy Forage Research Center, Madison, WI 53706, remuck@wisc.edu

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Abstract. *Aerobic stability of corn silage can be a major problem for farmers particularly in warm weather. Silage inoculants, while the most common type of silage additive, have not been effective at improving aerobic stability. This study investigated new and proposed inoculant products over three years on corn silage in mini-silos. Three new approaches were tested: enhanced homofermentative inoculants, a standard inoculant plus sodium benzoate, and heterofermentative lactic acid bacteria (*Lactobacillus buchneri*). These approaches were compared with untreated as well as four standard homofermentative lactic acid bacterial inoculants. The standard inoculants on average reduced aerobic stability 17 h relative to untreated silage. The best enhanced inoculant increased stability one year in three. The standard inoculant plus sodium benzoate increased stability but was only tested in one year. The *L. buchneri* inoculants improved stability consistently all three years except in one case where one of these products had low viability. Overall, the *L. buchneri* products appear to be most consistent at improving the aerobic stability of corn silage of those commercially available.*

Keywords. Silage, aerobic stability, inoculant, lactic acid bacteria, corn

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Introduction

Inoculants are the most common additives used in making silage in the U.S. These products provide selected lactic acid bacteria to supplement the natural population of lactic acid bacteria on the crop and ensure a rapid and efficient silage fermentation. While these products have provided improvements in dry matter (DM) recovery and animal performance, aerobic stability (the time until the silage begins to heat during feed out) has been made worse by inoculants approximately one-third of the time (Muck and Kung, 1997). The majority of the reductions in aerobic stability have occurred in corn and other whole-crop grain silages, silages that frequently have aerobic stability problems even with good silage management.

Inoculant manufacturers are aware of this problem and have been working on developing inoculants that more consistently improve aerobic stability. They have been looking at three approaches. The first is to find homofermentative lactic acid bacteria that can inhibit yeasts, the frequent initiators of spoilage and heating in silages at feeding (Woolford, 1990). Such bacteria would presumably work like current inoculants relative to fermentation and animal performance. Currently there is one new product on the market in this category. The second approach is to inoculate the crop with *Lactobacillus buchneri*, a heterofermentative lactic acid bacteria. In this approach acetic acid production would be increased. Because acetic acid is a better inhibitor of yeasts than lactic acid (Moon, 1983) at a given pH, yeast counts would be lowered improving stability. Two products are now available in this category. A third approach is including a chemical yeast inhibitor with standard homofermentative inoculant. Currently there are no products in this category.

The objective of this study was to test both standard and new inoculants for their efficacy in improving the aerobic stability of corn silage.

Materials and Methods

Experimental Design

Whole-crop corn was harvested with a forage harvester in each of three years. The chopped corn was ensiled in 60 x 10 cm dia. PVC silos sealed with a rubber end cap on one end and with black plastic secured with duct tape on the other. Depending on year, 1.5 or 2 kg fresh crop were placed in each silo. Four silos were ensiled per treatment. The number of treatments varied from year to year depending on product availability, but most of the products were tested all three years. Treatments included an uninoculated control, four standard corn silage inoculants available in the market, a new product with improved homofermentative strains, several new products with the heterofermentative species *Lactobacillus buchneri*, and a prototype product (1 year only) with a standard inoculant plus a chemical spoilage inhibitor (sodium benzoate). All products were applied at labeled rates but were diluted with water such that each treatment was applied at 1 g/50 g crop. The control received 1 g water/50 g crop. Silos were weighed before and after filling. Periodically through the filling process samples of untreated corn (four altogether) were taken for analysis, and all inoculants were analyzed for lactic acid bacteria counts.

The silos were opened after a minimum of 90 days ensiling. Silos were weighed prior to emptying. The spoiled silage on the top was removed and weighed. The rest of the silage was removed, mixed and analyzed for microbial groups, pH, fermentation products, crude protein, moisture content and ash. The remainder was placed in Styrofoam buckets. A type T thermocouple was placed approximately 10 cm into the silage, and average silage temperatures

were recorded hourly until heating occurred. Aerobic stability was defined as the time after opening for silage temperature to reach 2°C above ambient.

Analyses

Initial samples of chopped corn and silages were analyzed for the same constituents with the exception that fermentation products were only determined on silages. Duplicate subsamples were taken for moisture determination by freeze drying. After moisture determination, the duplicate freeze-dried subsamples were ground together and analyzed for crude protein by a Leco FP-2000A nitrogen analyzer and ash at 550°C for 3 h. Another portion of wet sample was diluted 10:1 with autoclaved distilled water and blended for 30 s. The diluted sample was enumerated immediately for acid-tolerant lactic acid bacteria (LAB) using Rogosa SL agar, acetic acid bacteria (ethanol-yeast extract agar), bacillus spores (nutrient agar), and yeasts and molds (malt agar) (Muck et al., 1992). The remainder of the diluted sample was strained through cheesecloth and analyzed for pH and fermentation products (lactic, acetic, propionic, butyric and succinic acids, ethanol, and 2-3 butanediol as per Muck and Dickerson, 1988).

The effects of the inoculants were tested within a year using SAS using PROC GLM.

Results

Initial Characteristics

The characteristics of the corn at ensiling are shown in Table 1. The corn in 2000 was more mature and drier than the other two years. The year 2000 corn also had the lowest crude protein concentration and epiphytic lactic acid bacterial count while numerically having the highest counts of spoilage microorganisms at ensiling.

The levels of LAB applied by the various treatments are given in Table 2. In 1999, the inoculants were similar to approximately one order of magnitude less than the epiphytic LAB population. The lone exception was the *L. buchneri* B treatment, which was 1.5 log(colony-forming units[cfu]/g fresh crop) less. In 2000, LAB application rates were similar to or higher than the epiphytic population with the exception of Standard C and *L. buchneri* B. The viability of these latter two were far less than anticipated for unknown reasons. Conversely, the LAB in Standard D and Enhanced B were at substantially higher levels than anticipated. In 2001, the inoculant LAB rates were more uniform and approximately 0.5 log units above or below the epiphytic population.

Silage Characteristics

The aerobic stabilities of the silages in all three years are summarized in Table 3. In 1999 numerically, the corn silage treated with standard inoculant A was more stable than the untreated corn silage whereas the other two standard inoculants produced less stable silages. The Enhanced A was numerically less than untreated as well. However, none of these differences were statistically significant ($p = 0.05$). The *L. buchneri* treatments and the Standard D + sodium benzoate treatment significantly improved aerobic stability.

In 2000, only the *L. buchneri* A treatment significantly improved aerobic stability compared with the untreated control. Standard D, Enhanced B and their combination significantly reduced aerobic stability; however, within that group the trend was that Enhanced B was the most stable and Standard D the least with the combination being intermediate. All other treated silages were not statistically different from the untreated silage.

In 2001, the Standard A silage was significantly less stable than untreated whereas the Enhanced B and *L. buchneri* treated silages were all significantly more stable than the untreated.

The pH and the principal fermentation products are given in Tables 4 to 6. The pHs of the untreated silages in all three years (3.64 to 3.87) were typical of corn silages observed in Wisconsin. Across the three years, no standard or enhanced inoculant treated silage had a pH that was significantly different from that of the corresponding untreated silage. The *L. buchneri* treated silages produced the only significant differences. These silages were typically 0.1 to 0.3 pH units higher than the pHs of the untreated silages.

The fermentation profiles for the standard, enhanced and standard plus sodium benzoate inoculants were generally similar to those of the untreated silages (Tables 4 to 6). The exceptions were the Standard D, Enhanced B and their combination in 2000, which were applied at higher levels than the other treatments. These three treatments had higher lactic acid concentrations, lower acetic acid concentrations (in two cases), and elevated ethanol concentrations.

The *L. buchneri* treatments had shifted fermentation profiles. The *L. buchneri* A and C inoculants consistently produced silages of lower lactic acid and higher acetic acid concentrations than those of the untreated silages. There were trends for higher ethanol, and in 2001 propionic acid was also measured in these treatments at 1.1 and 0.2 g/kg DM, respectively. The *L. buchneri* B in 1999 only had a trend toward lower lactic and higher acetic relative to the untreated, but it had a significantly higher ethanol concentration than the untreated silage. In 2000, the fermentation in *L. buchneri* B was not different from that observed in the untreated.

The microbial characteristics of the silages at opening for the three years are shown in Tables 7 to 9. In 1999 (Table 7), there were no significant trends with the exception of the yeasts and molds. (The high bacillus spore count for the untreated appears to have been due to a failure in the heat-shocking process and should be regarded as not a true value.) Yeast counts in the *L. buchneri* treatments and the Standard D plus sodium benzoate were reduced compared to those in the untreated whereas Standard C and Enhanced A had elevated yeast counts. Mold counts were generally similar to yeast counts within a treatment.

In 2000 (Table 8), there were no trends relative to acetic acid bacteria and bacillus spores except that Enhanced A had a higher acetic acid bacterial population than most of the other treatments. The LAB in the *L. buchneri* A and C treatments were approximately 3 log units higher than those in all other treatments, and their yeast and mold counts were lowest numerically even though not statistically lower than those in the untreated. The Standard D, Enhanced B and their combination had higher yeast and mold counts than did the untreated.

In 2001 (Table 9), Standard A had higher counts of LAB, yeasts and acetic acid bacteria than the untreated silages. The Enhanced A and *L. buchneri* treatments had lower yeast counts than the untreated silages whereas *L. buchneri* C had a higher acetic acid bacterial count than the untreated.

Dry Matter Losses

The average DM losses from the silos for all three years are given in Tables 10 to 12. There was considerable variability among replicates. Thus there were no significant differences within a year. The standard inoculants tended to have reduced gaseous (1999) and spoilage (1999, 2001) losses than the untreated leading to trends for reduced total losses in both years. The Enhanced A treatment had the numerically lowest losses in all three categories in 1999, but the

trends for this treatment in 2000 and 2001 were for numerically higher losses than the untreated. Numerically, the *L. buchneri* treatments generally ranked as having some of the highest gaseous and spoilage losses in 1999 and 2001 whereas in 2000 their losses were indistinguishable from those of the other treatments. The Standard D plus sodium benzoate in 1999 produced nearly identical losses to those measured in the untreated.

Discussion

Standard Inoculants

An extensive survey of inoculant studies (Muck and Kung, 1997) found that approximately one-third of studies reported that homofermentative LAB inoculants reduced aerobic stability and that most of these reductions occurred in whole-crop corn and small grain silages. They speculated that the reason for this was that a reduction in pH by inoculation was more difficult to achieve in such silages because they are typically low in pH plus the inoculant bacteria would shift fermentation toward lactic acid and away from acetic acid. Because acetic acid is a better inhibitor of yeasts than lactic acid at a given pH (Moon, 1983), the shift in fermentation by such inoculants should be detrimental to aerobic stability, especially because yeasts are the most frequent initiators of heating in silages (Woolford, 1990).

In the present study with four standard inoculants (10 comparisons), only once was there a positive trend (Standard A in 1999) in aerobic stability by comparison with that of the untreated. The rest were negative, two of which were significantly worse (Standard A, 2001; Standard D, 2000). On average, the standard inoculants reduced aerobic stability 17 h. This is a relatively small reduction but certainly could be important when feeding corn silage under warm conditions.

The reason for this reduced stability appears tied to fermentation. In the two treatments that were significantly less stable, only one (Standard D, 2000) had a much higher lactic to acetic acid ratio than that of the untreated silage. However, both had the highest ethanol concentrations in their respective years along with high yeast counts, a possible cause of the ethanol (McDonald et al., 1991). This suggests that the more efficient fermentation of inoculants may create conditions that are more conducive to yeasts so that yeast populations are higher when the silo is opened and thus more susceptible to spoilage and heating. Overall, the results with the standard inoculants in the present study are in line with those observed in previous studies with inoculated corn silage.

Enhanced Inoculants

Only one enhanced homofermentative inoculant (Enhanced A) was tested in all three years. In general, Enhanced A behaved similarly to the standard inoculants. It tended to produce a more homofermentative silage compared to the untreated in each of the three years although pH was not reduced. Consequently from a pH and fermentation profile perspective, Enhanced A appeared similar to the standard inoculants. In two years, aerobic stability relative to the control was similar to that observed for the standard inoculants, an average reduction of 25 h. In one case, the yeast population relative to the untreated was high; in the other, acetic acid bacterial counts were elevated relative to the untreated. In 2001 however, aerobic stability was improved significantly, 29 h, relative to the untreated silage. Overall, Enhanced A works from a fermentation perspective like a standard inoculant while, at least in one instance, improving aerobic stability. Certainly three trials in small-scale silos is insufficient to know how frequently and under what conditions this product will improve stability.

Enhanced B was used only in one year (2000) and produced a silage with reduced aerobic stability relative to the untreated silage. However, this inoculant was applied at a very high level, shifting fermentation toward lactic acid. This appeared to be conducive to yeasts developing in the silage, making it less stable after opening. On the other hand, this inoculant produced a more aerobically stable silage (23 h) than the standard product (D) from the same company. Perhaps if Enhanced B had been applied at more normal rates (10^5 to 10^6 cfu/g), it would have been more successful relative to aerobic stability.

Standard Plus Sodium Benzoate

The Standard D plus sodium benzoate was tested only in one year. This product had no effect on fermentation but did reduce yeast and mold counts relative to the untreated silage, producing more than a three-day improvement in aerobic stability. Benzoates are common food additives for inhibiting spoilage so enhanced aerobic stability was expected. More research on this concept is needed to both ascertain how consistently the concept works and determine the optimum level of chemical inhibitor, balancing cost and effectiveness.

Lactobacillus buchneri

Lactobacillus buchneri departs substantially from other silage inoculants because it is a heterofermentative LAB. It was only approved by the FDA in 2001 as a silage additive and thus is only in its second year in the U.S. market. This species has been tested relative to aerobic stability in a variety of crops: corn (Driehuis, et al., 1999a, 1999b; Ranjit and Kung, 2000; Ranjit et al., 2002), grass (Driehuis et al., 2001), alfalfa (Kung et al., 2002), whole-plant wheat (Weinberg et al., 1999), whole-plant barley (Kung and Ranjit, 2001; Taylor et al., 2002), high moisture shelled corn (Kendall et al., 2002) and sorghum (Weinberg et al., 1999). All of these studies have been carried out with the bacteria in the *L. buchneri* A treatment of the present study. This strain has shown itself to be remarkably robust and consistent in improving aerobic stability across a range of ensiled crops. The level of improvement in aerobic stability has been variable but consistently positive from less than a day to weeks greater than that of the untreated control. Similar to these other studies, *L. buchneri* A improved stability in all three years, 100 to 811 h relative to untreated.

The other two *L. buchneri* strains were also effective when applied at sufficient levels. *L. buchneri* B failed to improve stability when applied at 2 log(cfu/g) less than the epiphytic LAB population, but increased stability 103 h when applied at 1.5 log units below. *L. buchneri* C increased stability 22 and 454 h in 2000 and 2001, respectively. Thus while the *L. buchneri* A strain has shown itself to be an effective microorganism across a range of crops, the other *L. buchneri* strains tested here were effective on corn silage although their increases in aerobic stability were somewhat less than that provided by *L. buchneri* A.

The reason for *L. buchneri*'s effectiveness appears to be due to reducing yeast populations. As shown in Fig. 1, stability across all treatments and years was highly correlated with yeast counts, and the *L. buchneri* treatments were the most effective at reducing yeast counts (Tables 7 to 9). At least in part, this may be due to the conversion of lactic to acetic acid by *L. buchneri* (Oude Elferink et al., 2001), considering that acetic acid is a better inhibitor of yeasts than lactic acid (Moon, 1983). However, the increased stability by *L. buchneri* B in 1999 in absence of significant shifts in fermentation products and very long stabilities by *L. buchneri* A and C in 2001 suggest that possibly other mechanisms may also be involved. Both of these latter cases contained small amounts of propionic acid that would contribute to stability but not to the extent observed.

Overall, the current study together with research by others suggest that *L. buchneri* is a consistent performer in terms of improving aerobic stability and makes a good alternative for farmers wishing to improve the aerobic stability of corn silage at a lower cost than propionic acid and with a greater ease and level of safety than afforded by acids or anhydrous ammonia.

The major concern with *L. buchneri* may be how livestock will perform with high levels of acetic acid in the silage. Research is just beginning to be reported in this regard (Driehuis et al., 1999b; Kendall et al., 2002; Kung et al., 2002; Ranjit et al., 2002; Taylor et al., 2002). No reduction in intake has been observed, and milk production has generally been similar to untreated silages. However, more animal performance research is needed where the untreated silage is unstable in order to better gauge the economic value of *L. buchneri*.

Conclusions

Of the new corn silage inoculants available to farmers, the *L. buchneri* inoculants provided the most consistent improvement in aerobic stability. An enhanced standard inoculant was better than standard inoculants in one year of three relative to aerobic stability. A standard inoculant plus sodium benzoate was effective in improving aerobic stability in the one year it was tested; however, no commercial product is available with such a formulation. At this stage of testing, selection of a corn silage inoculant appears to hinge on the most important goal(s) of the farmer. If poor aerobic stability in corn silage and its effect on animal performance are consistent problems that have not been solved by improved silo management, then the *L. buchneri* products show the most promise. However, if the primary goals are improved animal performance and dry matter recovery, then the enhanced standard and conventional homofermentative inoculants appear more likely to achieve success.

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Table 1. Initial characteristics of the corn ensiled in each year.

Characteristic	1999	2000	2001
Dry Matter, g/kg	327	458	367
pH	5.90	5.98	5.79
Crude Protein, g/kg DM	90	64	71
Lactic Acid Bacteria, log(cfu/g)	6.49	5.04	6.02
Yeast, log(cfu/g)	6.28	8.02	7.40
Mold, log(cfu/g)	4.49	6.62	6.17
Acetic Acid Bacteria, log(cfu/g)	6.48	6.86	6.83
Bacillus Spores, log (cfu/g)	5.18	ND*	ND

* ND – not determined.

Table 2. Lactic acid bacteria applied [log(cfu/g crop)] by treatments in each year.

Treatment	1999	2000	2001
Standard A	5.26	5.00	5.87
Standard B	5.44	5.08	5.84
Standard C	6.43	2.63	5.80
Standard D	-*	7.80	-
Enhanced A	5.88	5.63	6.01
Enhanced B	-	7.47	-
Standard D + Enhanced B	-	7.43	-
L. buchneri A	6.35	6.10	6.59
L. buchneri B	4.93	3.05	-
L. buchneri C	-	6.07	6.57
Standard D + NaBenzoate	5.29	-	-

* - Treatment was not done in that year.

Table 3. Aerobic stabilities (h) of silages in all three years.

Treatment	1999	2000	2001
Untreated	75	97	75
Standard A	91	84	36
Standard B	71	77	69
Standard C	50	91	66
Standard D	-*	36	-
Enhanced A	51	70	104
Enhanced B	-	59	-
Standard D + Enhanced B	-	45	-
L. buchneri A	217	197	886
L. buchneri B	178	96	-
L. buchneri C	-	119	529
Standard D + NaBenzoate	152	-	-

* - Treatment was not done in that year.

Table 4. The pH and fermentation characteristics (g/kg DM) of silages in 1999.

Treatment	pH	Lactic	Acetic	Ethanol
Untreated	3.82	55	23	9
Standard A	3.85	52	22	7
Standard B	3.84	52	24	9
Standard C	3.83	60	26	12
Enhanced A	3.81	55	21	7
L. buchneri A	4.01	44	38	11
L. buchneri B	3.90	52	26	31
Standard D + NaBenzoate	3.83	59	23	9

Table 5. The pH and fermentation characteristics (g/kg DM) of silages in 2000.

Treatment	pH	Lactic	Acetic	Ethanol
Untreated	3.87	53	10	4
Standard A	3.89	58	11	5
Standard B	3.90	55	11	5
Standard C	3.90	59	11	5
Standard D	3.88	60	6	11
Enhanced A	3.90	59	11	6
Enhanced B	3.90	65	10	10
Std D + Enh B	3.90	65	6	11
L. buchneri A	4.11	36	24	8
L. buchneri B	3.90	60	11	5
L. buchneri C	4.06	42	23	8

Table 6. The pH and fermentation characteristics (g/kg DM) of silages in 2001.

Treatment	pH	Lactic	Acetic	Ethanol
Untreated	3.64	73	18	9
Standard A	3.71	89	23	20
Standard B	3.65	81	20	13
Standard C	3.62	75	16	10
Enhanced A	3.64	82	18	9
L. buchneri A	4.01	38	70	11
L. buchneri C	3.84	65	55	12

Table 7. The microbial characteristics [log(cfu/g)] of silages in 1999.

Treatment	Lactic Acid Bacteria	Yeasts	Molds	Bacillus Spores	Acetic Acid Bacteria
Untreated	8.53	3.15	3.80	10.46	4.27
Standard A	8.79	2.90	2.77	2.85	4.43
Standard B	8.42	3.27	2.62	2.43	4.64
Standard C	8.31	4.24	4.07	2.56	4.35
Enhanced A	8.53	4.13	4.04	2.77	4.43
L. buchneri A	8.76	1.00*	1.99	2.66	4.88
L. buchneri B	8.59	1.55	1.18	2.83	4.43
Standard D + NaBenzoate	8.59	2.38	2.41	2.63	4.46

* The detectable limit was 2 log(cfu/g); samples below detectable level were set to 1 log(cfu/g).

Table 8. The microbial characteristics [log(cfu/g)] of silages in 2000.

Treatment	Lactic Acid Bacteria	Yeasts	Molds	Bacillus Spores	Acetic Acid Bacteria
Untreated	6.54	3.42	3.23	2.28	4.75
Standard A	6.40	3.77	3.56	2.28	5.03
Standard B	6.48	3.87	3.15	2.14	4.65
Standard C	6.29	3.92	3.61	2.29	4.81
Standard D	5.95	5.01	5.07	2.22	4.57
Enhanced A	6.45	3.75	3.76	2.53	5.41
Enhanced B	5.99	4.71	4.67	2.44	4.53
Std D + Enh B	5.75	4.77	5.29	2.59	4.29
L. buchneri A	9.30	2.65	2.89	2.51	4.32
L. buchneri B	6.79	3.74	3.74	2.29	4.66
L. buchneri C	9.19	2.93	2.89	2.35	4.40

Table 9. The microbial characteristics [log(cfu/g)] of silages in 2001.

Treatment	Lactic Acid Bacteria	Yeasts	Molds	Bacillus Spores	Acetic Acid Bacteria
Untreated	6.17	3.63	2.57	3.51	4.82
Standard A	7.60	5.43	2.66	4.35	6.44
Standard B	6.75	3.08	1.87	4.11	4.99
Standard C	5.47	3.06	2.16	3.66	4.39
Enhanced A	5.85	2.73	2.18	4.08	4.67
L. buchneri A	7.00	1.54*	2.71	4.34	4.98
L. buchneri C	7.20	1.00	1.77	4.08	5.99

* The detectable limit was 2 log(cfu/g); samples below detectable level were set to 1 log(cfu/g).

Table 10. Dry matter losses in 1999 silages.

Treatment	Gaseous Loss, %	Spoilage Loss, %	Total Losses, %
Untreated	10.3	22.5	32.8
Standard A	7.6	21.9	29.4
Standard B	8.7	18.0	26.7
Standard C	9.1	16.7	25.8
Enhanced A	7.0	17.5	24.5
L. buchneri A	10.5	19.1	29.5
L. buchneri B	9.7	22.7	32.4
Standard D + NaBenzoate	9.2	22.8	32.1

Table 11. Dry matter losses in 2000 silages.

Treatment	Gaseous Loss, %	Spoilage Loss, %	Total Losses, %
Untreated	11.4	4.7	16.2
Standard A	13.6	6.3	19.8
Standard B	13.3	5.1	18.4
Standard C	12.0	4.5	16.5
Standard D	13.4	5.4	18.8
Enhanced A	14.5	6.4	20.9
Enhanced B	11.4	5.0	16.4
Std D + Enh B	16.0	4.5	20.5
L. buchneri A	13.5	4.6	18.1
L. buchneri B	14.8	4.7	19.6
L. buchneri C	12.1	5.0	17.1

Table 12. Dry matter losses in 2001 silages.

Treatment	Gaseous Loss, %	Spoilage Loss, %	Total Losses, %
Untreated	8.1	6.3	14.3
Standard A	9.5	4.2	13.7
Standard B	9.1	2.9	12.0
Standard C	7.9	5.9	13.8
Enhanced A	8.7	7.9	16.6
L. buchneri A	9.8	7.4	17.2
L. buchneri C	13.6	6.9	20.5

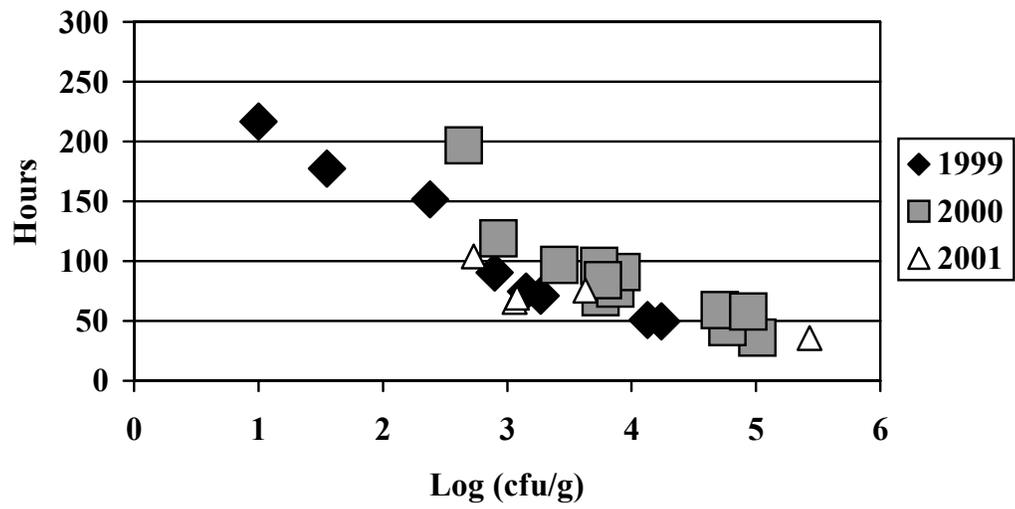


Fig. 1. Aerobic stability (h for temperature to reach 2°C above ambient) for all treatments as correlated with yeast count at silo opening.