CHEMICAL AND BACTERIOLOGICAL CHANGES IN GRASS SILAGE DURING THE EARLY STAGES OF FERMENTATION.

I. CHEMICAL CHANGES

C. W. LANGSTON, H. G. WISEMAN, C. H. GORDON, W. C. JACOBSON, C. G. MELIN, AND L. A. MOORE
Dairy Cattle Research Branch

AND

J. R. McCALMONT
Agricultural Engineering Research Branch
USDA, Beltsville, Maryland

SUMMARY

Important differences were observed between aerated and sealed silages examined in this study. Aerated silages showed high temperatures and generally poorer quality than the sealed silages. The sealed silages which were tramped and weighted gave few temperature increases.

Chemical composition of the silages usually varied according to treatment. In the poorer aerated silages high pH values were observed, along with increased amounts of butyric acid and ammonia nitrogen. Lactic acid always decreased as the fermentation progressed.

The rate and development of organic acids could not be correlated with the treatment of the forages. Some silages contained a small amount of acid after 24 hr and others practically none. Most of the acids formed were observed after the most active period of bacterial growth and when the sugar was depleted.

The initial levels of sugars in the forages appeared to have little effect on the amount of acids formed in the silages. The total acids were higher in sealed than aerated, which suggested that some of the substrate initially present was destroyed as a result of aeration. High levels of sugars did not ensure silage of superior quality unless the forage was properly ensiled.

Since considerably more acids were formed than could be accounted for by the initial sugar content, it was suggested that other substrates are available and utilized by silage bacteria as a source of energy when the sugar is depleted. Among these were included the hemicellulose fraction of plants and plant acids.

The general chemical changes in silage, as a result of plant and bacterial enzymes, are the conversion of carbohydrates and other compounds into organic acids and gases and the partial breakdown of protein into nonprotein and other more soluble compounds. The extent and rate at which these changes occur during the early stages of ensiling may be decisive in the outcome of the silage fermentation. The chemical transformation which occurs results from interaction of a number of species of bacteria and is greatly influenced by the amount and kind of substrate.

One of the most important factors which affects the chemical composition of forage and the competitive ability of organisms is the extent of oxygen inclusion in the mass during the early stages of ensiling. Vital nutrients are reduced as a result of oxidation and metabolism of bacteria may be limited because of high temperatures.

A study of orchardgrass and alfalfa silages by Langston et al. (16) revealed certain bacteriological and chemical variations which determined the course of fermentation during the early stages of ensiling. Silages of good quality showed rapid production and high levels of lactic acid. Poor silages exhibited comparatively large numbers of sporeforming anaerobes and high levels of butyric acid and ammonia nitrogen. The latter silages were further characterized by low rates of nutrient preservation.

A previous study (12) of several hundred bacterial cultures obtained from the silages at

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different stages of fermentation showed that better-quality forages were obtained when the early flora consisted mainly of cocci. Three species of bacteria evolved in the good-quality silages as the dominant population (*Pediococcus, Lactobacillus plantarum,* and *Lactobacillus brevis*). The poor silages contained the species mentioned above and also a fourth species [*Lactobacillus casei* (variable)] which produced submaximal amounts of acid.

The viable bacterial counts from the silages gave an interesting pattern, because the total numbers of lactic acid bacteria present were similar regardless of quality. The important difference in bacterial types was the high numbers of sporeforming anaerobes found in poor silages.

Experiments have shown that the silage fermentation is carried out by a small proportion of microorganisms initially present. These organisms must overcome rather great odds and show superior competitive ability in their search for substrates.

Because of the importance of changes which occur during the early ensiling period, it seemed advisable to follow closely the development of bacteria in these first stages of the fermentation process and attempt to relate their activities to the chemical changes. Aeration was employed in one set of silos to obtain silages of differing quality.

METHODS

The forages used in the experiments were harvested in 1957 and 1959. The 1957 crop included both first and late second-cutting orchardgrass and alfalfa. The first-cutting orchardgrass was taken from a field fertilized with 500 lb 0-10-20 fertilizer and 100 lb ammonium nitrate per acre. The alfalfa was in full bloom. The forages were chopped by a direct-cut forage harvester set at 5/16-inch theoretical cut.

The second-cutting orchardgrass and alfalfa were obtained from a late-harvest crop. The orchardgrass was fertilized with 100 lb ammonium nitrate per acre in early August, but did not respond until September because of drought conditions. It was green and vegetative except for dead leaves at the plant base. The alfalfa was in full bloom, rather weedy and drought-stunted.

The 1959 studies included only second-cutting orchardgrass chopped with a direct-cut forage harvester set at 3/4-inch theoretical cut.

In each experiment, and for each cutting and type of forage, sealed and aerated silages were prepared. The ensiling methods were essentially the same as those described earlier (16). The sealed silages were tramped, weighted (500-lb wt), and sealed immediately. The aerated silages were not tramped, weighted, or sealed and air was pumped into the bottom of the silo for 5 to 6 hr after filling.

Samples for bacteriological analysis were obtained from the fresh crop, at 2-hr intervals over a 48-hr period, usually at 1 and 2 wk and at 62 days. Samples for chemical analysis were taken at 6-8 hr intervals and later as indicated in the results.

Procedures used in sampling and studying the chemical and bacteriological changes of the silages were described in earlier publications (12, 16).

RESULTS AND DISCUSSION

Temperatures of the silages examined in this study are shown in Table 1. Aeration caused temperatures to increase markedly in the sil-

TABLE 1

Silage temperatures (C)

Days after ensiling	Orchardgrass First-cutting 1957		Orchardgrass Third-cutting 1957		Alfalfa First-cutting 1957		Alfalfa Third-cutting 1957		Orchardgrass Second-cutting 1959	
	S-4 Sealed	S-6 Aerated	S-4 Sealed	S-6 Aerated	S-8 Sealed	S-5 Aerated	S-8 Sealed	S-5 Aerated	S-4 Sealed	S-6 Aerated
0	18.3	18.9	30.0	49.0	22.8	23.9	27.2	48.0	31.7	50.0
1	18.3	20.0	31.7	57.0	24.4	27.2	25.6	50.0	32.2	49.0
2	18.9	22.2	32.8	55.0	26.7	31.1	28.3	50.0	32.2	48.0
3	19.4	22.8	32.2	51.0	26.7	35.0	29.4	48.0	31.7	47.0
4	18.9	23.3	31.7	48.0	26.7	35.6	28.9	47.0	31.1	45.0
7	18.3	27.2	28.9	39.0	23.9	32.8	26.7	39.0	28.9	43.0
14	21.7	28.3	21.7	27.0	22.8	32.8	23.9	39.0	27.8	47.0
21	20.0	25.0	16.1	23.0	21.1	32.8	23.9	48.0	28.3	47.0
30	20.6	28.9	12.8	22.0	22.2	33.9	22.8	49.0	27.2	48.0

ages, although some responded faster and showed greater increases than others. A short time after aeration ceased (the 0-day figures show the peak temperatures in the silos 12 to 15 hr after ensiling), temperature increases of 14 to 20.8 C were observed in the aerated as compared to the sealed silages. The high peak temperature in the aerated alfalfa silage (S-5, third cutting, 1957) might be attributed to lower moisture content (Table 2), but silages of higher moisture content similarly treated showed comparable temperature increases. Most of the aerated silages reached maximum temperatures within one day, then gradually decreased. The temperature differences were not as pronounced in the first-cutting orchardgrass (S-6, 1957) and first-cutting alfalfa (S-5, 1957), even though they were aerated. For some reason these forages failed to respond immediately to oxygen but, as the fermentation progressed, they did show higher temperatures than the sealed silages. The sealed silages generally did not increase more than a few degrees in temperature during the storage period.

The aerated silages examined in this study proved to be inferior in quality. These results are in agreement with several who have studied the influence of temperature on silage quality (6, 7, 19, 21, 22, 26). It should be mentioned that this work may not be a true comparison, because we used aeration as a means of increasing temperature and other investigators have used controlled temperature chambers probably with little oxidative effect. Some workers, however, have reported experiments which suggest that temperature may not be a factor in reducing silage quality. Nilsson, Tóth, and Rydin (23), working with small silos in constant-temperature chambers, showed increases in pH, ammonia nitrogen, and butyric acid when they increased temperatures ranging from 20 to 37 C. Below 20 C good silage was obtained. Kempton and San Clemente (7) reported well-preserved silages with low pH and no butyric acid which had heated to 46 and 55 C. Another silage which reached 59 C was inferior. Murdoch's (22) work generally supports the low-temperature theory. His studies with treated grass-clover silages held at 26 C were of satisfactory quality, but a nontreated alfalfa-orchardgrass silage in the same temperature range was poor. Other untreated forages examined showed variability with the type of crop ensiled, but the high-temperature ones (39-47 C) were usually inferior. Murdoch concluded that although improved fermentation was observed at low temperatures, other conditions must also be met to ensure high-quality forage. Previous work at this station (16) has shown high losses with increased temperatures, although some crops were more tolerant to high temperatures than others.

Most workers now agree that because of the large nutrient losses resulting from oxidation and the probability of an undesirable fermentation, attempts should be made to maintain low temperatures in the silage fermentation by means of air exclusion.

The chemical data of the silages presented in Table 2 demonstrate that aeration had a dominant influence on final composition. Minor pH changes were observed during the first few hours of fermentation, but the final pH revealed major differences. The pH values usually corresponded with changes in organic acids and ammonia nitrogen. When lactic acid decreased, pH, butyric acid, and ammonia nitrogen increased. These changes are a reflection of the large number of sporeforming anaerobes which convert the stronger acid (lactic) to the weaker acid (butyric) and the increase in nitrogenous compounds which further neutralize the acidity by virtue of their basic action. Only one of the aerated silages reached what is considered an acceptable pH before reversing its trend and subsequently making poor silage. Similar observations were made in other trials (14) and the authors concluded that pH alone is not always a good criterion of silage quality. Based on pH, few of the silages examined were of superior quality. The first-cutting orchardgrass (S-4, 1957) dropped to pH 3.8 and remained at this figure. Two other sealed silages (alfalfa, first-cutting, S-8, and alfalfa, third-cutting, S-8, 1957) reached pH values of 4.5 or above. It should be emphasized, however, that a low moisture or wilted silage with pH values of 4.5 to 4.8 may be of good quality.

Some workers consider the ammonia nitrogen content of silage one of the best criteria of silage quality. But, like pH, variability of this compound in silage of different quality has been observed. Generally, high levels of ammonia nitrogen are associated with deterioration of silage and proof of considerable breakdown of amino acids into more soluble products. Results in Table 2 show relatively higher ammonia nitrogen values in aerated than in sealed silages and these values usually parallel increased pH and butyric acid and destruction of lactic acid. The mechanism involved in the formation of large amounts of ammonia nitrogen in poor silage has sometimes been misinterpreted. Some workers continue to speak of this compound in terms of proteolysis. When proteolytic enzymes are active in silage the

TABLE 2

Chemical composition of orchardgrass silages
First-cutting 1957

Time after ensiling	Composition of dry matter							
	Aerated silo S-6 [a]				Sealed silo S-4 [b]			
	pH	Ammonia nitrogen	Acetic Acid	Lactic Acid	pH	Ammonia nitrogen	Acetic Acid	Lactic Acid
0 hr	5.8	0.08	0.29	0.05	5.7	0.09	0	0
6 hr	5.7	0.20	0.52	0.13	6.1	0.15	0.46	0.15
14 hr	6.1	0.15	0.60	0.23	5.9	0.21	0.53	0.13
22 hr	5.2	0.24	0.48	0.30	5.6	0.21	0.47	0.10
14 days	3.9	0.82	1.28	9.00	3.8	0.84	1.39	9.32
23 days	4.7	1.65	3.06	4.28	3.8	1.17	1.49	8.75

Chemical composition of alfalfa silages
First-cutting 1957

	Aerated silo S-5 [c]				Sealed silo S-8 [d]			
0 hr	5.8	0.12	0.65	0.14	5.8	0.12	0	0
8 hr	5.8	0.19	0.92	0.46	5.8	0.29	0	0
16 hr	5.0	0.40	1.48	1.73	5.4	0.29	0.99	0.93
24 hr	4.8	0.68	1.96	2.89	4.8		1.27	2.86
18 days	4.6	1.87	3.80	5.46	4.2	1.70	2.93	8.12
40 days	4.9	3.72	4.69	3.02	4.5	2.35	3.99	6.58

Chemical composition of orchardgrass silages
Second-cutting 1957

	Aerated silo S-6 [e]				Sealed silo S-4 [f]			
0 hr	5.9	0.08	0	0	5.8	0.65	0	0
7 hr	5.7	0.40	0.31	0.16	5.5	0.31	0.41	1.23
15 hr	6.1	0.46	0.70	0.31	5.2	0.39	1.16	2.05
23 hr	6.0	0.89	1.17	0.79	4.9	0.71	0.37	2.63
47 hr	5.7	1.28	0.80	0.89	4.8	1.04	1.68	3.73
14 days	5.6	3.58	2.86	0.29	4.6	1.80	3.01	4.49
221 days	5.4	4.96	3.95	0.18				
181 days					4.5	2.16	4.19	6.45

Chemical composition of alfalfa silage
Third-cutting 1957

	Aerated silo S-5 [g]				Sealed silo S-8 [h]			
0 hr	5.7		0	0	5.7		0	0
8 hr	5.6		0.60	0.17	5.7		0.53	0.14
16 hr	5.6		0.64	0.17	5.7		0.73	0.78
24 hr	5.3		0.62	0.68	5.5		0.96	0.81
48 hr	4.7		0.86	2.24	4.6		1.20	2.33
6 days	4.7		0.81	2.86	4.6		1.58	4.70
9 days	4.7		0.78	2.60	4.6		1.57	3.83
14 days	5.0		2.53	1.85	4.2		1.83	4.50
247 days	....		.....	.....	4.6		3.07	5.49

Chemical composition of orchardgrass silages
Second-cutting 1959

	Aerated silo S-6 [i]				Sealed silo S-4 [j]			
0 hr	6.2	0.10	.....	.....	6.1	0.10	.....	.....
8 hr	6.1	0.22	0.83	.04	6.0	0.18	0.77	0.73
16 hr	6.0	0.33	0.71	.07	5.7	0.36	1.09	0.74
24 hr	5.6	0.60	0.91	0.50	5.2	0.56	1.21	0.31
40 hr	6.2	0.97	1.08	0.05	5.2	0.88	1.45	1.60
48 hr	5.7	0.78	1.13	0.70	5.1	1.01	1.52	1.64
7 days	4.9	1.36	1.96	2.42	4.6	1.40	2.26	3.59
14 days	5.4	2.78	3.49	1.15	4.5	1.70	2.44	4.77
62 days	7.0	6.38	4.79	0.35	4.9	2.07	1.88	2.54

See footnotes next page.

breakdown of protein does not go past the amino acid stage (17). Actually, some of the lactic acid bacteria (streptococci and lactobacilli) contain weak to strongly active proteolytic enzymes. The presence of high levels of ammonia nitrogen in silage is a reflection of amino acid breakdown and is properly termed putrefaction. Decarboxylases have been described for a number of amino acids and McDonald et al. (21) point out that basic compounds formed from decarboxylation may be important in neutralizing acids formed in silage.

In the poorer (aerated) silages the organic acid pattern was similar to results previously obtained (16), although the appearance of butyric acid was slower. Decrease in lactic acid and the increase in butyric acid was consistent. There was not, however, always a positive correlation between pH and butyric acid. This is illustrated in the first-cutting orchardgrass (S-6, 1957) which had a pH of 4.7 and 2.77% butyric acid, and the third-cutting alfalfa (S-5, 1957) with a pH of 5.0 and only 0.56% butyric acid. Furthermore, the first-cutting orchardgrass (S-4, 1957) with a final pH of 3.8 had 0.45% butyric acid. Similar results have previously been observed and suggest that butyric acid–producing bacteria are not as sensitive to acid under some ensiling conditions as has been thought. The possibility also exists that organisms capable of producing butyric acid, other than the sporeforming anaerobes, have not been detected in silage. Although no positive data exist to substantiate this point, the probability that such organisms do exist was recently demonstrated by Kearney (6), who aerated forage for a period of 30 hr and found considerable accumulation of butyric acid during this period. Our present knowledge of the spore-forming anaerobes suggests that their activity would be severely, if not completely, limited under highly aerobic conditions.

The observation in Table 2, that the rate and development of organic acids proceed more rapidly in some silages than others, was not necessarily correlated with the treatment of the forages. Some silages contained considerable amounts of acids at 22-24 hr and others only limited amounts. In this respect, it should be mentioned that the greatest amounts of acids in the silages were formed after active bacterial growth had ceased and when the sugar was practically depleted. Gibson et al. (3) suggest that variations in acid production might be related to the rate of sugar liberation from plants as the temperature is increased. It might also be suspected that variation in the initial bacterial flora could account for some of the differences observed. However, the numbers and types of lactic acid bacteria present in the early stages of ensiling were not too different, regardless of treatment or type of forage ensiled (13).

It is generally agreed that the composition of forage, as a substrate for microorganisms, may be decisive in the outcome of silage fermentations, since it determines the quantity of vital nutrients available for their development. The lactic acid bacteria, as a group, are saccharolytic and it is assumed that to produce enough preserving acids in silage the sugar content must be high. The sugar content of legumes is usually lower than in grasses and some of the difficulties in ensiling legumes have been attributed to this deficiency. The data in Table

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Footnotes for Tables, preceding page.

[a] S-6, DM (0 day) 20.2; crude protein (0 day) 14.3 (23 days) 16.3; sugar (0 day) 9.03 (23 days) 0.20; butyric acid (23 days) 2.77.

[b] S-4, DM (0 day) 20.1; crude protein (0 day) 13.9 (23 days) 14.7; sugar (0 day) 9.94; butyric acid (23 days) 0.45.

[c] S-5, DM (0 day) 21.5; crude protein (0 day) 21.3 (40 days) 22.4; sugar (0 day) 1.76 (40 days) 0.07; butyric acid (40 days) 1.72.

[d] S-8, DM (0 day) 20.0; crude protein (0 day) 21.0 (40 days) 21.3; sugar (0 day) 1.85 (40 days) 0.19; butyric acid (40 days) 0.10.

[e] S-6, DM (0 day) 23.8; crude protein (0 day) 19.2 (221 days) 20.7; sugar (0 day) 1.76 (221 days) 0.06; butyric acid (221 days) 1.82.

[f] S-4, DM (0 day) 19.9; crude rpotein (0 day) 20.3 (181 days) 21.4; sugar (0 day) 1.32 (181 days) 0.05; butyric acid (181 days) 0.06.

[g] S-5, DM (0 day) 27.3; crude protein (0 day) 18.0; sugar (0 day) 2.13; butyric acid (14 days) 0.56.

[h] S-8, DM (0 day) 29.0; crude protein (0 day) 18.5 (247 days) 18.7; sugar (0 day) 2.42 (247 days) 0.28; butyric acid (247 days) 0.10.

[i] S-6, DM (0 day) 23.7; crude protein (0 day) 16.2 (62 days) 17.3; sugar (0 day) 3.03 (7 days) 0.18; butyric acid (62 days) 5.12.

[j] S-4, DM (0 day) 23.7; crude protein (0 day) 16.2 (62 days) 15.9; sugar (0 day) 3.03 (7 days) .12; butyric acid (62 days) 2.66.

2 revealed that the amount of sugar (80% alcohol-extractable) initially present in forage may not always be a limiting factor in the production of acids in silage. There appear to be other constituents which are also available as carbon or energy sources for bacterial growth. With the exception of the first-cutting orchardgrass (1957), all of the forages examined had unusually small amounts of sugars, but on final analysis of the silages the organic acid content was much higher than could be accounted for as sugar in the fresh plant. This finding was previously observed (1, 16) and suggested that compensating substrates other than sugar were being utilized. The compound(s) other than sugar was utilized to a greater extent when sugar was deficient in the plants. This is seen by comparing the first-cutting orchardgrass (1957) with the other silages in Table 2. The data also showed that the acid levels were higher in the sealed than aerated silages, which indicated that some of the initial substrate available for fermentation was destroyed by aeration.

Possibly too much emphasis has been placed upon the initial sugar present in forage without attention to other constituents and environmental conditions. That high sugar content does not necessarily ensure good quality unless the forage is properly ensiled is demonstrated by the results obtained from the aerated orchardgrass silage made in 1957 (first-cutting, S-6). This silage had a pH of 3.9 at 14 days but at 23 days increased to 4.7 and contained constituents indicative of poor silage.

Many workers have examined either the reducing sugars or the so-called available sugars in forage and concluded that poor-quality silage is attributable to low sugar levels. Our data do not dispute these findings but show that when sugar levels are low other substrates are available for bacterial metabolism. Several investigators have attempted to determine which compounds in plants are available to organisms other than simple sugars. The logical approach has been to examine the complex carbohydrates in forage. Recent work (21) strongly suggests that the hemicelluloses in plants are utilized by silage organisms as an energy source. The hemicellulose fraction contains a rather heterogenous group of substances in the cell wall of plants and upon hydrolysis yields galactans, pentosans, mannosans, and uronic acids. Various workers have evidence which suggests that lactic acid bacteria ferment pentosans (4, 18, 24) and galactan (20) in plants. Recent work by Langston (9) showed that hemicellulose from plants and also its hydrolytic products (glucuronic and galacturonic acids) are readily utilized by silage bacteria. According to Tóth, Rydin, and Nilsson (25), starch cannot be fermented by lactic acid bacteria. Contrary to their work, our results (10, 11, 14, 15) have shown that many of the silage organisms do ferment and hydrolyze starch. This was true especially among the high acid–producing lactobacilli.

Another source of energy for silage organisms appears to be the plant acids. Workers have shown some plants to contain fair amounts of malic, oxalic, citric, malonic, and quinic acids (5, 8, 16). These acids disappear soon after ensiling and it is probable that at least part of the acids are converted to lactic acid (8).

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