INTRODUCTION

Harvesting and ensiling corn as a high moisture product offers an excellent alternative to dry grain for the feeding of domestic livestock. Preservation of high moisture corn (HMC) typically is accomplished through ensiling, defined as the preservation of a perishable feedstuff for use at a later time. The predominant factor in the ensiling process is the reduction of pH due to production of organic acids, predominantly lactic acid, from soluble sugars due to bacterial fermentation. Numerous silage additives have been developed to aid ensiling and to reduce storages losses. Although various types of fermentation aids may prove useful in different situations, this review will focus on the addition of bacterial inoculants that can favorably affect the outcome of the microbial fermentation in high moisture corn.

THE ENSILING PROCESS

The ensiling process, although appearing quite simple, is a complex dynamic process encompassing a number of interrelated factors. In essence, various substrates (soluble sugars under ideal circumstances) are converted by bacteria to various products with loss of weight and energy. Specific fermentation (anaerobic) as well as oxidation (aerobic) reactions together with loss of mass and energy during conversion from substrates to products are shown in Table 1. The ensiling process can be divided into six different phases (Figure 1; McCullough, 1984).

<table>
<thead>
<tr>
<th>Fermentation type</th>
<th>Substrate</th>
<th>Product(s)</th>
<th>Weight loss, %</th>
<th>Energy loss, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homolactic</td>
<td>Glucose</td>
<td>2 Lactate</td>
<td>0.0</td>
<td>3.1</td>
</tr>
<tr>
<td>Acetic (Heterolactic)</td>
<td>Glucose</td>
<td>Lactate + acetate + CO₂</td>
<td>16.7</td>
<td>20.4</td>
</tr>
<tr>
<td>Propanediol (Heterolactic)</td>
<td>Glucose</td>
<td>Acetate + 1, 2 propane diol + CO₂</td>
<td>24.4</td>
<td>4.8</td>
</tr>
<tr>
<td>Butyric</td>
<td>Glucose</td>
<td>Butyrate + 2CO₂</td>
<td>51.1</td>
<td>22.1</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Glucose</td>
<td>Ethanol + 2CO₂</td>
<td>48.9</td>
<td>2.6</td>
</tr>
<tr>
<td>Oxidation</td>
<td>Any organic compound</td>
<td>CO₂, H₂O</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Phase 1 (Aerobic)

The first phase begins when the plant is harvested. During this phase, indigenous aerobic microorganisms convert water-soluble carbohydrates to carbon dioxide, water and heat. The production of carbon dioxide, water, and heat continues as long as respiration occurs in the harvested plant material and ceases when oxygen in the silage mass is depleted or the supply of water soluble carbohydrates is exhausted. Under optimal conditions, phase 1 is completed quickly, but it can last for several days depending on moisture, compaction, and the epiphytic microbial load. At surfaces unprotected from oxygen penetration, oxidation can continue with sizeable losses of both weight and energy.

Phase 2 (Anaerobic)

Oxygen becomes depleted either due to use by microbes or plant tissue or due to displacement by dense carbon dioxide. With depletion of oxygen, the initial aerobic ensiling phase ends and anaerobic heterofermentation of phase 2 begins. The term “hetero” refers to the assortment of fermentation end products that are generated by bacteria that can tolerate the heat produced during phase 1. These bacteria are inefficient (Table 1) and produce relatively small amounts of typical fermentation products (acetate, lactate, propionate and ethanol).
plus heat when compared to the nutrients consumed. Uneconomical fermentation by these organisms can result in a sizeable nutrient and energy loss from the silage mass.

<table>
<thead>
<tr>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
<th>Phase IV</th>
<th>Phase V</th>
<th>Phase VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell respiration. Production of CO₂ heat and water</td>
<td>Production of acetic and lactic acid. Ethanol</td>
<td>Lactic acid formation</td>
<td>Lactic acid formation</td>
<td>Material storage</td>
<td>Aerobic decay after exposure to oxygen</td>
</tr>
<tr>
<td>70°</td>
<td>90°</td>
<td>84°</td>
<td></td>
<td></td>
<td>115°</td>
</tr>
<tr>
<td>Temperature Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.0-6.5</td>
<td>5.0</td>
<td>4.0</td>
<td>7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic and lactic acid bacteria</td>
<td>Lactic acid bacteria</td>
<td>Lactic acid bacteria</td>
<td></td>
<td>Mold and yeast activity</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Six Phases of silage fermentation and storage.

The aerobic bacteria that are dominant during phase 2 are relatively active above pH 5, but their production of acid reduces the pH to near 5; this inhibits their activity. Depletion of oxygen coupled with the reduction in pH shifts metabolism to from homofermentative (“homo” referring to single product) bacteria which thrive at pH 5 and below.

**Phase 3 (Anaerobic)**

Phase 3 also is a short-lived transitional phase that usually last only about 24 hours. During phase 3 the populations of efficient homofermentative lactic acid-producing bacteria (Table 1) increase rapidly. These bacteria are considerably more efficient for conservation of energy, producing mainly lactic acid that drives the pH even lower. These organisms are less tolerant to heat than the anaerobic heterofermentors that dominant phase 2 but are more heat-tolerant than those dominating in Phase 4. As the silage mass cools, these organism give way to another group of homofermentative lactic acid-producing bacteria that continue to produce lactic acid but remain active at a lower pH and temperature.

**Phase 4 (Anaerobic)**

Often considered a continuation of phase 3, phase 4 is the period when silage temperature stabilizes and the predominant lactic acid bacteria (*Lactobacillus plantarum*) continue to convert water-soluble carbohydrates to lactic acid. The conversion of the carbohydrates to lactic acid is highly desired. The strongest of the organic acids produced during fermentation, lactic acid is efficient for reducing pH. This reduced pH conserves silage nutrients and the lactic acid present that can be utilized as a source of energy by ruminants.

Phase 4 continues until the pH is sufficiently low to limit the growth of all organisms. The limited growth
and metabolism of the silage organisms results in little further change in pH and the silage mass remains in a preserved state. Silage pH serves as an indicator that the crop has been stably preserved, but it tells nothing about the rate and quality of the fermentation.

**Phase 5 (Anaerobic)**

Phase 5 is considered the stable phase; it lasts throughout the duration that the silage is stored. Although often considered stable, phase 5 still is dynamic; changes continue to occur in the silage mass depending upon environmental conditions, the epiphytic populations present on the forage at harvest, and the number and activity of the dominant populations of lactic acid producing bacteria. The amounts of fermentation substrates remaining and the variety of fermentation acids present dictate what changes occur during this phase.

**Phase 6 (Aerobic)**

Phase 6, the final phase, occurs when silage is removed from storage for feeding. It begins when silage is exposed to air. Initially, organic acids including lactic acid will be catabolized by yeast and other aerobic organisms producing carbon dioxide and water (Table 1); this causes pH to rise that permits spoilage organisms such as molds and bacillus to proliferate.

Aerobic activity of the microorganisms in the silage mass causes silage to heat and reduces the palatability of silage and availability of nutrients. The degree of spoilage in the silage mass depends upon the number and activity of the spoilage organisms present and the amount of residual carbohydrates remaining. Spoilage can account for a loss of 1.5-4.5% of DM per day in affected areas of corn silage (Oude-Elferink, 2002).

**IMPROVING HMC WITH BACTERIAL INOCULANTS**

Bacterial inoculants can have a profound effect on fermentation of HMC. Microbial inoculants insure that a sufficient number of organisms with the proper activities are present to direct the fermentation and provide an environment suitable for long-term storage. The use of bacterial inoculants can alter the fermentation process in several ways to improve the feed value of the stored crop.

Generally, bacterial inoculants will compress phases 1-4. These phases can last as long as 3 weeks in uninoculated silage, but an appropriate inoculant can reduce this time to less than one week. Because terminal pH is reached sooner, the undesirable fermentation steps are avoided and preservation of the silage is enhanced. The more rapid reduction in pH provided by an inoculant will reduce respiration from harvested grain and limit the extent of inefficient fermentation of phases 1 and 2. Swift progression through the early phases causes more rapid transition to efficient lactic acid fermentation to preserve more nutrients and dry matter.

The most obvious response to the compression of the initial phases of silage fermentation is an increase in dry matter recovery (Bolsen et al., 1989a, 1989b; Hoffman and Muck, 1999). In a survey of 35 trials, corn silage dry matter recovery was increased an average of 1.7% by inoculation (Bolsen et al., 1989b). Similar improvements in dry matter recovery have been evident both with high moisture shelled corn and high moisture ear corn in controlled research settings (Pioneer, unpublished; Soderlund, 1997; Wardynski et al., 1993).

In addition to having effects at the onset of fermentation, inoculation can alter the later phases of fermentation. Phase 5, the storage phase, often is regarded as a maintenance phase when nutrients are preserved indefinitely with little, if any, microbial activity. However, advances in silage microbiology have shown that silage at phase 5 still is dynamic with shifting populations and changing metabolic activities. Nutrient utilization by livestock fed inoculated silage appears to be a result of alterations during phase 5 of the fermentation. Using an automated in vitro system adapted from Schofield and Pell (1995), we observed that inoculant-treated ground HMC ensiled at 29% moisture had a slightly slower rate of gas formation but a considerably higher extent of fermentation than untreated HMC similar in moisture content (Figure 2). Fellner et al. (1993) also have shown detected differences in ADF digestibility between inoculated and uninoculated high moisture ear corn.
Figure 2. Effect of inoculation on high moisture shelled corn in vitro digestion. Samples of high moisture shelled corn were ensiled at 29% moisture in 4” x 14” PVC silos for 88 days. Gas production was evaluated via an automated in vitro system according to the methods of Schofield and Pell (1995). Microbial inoculant was applied at $1 \times 10^4$ colony forming units per gram forage ensiled. Uninoculated controls were treated with equal volume of water.

It has been observed that starch availability continues to increase in ensiled corn with longer ensiling periods. It is not known if this increased starch availability is a function of the chemical action of high acid content and low pH or if it is an active process facilitated by the ever-changing metabolic activities of the microorganisms present. (Benton et al., 2004; Pringe, 1976).

The most recent development in microbial inoculants addresses one of the most challenging areas of silage fermentation, deterioration of the silage mass when exposed to air. In the past, aerobic deterioration upon exposure of silages to air was prevented by adding specific chemicals, typically short chain organic acids such as acetic and propionic acid (Phillip and Felner, 1992; MacDonald et al., 1991; Weinberg and Muck, 1996; Kung et al. 2004). In the past 10 years, attention has been focused on the use of the heterolactic bacteria, Lactobacillus buchneri, for the prevention of aerobic deterioration in silage. Inhibition of aerobic spoilage by this organism appears to be due to this organism’s ability to convert lactic acid to acetic acid and 1,2 propanediol. These in turn significantly reduce the yeast population of silage (Driehuis et al., 1997, 1999; Oude Elferink et al., 2001). The inhibition of yeast growth during exposure to air can extend phase 6 from as little as 24 hours to as long as 5 days before silage begins to heat. Reduced heating and lower yeast and mold counts result in cooler silage with less aerobic losses than either untreated or silage inoculated with traditional homofermentative silage inoculants.

Improvements in high moisture corn aerobic stability have been reported following the use of L. buchneri inoculants. Taylor and Kung (2002) observed a marked increase in the aerobic stability and reduced population of yeast that was proportional to an increased acetic acid content. Data from our laboratory with HMC using L. buchneri combined with selected strains of L. plantarum indicate that total dry matter loss is decreased (Figure 3) and that populations of yeasts and molds were reduced by nearly 100-fold (Figure 4).
Concerns have been raised about the use of heterolactic rather than homolactic inoculants because their less efficient metabolism could lead to excessive dry matter losses. In addition, high concentrations of acetic acid in the silages might depress animal intake. Current research has not supported these concerns. The increases in dry matter loss have been small and no negative effects on animal intake or performance have been observed among cattle fed high moisture corn treated with *L. buchneri* (Kendall, et. al., 2002; Combs and Hoffman, 2003). Indeed, except for lactic acid, ruminal concentrations and yields of organic acids far exceed concentrations in fermented silages. For example, dilution of 10 kg of DM from corn silage in 50 L of ruminal contents would add 0.25 mM lactate, 0.1 mM acetate, and 0.03 mM propionate. Typical ruminal concentrations of these acids are about 5 mM lactate, 60 mM acetate, and 30 mM propionate.

Alterations in fermentation by microbial inoculants results in improvement in other important attributes of the silage. The target for inoculants is to enhance the productivity of livestock fed silages. Kung and Muck (1996) have reported that positive responses to microbial inoculants on gain and milk production in studies comparing treated and untreated silages were detected in approximately 50% of the trials reviewed and seldom if ever were negative effects detected. These responses were observed across all silage crops, dry matters and inoculation levels.

Positive animal production responses also have been noted for high moisture corn treated with microbial inoculants. Fellner and co-worker (2001) found that weight gain was greater for steers fed inoculated high moisture ear corn than for steers fed an untreated control. A summary of 10 feeding trial with high moisture shelled and ear corn inoculated with microbial inoculants have show an average

![Figure 3. Reduced total dry matter loss in high moisture shelled corn with Lactobacillus buchneri combination inoculants. Comparisons were made between inoculated and uninoculated high moisture corn ensiled at 30% moisture for 60 days. Total dry matter loss is the sum of the losses occurring throughout ensiling plus the aerobic loss upon exposure to air as determined by the methods of Honig (1985). L. buchneri combination (mixture of *L. buchneri* and *L. plantarum*) was applied at a rate of 1x10^4 colony forming units per gram forage ensiled and compared to an uninoculated control treated with an equal volume of water.](image-url)
improvement in daily gain of more than 8% and a feed efficiency improvement of more than 6% (Pioneer, unpublished). These performance responses are above and beyond the increase in dry matter recovery usually seen with bacterial inoculants.

**Figure 4.** Reduction of yeast and mold level in high moisture shelled corn by treatment with Lactobacillus combination product. L. buchneri combination (mixture of *L. buchneri* and *L. plantarum*) was applied at a rate of 1x10⁴ colony forming units (CFU) per gram forage ensiled and compared to an uninoculated control treated with an equal volume of water. Yeast and mold levels were determined according to Taylor and Kung (2002).

**SUMMARY**
Microbial inoculants can consistently improve high moisture corn preservation and feeding value. Traditional homofermentative inoculants improve dry matter recovery primarily by accelerating the early fermentation process and can improve the availability of nutrients from the ensiled feedstuff. The newer *L. buchneri* inoculants have proven to effectively reduce the aerobic deterioration and heating that occurs upon exposure of silage to air during feeding. Combined, changes in the fermentation process achieved with active and effective inoculants can increase efficiency of energy conservation and the efficiency of livestock production.

Microbial fermentation aids are no substitute for proper silage management. The key to success in the use of microbial inoculants is attention to proper management. These would include harvesting and ensiling at the proper moisture, adequate packing of the silage mass to exclude as much air as possible, and use of a suitable cover to protect the ensiled grain from air and the environment. Strict attention to proper silage management techniques can maximize the beneficial effects of microbial additives.

**LITERATURE CITED**
http://www.oznet.ksu.edu/historicpublications/Pubs/SRP567.pdf
http://beef.unl.edu/beefreports200510.shtml
http://www.fass.org/phoenix03/abstracts/230.pdf