The Importance of the Aerobic Stability of Silages

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INTRODUCTION

Woolford (1990) stated that "the single most important factor which influences the efficiency with which forage crops are conserved as silage is the degree of anaerobiosis achieved in the completed silo". Air (because it contains oxygen) is a problem for forages at the start of ensiling, during storage, and at feed out. The presence of air at the start of fermentation prolongs the metabolism of undesirable microbes that thrive in air and delays the growth of beneficial bacteria that produce lactic acid needed to decrease the pH, which helps to stabilize the ensiled crop. This can lead to poor fermentations and a loss in nutritive value. During storage and feed out, infiltration of air into the silage mass can result in aerobic spoilage that often is responsible for the majority of total DM lost in forage conserved as silage and may be as high as 15 to 25% and even higher in areas of poor silage compaction.

"Aerobic stability" is the term used to describe the ability of a silage to remain stable and not spoil when exposed to air. Most researchers measure how long it takes for a silage to spoil under controlled conditions (e.g., at about 75^{o}F) when exposed to air. The number of hours or days a silage remains cool before about a 3 to 4^{o}F rise in temperature is a measure of its aerobic stability. Silages that have poor aerobic stability usually heat and spoil in less than 12 to 24 h. Conversely, a silage with an aerobic stability of more than 100 hours would be considered highly stable. Silages with good aerobic stability are especially desirable when fed during hot weather, which can accelerate spoilage caused by air and when fed they can negatively affect animal intake and performance. This review will briefly focus on the primary processes involved during aerobic spoilage of silages during storage and feed out, its potential consequences on animal productivity, and some ways to improve aerobic stability.

AEROBIC SPOILAGE BY LACTATE-ASSIMILATING YEASTS

When the active stage of ensiling is complete, the remaining microorganisms (bacteria and fungi [yeasts and molds]) in the silage mass are relatively dormant because of a low pH, an absence of air, or both. However, it is inevitable that silage will be ultimately exposed to air for a number of reasons. If lactate-assimilating yeasts are present in the silage mass when exposed to air, the result is a chain reaction of events (Figure 1). They initiate the aerobic spoilage of silages. Examples of this type of yeasts include *Issatchenkia orientalis* (*Candida krusei), Pichia anomala* and *Pichia membranifaciens* (*C. valida*) (Woolford, 1990, Inglis et al., 1999). These undesirable yeasts should not be confused with "beneficial" yeasts that are used in direct fed

microbials. They use lactic acid as an energy source, grow in numbers causing an increase in pH of the silage to a level that allows opportunistic bacteria (e.g., *Bacilli*) and molds (e.g., *Aspergillus, Fusarium,* and *Pencillium*) with the potential to mycotoxins and other noxious compounds to then become active, adding to the spoilage process (McDonald et al., 1991). For example, silages sampled from the top layers of silos (where pack density was poor) and silage that was loose on the bunker floor had higher levels of *A. fumigatus* than silage sampled from the intake silo face (Prince Agri, 2007). This is of particular concern because this organism has been linked with hemorraghic bowel syndrome in ruminants (Forsberg and Wang, 2006). Heating accompanies aerobic spoilage and is an obvious loss of energy. Ultimately, aerobic spoilage results in a loss of energy, a loss of dry matter, a decrease in nutritive value, and accumulation of unwanted compounds. In some cases, bacteria from the genus, Acetobacter may initiate aerobic spoilage in corn silages, but they are not dominant in North American silages (Muck and Pitt, 1994).

Current tests from analytical laboratories only measure the number of total yeasts in silages and do not differentiate between those that are lactate-assimilators and those that are not. Even so, the total number of all kinds of yeasts in silage is highly negatively correlated with aerobic stability. Natural populations of yeasts are found on all forage crops in the field usually in the range of a few hundred thousand colony forming units per gram of wet forage weight but their numbers are not well correlated to the aerobic stability of silages because the ensuing fermentation and level of silage management ultimately determines the number of lactate-assimilating yeasts that may survive ensiling. Porous silage masses, breaks in integrity of plastic and the concentrations of antifungal compounds added at ensiling (e.g., antifungal acids), or produced during fermentation (e.g., from various inoculant additives) can have profound effects the number of yeasts in the final fermented silage. High concentrations of lactic acid and/or a low pH have minor effects on the numbers of yeasts in silages as these organisms are relatively acid tolerant and lactic acid has poor antifungal characteristics. In contrast, relatively high concentrations of acetic and/or other acids (e.g., propionic acid, sodium benzoate, or potassium sorbate) can reduce their numbers because of their antifungal properties (Woolford, 1975). The concentration of acetic acid in silages can be especially high in silages with high moisture contents because the microorganisms that can produce this acid in silages (e.g., enterobacteria and heterolactic acid bacteria) thrive in wet conditions (more than 70% moisture). Thus, wet silages with high concentrations of antifungal organic acids tend to have low numbers of yeasts and are relatively stable when exposed to air. Ironically, one of the most antifungal acids sometimes produced in high moisture silages is butyric acid. This product of clostridial fermentation is very active in inhibiting the growth of yeasts but is certainly undesirable because of the other detrimental factors associated with this type of fermentation (i.e., large dry matter loss and degradation of protein, and production of biogenic amines). Conversely, low moisture silages (less than 60% moisture) undergo restricted fermentations and thus produce low concentrations of organic acids and pack poorly, often resulting in high numbers of yeasts. Ammonia also has good antifungal activity, but it is doubtful that natural concentrations of this compound effects populations of yeasts in silages.

The ambient temperature around the silage mass affects the rate of aerobic spoilage. When temperatures are relatively cool (<40-50°F), all microbial activity in silage is slowed or even stopped (e.g., in freezing weather) even when air is present. In contrast, warm temperatures stimulate microbial activity and thus, it is the primary reason that more spoilage typically occurs in the summer than in the winter. High concentrations of residual sugars in silage can also lead to a higher probability of aerobic spoilage. For example, sugarcane silage has very poor aerobic stability because of its high concentrations of sucrose and population of yeasts. A list of some factors affecting the aerobic stability of silages is shown in Table 1.

FEEDING AEROBICALLY SPOILED SILAGES NEGATIVELY IMPACTS RUMINANTS

Feeding aerobically spoiled silage to ruminants has resulted in depressed dry matter intake and decreased production Hoffman and Ocker (1997) reported a negative correlation between numbers of molds in the diet and milk production when cows were fed a TMR with soiled HMC. Whitlock et al., (2000) observed lower dry matter intake and average daily gain when steers were fed increasing levels of spoiled corn silage. Similarly, Windle et al. (2013) reported that heifers fed a spoiling TMR containing more than 66,000,000 yeasts per g of TMR ate less DM than heifers fed the same TMR that was fed fresh and contained only about 110,000 yeasts/g. Gerlach et al. (2013) reported that a depression in DM intake in goats fed aerobically spoiled silage was highly negatively correlated with the change in temperature of the spoiling silage mass. Bruning et al. (2018) found that corn silage exposed to air had a stronger negative effect on dry matter intake from goats than did delayed filling or silo compaction. When animals consume spoiled silages, the exact causes of reduced intake and/or performance are not fully understood. Oxidation of nutrients reduces the nutritive value of spoiled silages. However, detrimental yeasts in silage may also compete with rumen microbes for nutrients and may produce unknown end products that might alter rumen fermentation. For example, Santos et al. (2011) reported that adding high levels of *I. orientalis*, a lactate-assimilating yeast, to in vitro ruminal fermentations reduced NDF-D. Effects on immune function may occur but are not well documented. Spoiled silage may also be unpalatable to animals.

METHODS TO IMPROVE THE AEROBIC STABILITY OF SILAGES WITH SILO MANAGEMENT

Yeasts are able to grow in the presence and absence of air (oxygen) but their numbers can multiply more quickly when air is present. Filling silos quickly with sufficient pack weight to maximize density and minimize porosity can minimize air in a silo. Even distribution of forage in the storage structure, chopping to a correct length and ensiling at recommended dry matters (DM) for specific storage structures aids in this process. After filling, silage should be covered with plastic as soon as possible and weighed down with tires (tires should be touching) or gravel bags to exclude air. Split tires are a good alternative because they are easier to handle, do not accumulate water (thus less breeding grounds for mosquitoes that could carry the West Nile Virus), and are undesirable for animals to nest in. The return on investment (labor and plastic) is extremely high for covering bunk and pile silos (Bolsen et al., 1993). Oxygen barrier plastics are also now available for use (Borreani et al., 2007). Extreme care should be taken to prevent air from penetrating between the plastic and reaching the silage mass during feed out

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and storage and this can be accomplished by stacking tires, or lining gravel bags on the plastic at the leading edge of the feeding face. Such practices can reduce the number of yeasts in silages and improve aerobic stability.

Proper management for removal of silage from silos at the feed bunk can help producers to maximize profits and production. Enough silage should be removed between facing to minimize aerobic spoilage. Common removal rates range from 8 to 12 in per day, but lesser amounts may be removed in areas where ambient temperatures remain cool during the winter months. Removal of silage should be such to minimize disruption of the silage face and loose silage on the ground between feedings. Steele et al. (2018) reported that incorporating as little as 10% of spoiled for unspoiled corn silage in a TMR was enough to destabilize the entire TMR and cause spoilage.

IMPROVING AEROBIC STABILITY WITH ADDITIVES

Chemical additives. Various chemical additives with antifungal properties have been used to enhance the aerobic stability of silages. The most common are organic acids, specifically propionic acid. Buffered propionic acid-based products are commonly used because of they are less corrosive and safer to handle than the straight acid. It is the undissociated form of organic acids that is responsible for their antifungal properties and its prevalence is dependent on pH. This fact unfortunately means that more acid is needed to be effective in crops that are naturally limiting in acids from silage fermentation (e.g., crops with more than 40% DM). At the pH of a standing crop of alfalfa (about 6) only about 1% of propionic acid is in the undissociated form whereas, at a pH of 4.8, about 50% of the acid is undissociated. The undissociated acid functions both by staying active on the surface of microorganisms and competing with amino acids for space on active sites of enzymes and by altering the cell permeability of microbes. Undissociated acids also can penetrate microbial cells and disrupt cytosolic functions because of the release of H+. Application of buffered propionic acid-based products ranges from about 1 to 6 lb/ton of forage depending on the specific situation. The efficacy of low application rates is questionable. For example, if 2 lb of a product that contained 65% propionic acid were added to 35% DM corn silage, this would increase the propionic acid content in that silage by 0.18% on a DM basis. In previous studies, we have found that, as expected, the effectiveness of propionic acid-based additives increases with higher application rates (Kung et al., 2000). Potassium sorbate, benzoate and acetic acid are commonly found as components of many antifungal formulations but are generally too expensive to be used alone in high concentrations. A commonly used additive to control yeasts and molds in the past has been anhydrous ammonia (5-7 lbs/ton of forage). The major drawback with ammonia was operator safety during application. Urea is generally less effective at improving the aerobic stability of silages than ammonia is.

Microbial inoculants. Bacterial inoculants, based on homofermentative lactic acid bacteria are commonly added to silages to improve fermentation and increase DM and energy recovery. However, most of these inoculants do not consistently inhibit wth of yeasts because they tend to maximize the production of lactic acid (poor antifungal activity) and decrease the

accumulation of other organic acids that have good antifungal activity. In fact, Muck and Kung (1997) reported that a summary of research studies showed that treatment with classical homolactic acid-based inoculants had no effect in about 33% of the studies and actually made aerobic stability worse in another 33% of studies.

Microbes from the *Lentilactobacillus buchneri* family of organisms are obligate heterolactic acid bacteria used to specifically improve the stability of silages. The organism *L. buchneri* has been most widely researched but recent studies also support the use of *L. diolivorans* and *L. hilgardii*. All of these bacteria have the ability to convert moderate amounts of lactic to acetic acid under anaerobic conditions. Acetic acid has good antifungal (yeast killing) activity and thus silages treated with these organisms have slightly lower lactic acid but more acetic, fewer yeasts, and better stability than untreated silage. The amount of acetic acid produced from these organisms is roughly equivalent to 5 to 8 lb. of acid per ton of wet silage weight. Practical recommendations in the field have suggested a desirable lactic:acetic ratio of more than 3:1 (Kung et al., 2018), which would be an indication of a more dominant homolactic fermentation. However, it is now known that silages treated with *L. buchneri* organisms should not be held to this standard. In general, improvements in aerobic stability from using *L. buchneri* has required about 60 days of ensiling. However, combining *L. hilgardii* and *L. buchneri* appears to result in a synergistic effect such that improvements in stability can be detected as early as 15 days after ensiling in some crops (da Silva et al., 2020). Combining *L. diolivorans* and *L. buchneri* has also resulted in accelerated improvements in quick stability (Thaysen and Kramer, 2018). To date the exact mechanism(s) of the synergistic effects is (are) unknown. *Lentilactobacillus diolivorans* has the capability of also producing moderate amounts of propionic acid, which also has strong antifungal attributes.

Concerns relative to the potential of large losses of DM from silages treated with *L. buchneri* because of its heterolactic nature have not been substantiated (Kleinschmit and Kung, 2006). The loss of DM in corn silage by the higher application of *L. buchneri* was 1 percentage point more than for untreated silage. Relative to the potential beneficial effects of improved aerobic stability during storage and feeding, this loss is small. Although some have suggested that high levels of acetic acid in silages may depress intake, research studies have shown that ruminants fed silages treated with *L. buchneri* consume the same amount of DM when compared to counterparts fed untreated silages (Dreihuis et al., 1999b, Kung et al., 2003, Ranjit et al., 2002, Taylor et al., 2002). Most research on improving the aerobic stability of silages has dealt with the stability of the silages alone. However, there is good evidence that if silages are stable this benefit is transferred to the TMR. In two studies, TMR that were made with silages treated with *L. buchneri* were more stable than TMR made with untreated silages (Kung et al., 2003, Taylor et al., 2002).

When Should Additives Be Used to Improve Aerobic Stability? In some circumstances, silages are moved between storage structures resulting in air interacting with the silage mass. Moving silages quickly and in cool weather minimizes the potential for aerobic spoilage. On many large dairies it is now common to find several days' worth of silage fed from temporary piles (brought in from other farms or silos and stored at a staging area). The chance of aerobic spoilage

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increases with this practice especially in warm weather. Such silages are good candidates for additives to improve stability. Additives for improving aerobic stability can also be justified if silages have slow feed out rates, are packed poorly, and or have high dry matter contents. Note that microbial-based additives are ineffective on silages that have already fermented and should be added at the time of ensiling.

Improving Aerobic Stability in TMR. Because silages are often incorporated into TMR, their stability is also a challenge on many farms. In a small survey of TMR sampled in DE, PA and MD over two years, more than 50% of 30 TMR that were sampled within 1 hour of being made, spoiled in less than 12 h when incubated at a controlled laboratory temperature of about 72°F (Kung, Mulrooney and Morges, unpublished data Univ. of Delaware). These TMR would have spoiled even quicker if they were incubated at the ambient temperatures encountered during an average summer day (high 80 to 90 °F). Thus, these TMR had the potential to spoil in the feed bunk even if the farms were feeding twice daily. If nothing can be done to alleviate the primary causes(s), additives (commonly referred to as "TMR-savers") containing antifungal compounds can be added directly to the TMR to improve aerobic stability. The degree that silages have spoiled in the silo and ambient temperatures will determine the doses required to stop further spoilage in the feed bunk (perhaps 4 to 8 lb of additive per ton of TMR may be required to prevent further spoilage in challenging conditions). When using TMR-savers, it is best to start with a high dose for several days. If stability in the bunk has been achieved, a lower level can be used that keeps the TMR from heating in the bunk. TMR-savers can be helpful but they are not economical for long-term use because the rates of addition are very high. For example, even added only at 4 lb/ton of TMR, the equivalent would be adding 8 lb of the product per ton of forage. In addition, stopping further heating and spoilage in the feed bunk does nothing to stop the initial heating and loss of nutrients that may have occurred in the silo. Data from our lab suggests that it is better to control yeasts at that time of ensiling rather than after the fact in a TMR. The more yeasts that are present in the silage and TMR, the higher the dose of a TMR-saver will be needed to keep the feed from spoiling.

Diagnosing Problems with Aerobic Stability. Because silages heat during aerobic spoilage, the length of time that silage remains cool when it is exposed to air is often used as a measurement of aerobic stability. Many research studies determine aerobic stability by assessing the time it takes for a silage mass to increase about 3-4 degrees F above ambient or baseline temperatures after it is exposed to air. On the farm, warm silage is not always an absolute indicator of spoilage because large silos often retain relatively high core temperatures even in the winter. In a recent survey, we noted core silage temperatures as high as 90 to 95 °F in some silos for as long as three months. Thus, steam coming from the silage mass during silo removal during the winter, is not necessarily a sign of aerobic spoilage. In contrast, aerobically spoiled silage can often reach temperatures as high as 120-150°F for short periods of time. Signs that silage is aerobically spoiling on the farm include measuring temperatures in excess of 100-105 °F in fermented silage and reheating in the bunk.

The number of yeasts and molds in a silage sample can sometimes be used to assess whether a silage sample has spoiled or has the potential for rapid spoilage. Care should be taken to

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ensure that the sample is representative of that being fed. In addition, samples for microbial analyses should be kept refrigerated (not frozen) and sent to the laboratory as quickly as possible (preferably stored with ice packs). This will minimize the growth of yeasts and molds that could grow during transit. Unspoiled silages usually contain about 1,000 to 250,000 yeasts per gram of wet silage. Samples containing more than 500,000 yeasts per gram have a high probably or spoiling rapidly in warm weather and/or have already started to aerobically deteriorate. It is not uncommon to find spoiled silages with more than 100,000,000 yeasts/g of silage.

Visible signs of molds lack of a sharp or sweet smell to the silage and/or a flat or moldy/musty smell are also indicators of aerobic spoilage. If a pH meter is available, a moldy smell coupled with a high pH may also be a good indicator that a feed has undergone aerobic deterioration. Measuring the production of CO2 and the time it takes for silage pH to rise have also been used to assess aerobic stability but these techniques are not well suited for use on the farm.

CONCLUSIONS

Most aerobic spoilage of silage in the US is initiated by lactate-assimilating yeasts. Aerobically spoiled silage is undesirable because of losses in nutrients and potential negative effects on animal performance and health. Good silo management and the use of various additives can help to minimize the incidence of aerobically spoiled silage and improve net farm income.

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Figure 1. The effect of air on the aerobic spoilage of silages.

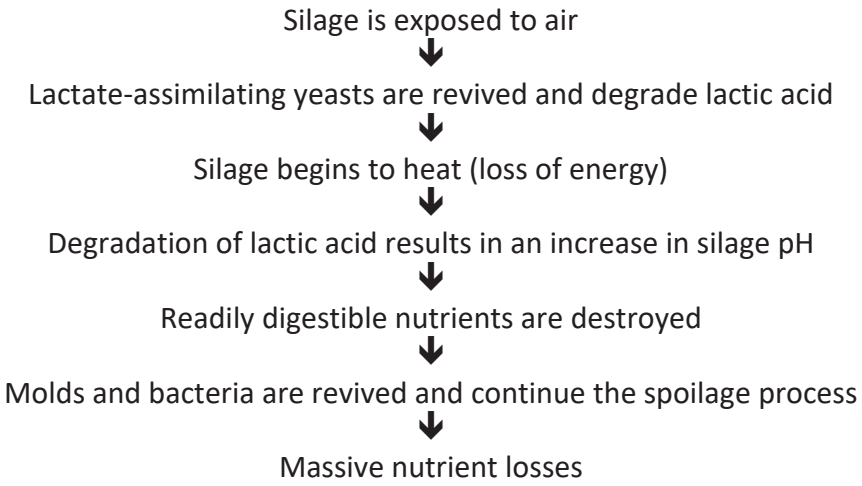

Table 1. Some factors that may make silages more prone to aerobic spoilage by silage yeasts.

Factor	Effects	Examples
High DM content	a) High DM restricts fermentation and reduces acids that could minimize the numbers of yeasts b) High DM crops are more difficult to pack and allow infiltration of air into the mass	a) Haylage ensiled > 45 to 50% DM b) Corn silage ensiled > 40% DM
Poor pack density/porosity	Allows penetration of air into the silage mass	a) Fill rate too fast b) Insufficient pack ractor weight and time
Poor feeding face management	Allows penetration of air into	a) Slow silage removal

	the silage mass	b) Loose silage c) Uneven silage face d) Intermediate feeding piles e) Moved silage
Poor management of plastic and weights	Allows penetration of air into the silage mass	a) Torn bag silos b) Torn silo covers c) Insufficient weight on plastic d) Plastic pulled back too far in advance
High ambient temperatures	Spoilage organisms grow faster in warmer weather	More spoilage in the summer than winter months
Addition of spoiled feeds to a TMR	Spoiled feeds bring spoilage organisms to the TMR	Spoiled wet distillers grains, left over TMR
Overly dominant homolactic acid fermentation	Limited production of organic acids that have antifungal properties	An extremely dominant homolactic acid fermentation caused by microbial inoculation