Radin

Fly to Future

BioMould®

Silage Preservative

Proper Fermentation Stimulant

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Ensiling is the preservation of forage based on lactic acid fermentation under anaerobic conditions. The lactic acid bacteria ferment water-soluble carbohydrates in the crop to lactic acid, and to a lesser extent to acetic acid. The production of these acids reduces the pH of the ensiled forage which inhibits spoilage microorganisms' growth and proliferation.

Making silage is classified in four phases:

1- Aerobic phase
   Oxygen + Sugar → CO_2 + Heat + Water (protein degraded)

2- Fermentation phase
   Sugar → Acetic Acid

3- Stable phase
   Sugar → Lactic acid, acetic acid, ...

4- Feed out phase or aerobic spoilage phase (Stable state until silage is exposed to oxygen)

Silage stability is a significant factor for preserving silage quality. Yeasts can metabolize lactic acid in presence of oxygen which leads to PH reduction in silage. When PH increases, the population of undesirable fungi and bacteria can grow which result in silage spoilage. The spoilage wastes dry matter which can be more than 10% in critical situations. One of the factors of a proper management for providing good silage is supplementation of that with some additive. Organic acids are used directly (all over or just on the surface) in the silage, in order to protect it by reduction in PH. Propionic acid and formic acid are mostly used for products containing more than 70% moisture and low in glucose such as maize which is high in moisture. Based on aforementioned conditions, a low PH is required to prevent Clostridia Spp. growth.

Table1- Different microorganism and their optimum PH

Microorganism	Optimum PH
Lactobacillus Spp.	5/4 –6/4
Escherchia coli	6-8
Salmonella Spp.	6/8-7/2
Campylobaster jejuni	6/8-7/2

Benefits of adding BioMould® to the silage

- BioMould® contains High concentration of formic acid and propionic acid (57%)
- Reduction in PH by applying BioMould® in silage results in Lactic acid bacteria propagation which subsequently induces resistance against Pathogenic molds, Butyric acid bacteria and Clostridia Spp.
- The carrier material releases the acids mainly as gas, so that the acid vapors are equally distributed throughout the silage to be reserved.
- It Increases nutritional value of silage by preserving its crude protein content.
- BioMould® induces quick lactic acid bacteria fermentation which results in protecting nutrients of silage.
- Consequently BioMould® boosts the durability of silage.

Formic acid improves protein preservation of silage by an immediate acidification, which inactivates protein-degrading bacteria.

Propionic acid improves DM recovery and feed-out stability of silage by inhibiting yeast and mold growth as yeast cannot assimilate propionic acid.

Formic acid, (43% in BioMould®) as a potential bactericide, improves protein preservation of silage by an immediate acidification which results in inactivating of protein-degrading bacteria.

Propionic acid, (16% in BioMould®), as a fungicide, improves dry matter recovery and feedout stability of silage by inhibiting yeast and mold growth as yeast cannot assimilate propionic acid.

An effective additive may help make good silage better, but it will not make poor silage good. The options to create better silage are manifold. One efficient method is surface treatment of silage.

Fermentation process in corn silage

Aerobic Phase	Anaerobic Phase				Stability Phase
Day 1	Day 2	Day 3	Day 4-7	Day 8-21	After day 21
Cellular respiration and production of CO_2, heat and water	Initiation of fermentation, acetic acid production and temperature reduction	Initiation of lactic acid production and continuous of acetic acid production	continuous of lactic acid production and temperature reduction	Continuous of lactic acid and PH reduction of silage and then PH stabilizing.	Termination in bacterial fermentation until silage expose to oxygen
Temperature					
$70^{o}F$	$95^{o}F$	$80\text{-}85\ ^{o}F$			Environmental temperature
PH	PH	PH			
6	5	PH reduces to 4 and stabilize in this level			

Surface and edge treatment of silage

The treatment of silo surface does not replace complete treatment at ensiling, but, if used properly, it is an efficient protection against losses at the especially endangered parts of the silo. Spreading BioMould® on top of the ready to ensile stock is possible, if the silage is sealed air tightly immediately afterwards. Due to the especial product formulation the acids diffuse slowly from the Vermiculite lattice (from the carrier) and concentrates below the silage-film. As the acidic vapors cannot escape, the acids concentrate in the upper layer of the silage and so provide efficient protection.

Table 2- Comparing Means (Test–Duncan) of bacterial cultures in samples treated with and without BioMould® (P<0.05)

	Lactobacilli (MRS) (CFU/g)			Aerobic bacteria (NA) (CFU/g)			Clostridia (Blood Agar) (CFU/g)		
Dilution of Microbial Suspension / Treatment	10^4	10^5	10^6	10^4	10^5	10^6	10^4	10^5	10^6
Surface Control (without BioMould®)	$(260.25 \times 10^5)^b$	$(270.00 \times 10^4)^b$	$(255.25 \times 10^3)^b$	-	$(325.75 \times 10^4)^a$	$(307.75 \times 10^3)^a$	Hemolysis (β)	Hemolysis (β)	Hemolysis (β)
Depth Control (without BioMould®)	$(334.50 \times 10^5)^a$	$(322.00 \times 10^4)^a$	$(324.00 \times 10^3)^a$	$(222.50 \times 10^5)^a$	$(213.25 \times 10^4)^b$	$(214.50 \times 10^3)^b$	Hemolysis (β)	Hemolysis (β)	Hemolysis (β)
Surface Treatment (with BioMould®)	$(167.50 \times 10^5)^d$	$(166.50 \times 10^4)^d$	$(168.75 \times 10^3)^d$	$(81.50 \times 10^5)^b$	$(83.50 \times 10^4)^c$	$(84.00 \times 10^3)^c$	Hemolysis (γ)	Hemolysis (γ)	Hemolysis (γ)
Depth Treatment (with BioMould®)	$(206.50 \times 10^5)^c$	$(204.50 \times 10^4)^c$	$(201.50 \times 10^3)^c$	$(14.75 \times 10^5)^c$	$(15.00 \times 10^4)^d$	$(11.50 \times 10^3)^d$	Hemolysis (γ)	Hemolysis (γ)	Hemolysis (γ)

Acid formic in BioMould® caused reduction in aerobic bacteria and clostridia growth and simultaneous increase in Lactobacilli population (table 2).

Table 3- Comparing Means (Test–Duncan) of yeast and mould cultures in samples treated with and without BioMould® (P<0.05)

	Yeast (CFU/g)			Mould (CFU/g)		
Dilution of Microbial Suspension / Treatment	10^1	10^2	10^3	10^1	10^2	10^3
Surface Control (without BioMould®)	$(148.70 \times 10^3)^a$	$(133.75 \times 10^2)^a$	$(121.50 \times 10^1)^a$	$(69.75 \times 10^3)^a$	$(61.00 \times 10^2)^a$	$(57.25 \times 10^1)^a$
Depth Control (without BioMould®)	$(110.00 \times 10^3)^b$	$(108.50 \times 10^2)^b$	$(100.50 \times 10^1)^a$	$(30.50 \times 10^3)^c$	$(26.00 \times 10^2)^c$	$(24.00 \times 10^1)^c$
Surface Treatment (with BioMould®)	$(95.50 \times 10^3)^b$	$(90.50 \times 10^2)^b$	$(86.25 \times 10^1)^{ab}$	$(42.50 \times 10^3)^b$	$(41.75 \times 10^2)^b$	$(40.50 \times 10^1)^b$
Depth Treatment (with BioMould®)	$(76.25 \times 10^3)^c$	$(69.75 \times 10^2)^c$	$(65.75 \times 10^1)^b$	$(22.00 \times 10^3)^d$	$(20.25 \times 10^2)^d$	$(16.00 \times 10^1)^d$

Reduction in yeasts and moulds growth demonstrates that volatile acids like propionic acid are better inhibitors for these organisms than lactic acid (table 3).

Table 4- Effects of Biomould® on Lactobacilli to aerobic bacteria ratio

	Lactobacilli to aerobic bacteria ratio		
Dilution of microbial suspension	10^4	10^5	10^6
Surface	-	2.406	2.426
Depth	9.146	9.029	11.600

In this study, applying BioMould® in the surface of silage lead to reduction in growth of aerobic bacteria and clostridia Spp. because of formic acid effects: reduction in PH and increase in Lactobacilli population growth.

The ratio of population of lactobacilli SPP. to population of aerobic species of silage is the most significant parameter for determining the quality of silage surface (table 4).

Table 5- Fermentation parameters of corn silage subsequent to adding Biomould®

Treatments/effects		mg/dL	g/100gFW*					
	PH	N-NH_3	Lactate	Acetate	Propionate	Butyrate	Isovalerate	Valerate
Control	4.095^a	2.077^a	1.921^a	2.090^a	0.167^a	0.054^a	0.039^a	0.041^a
BioMould®	3.924^a	1.770^a	2.055^a	2.624^a	0.280^a	0.023^b	0.014^b	0.079^a
Significance level	0.326	0.580	0.773	0.065	0.194	0.0103	0.046	0.459
standard error of the mean	0.0835	0.1068	0.1931	0.131	0.0427	0.0052	0.0056	0.0123
Surface	4.116^a	1.871^a	2.048^a	2.248^a	0.229^a	0.029^a	0.028^a	0.040^a
Depth	3.903^a	1.770^a	2.317^a	2.466^a	0.244^a	0.048^b	0.025^b	0.080^a
Significance level	0.235	0.718	0.321	0.421	0.869	0.093	0.828	0.430
standard error of the mean	0.0836	0.1369	0.2421	0.1312	0.0446	0.0051	0.0056	0.0245

FW: Fresh Weight

Table 6- Chemical analysis of corn silage subsequent to adding Biomould®

Treatments/ effects	°C	%							
	Temperature	DM	OM	CP	Ash	EE	NDF	ADF	NFC
Control	18.771^a	21.181^b	92.814^a	9.130^a	7.186^a	2.941^a	50.716^a	23.828^a	30.186^a
BioMould®	17.238^a	25.256^a	92.533^a	9.314^a	7.468^a	2.845^b	49.253^a	22.738^a	31.121^a
Significance level	0.366	0.0006	0.422	0.516	0.421	0.476	0.459	0.139	0.763
standard error of the mean	0.0418	0.4265	0.169	0.1458	0.168	0.0652	0.8072	0.331	1.0525
Surface	14.763^b	22.415^a	92.629^a	9.265^a	7.371^a	2.694^b	50.320^a	23.321^a	30.350^a
Depth	20.525^a	23.586^a	92.718^a	9.279^a	7.283^a	3.093^a	49.960^a	23.420^a	30.386^a
Significance level	0.0008	0.718	0.797	0.430	0.421	0.869	0.093	0.828	0.988
standard error of the mean	0.0836	0.1369	0.169	0.0245	0.1312	0.0446	0.0051	0.0056	1.1896

Adding BioMould® to silage resulted in lactate production due to increase in lactobacilli population. Domination of these species in the silage is directly related to the acidic environment provided by BioMould® (table 5). Wasting energy and protein in silage by Lactobacilli Spp. is lesser than other bacterial species. Simultaneously, BioMould® decreases PH of silage immediately by reducing buffer capacity. This is followed by inhibiting growth yeasts, molds and pathogenic bacteria like clostridia Spp. These microorganisms are butyrate producers and the main protein-degrading factors in the silage.

Ammonia reduction in BioMould®-treated silage is another indicator that demonstrates success in preserving the protein content of silage. Final products of fermentation not only indicate the quality of silage, but also demonstrate the kind of fermented sugars and dominated microorganisms in the silage. Accordingly, amount of lactic acid in the silage is a criterion for its soluble carbohydrate content that can help proper ensilaging.

In this study, adding BioMould® to the silage resulted in significant increase in dry matter content (table 6). It should be noted that propionic acid is the factor for preserving dry matter content and aerobic stability of silage by preventing from yeast and mold growth (propionic acid is a fungicide). Increase in none-fibrous carbohydrates and crude protein is a criterion for increase in dry matter of silage. Formic acid preserved protein content of silage by immediate acidifying and subsequently prevention of protein-degrading bacteria growth.

Discussion

In aerobic phase, the atmospheric oxygen present between the plant particles is reduced, due to the respiration of the plant material and aerobic and facultative aerobic microorganisms such as yeasts and enterobacteria. Furthermore, plant enzymes such as proteases and carbohydrates are active during this phase, provided the pH is still within the normal range for fresh forage juice (pH 6.5-6.0). During this period, plant cells and microbes will metabolize sugars and starch in the presence of oxygen, generating carbon dioxide, water and heat in the process.

Oxygen should be eliminated from the silage by good packing and sealing as much as possible. Therefore, it is critical to ensure good compaction, proper moisture, and good sealing, all of which lead to a rapid transition to anaerobic conditions. Despite the best management, some oxygen will remain in the silage. Aerobic bacteria also use up oxygen as they degrade the silage at this time. Long aerobic phases are a problem. They result in higher silage fiber (ADF and NDF) and less energy (NEl). Digestible sugars are used and wasted by the bacteria.

After the oxygen is used up in the aerobic phase, in second phase, plant cells are broken down and used as a food source by bacteria. Plant enzymes break down complex carbohydrates (starch and fiber) into simpler sugars (glucose, fructose, sucrose) that are easy for bacteria to use. Enzymes also break down plant proteins at this time, making the protein more soluble.

Rapid decrease in pH prevents breakdown of plant proteins and helps inhibit growth of spoilage microbes. Consequently, lactic acid production is preferred to ensure a low silo shrink.

Adding BioMould® resulted in acceleration in silage acidification which leaded to a rapid transition to anaerobic conditions by immediate terminating of aerobic and fermentation phase. Consequently, preserving the dry matter content of silage due to reduction in fermentation level was seen. Table 5 demonstrates the reduction in none-fibrous carbohydrates and preservation of protein content of corn silage following to treating silage with BioMould®. In conclusion, BioMould® could increase the silage quality by preserving its nutrients for dairy cow consumption.

In conclusion, adding BioMould® inhibits the growth of protein-degrading bacteria, yeasts and molds and increases the growth of lactic acid bacteria by rapid reduction in silage PH, increase in lactic acid content of silage and decline in butyric acid production. All these reduce degradation of dry matter and nutrients of silage, mainly proteins and finally result in high-quality silage with high available nutrients.

Figure 1- Comparing Means (Test–Duncan) of aerobic bacteria culture with 10^5 dilution factor in samples treated with and without BioMould® ($P<0.05$)

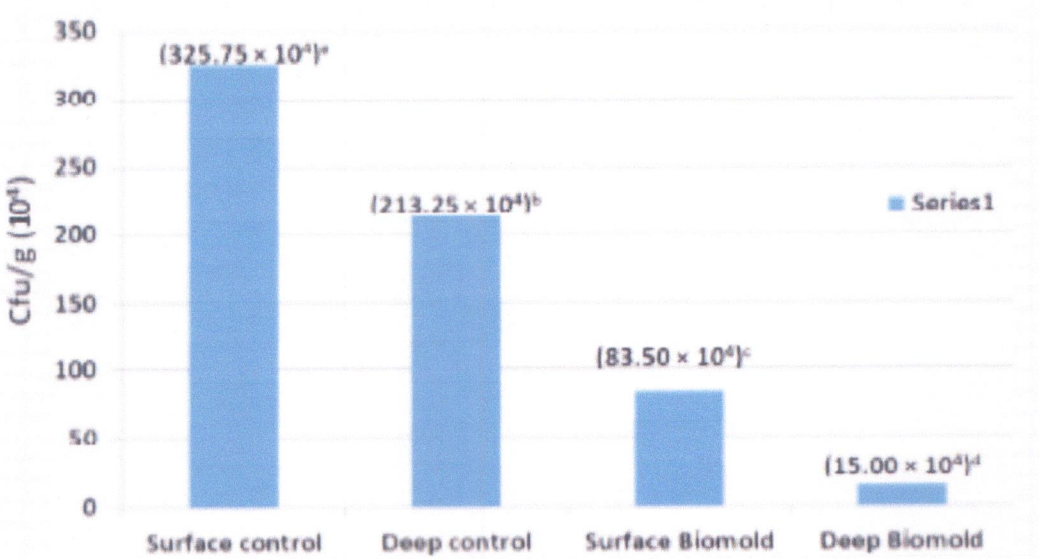
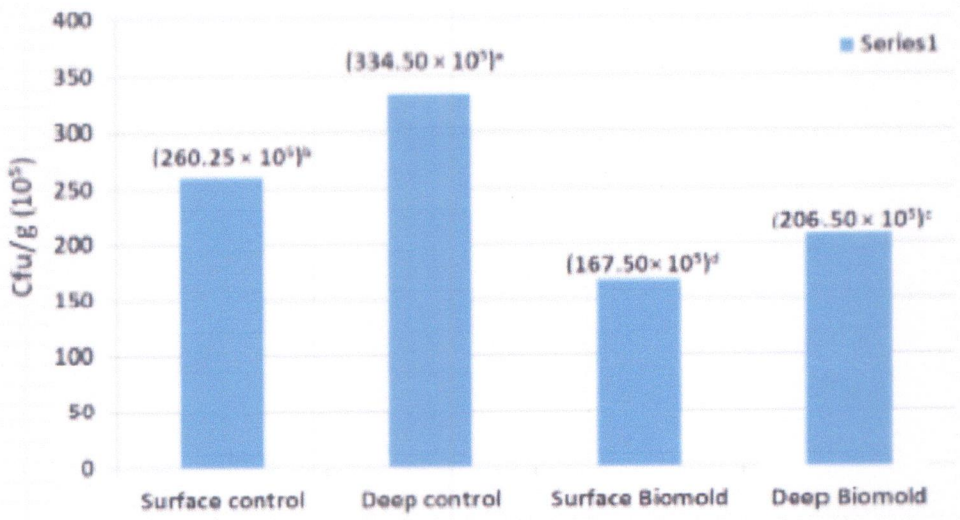
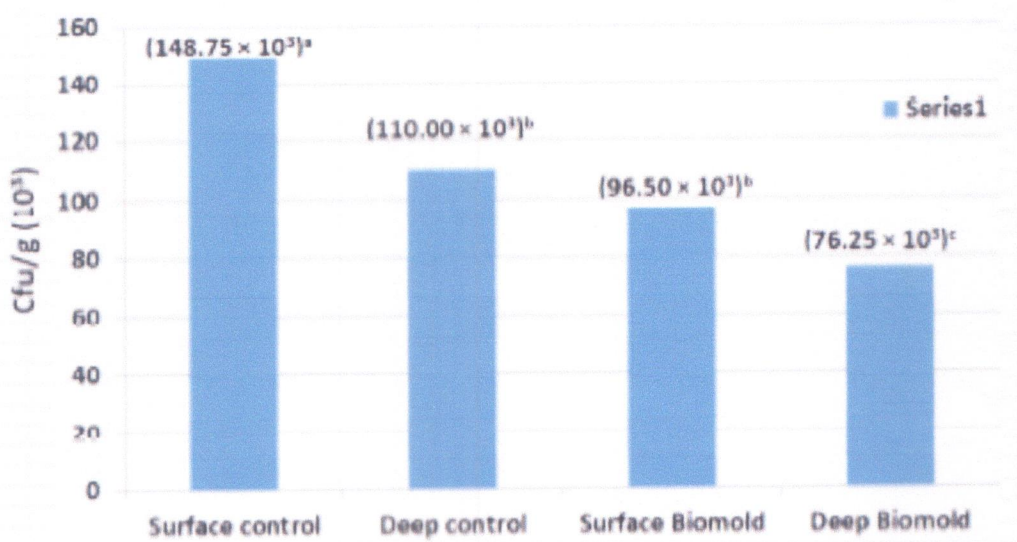


Figure 2- Comparing Means of Lactobacilli Spp. culture with 10^4 dilution factor in samples treated with and without BioMould® ($P<0.05$)



Comparing Means of yeast culture with 10^1 dilution factor in samples treated with and without BioMould® ($P<0.05$)



Comparing Means of mould culture with 10^1 dilution factor in samples treated with and without BioMould® ($P<0.05$)