J. Agric. Sci. Mansoura Univ., 28 (6): 5021 – 5029, 2003

EFFECT OF WHEY AND EFFECTIVE MICROORGANISMS ON CHEMICAL AND MICROBIAL CHARACTERISTICS OF SILAGE

Marcos, Mona M .; K. A. Abdel-Kawi and G. G. Antoun
Microbiol. Res. Dept., Soil, Water and Environ. Res. Inst., Agric. Res. Center, Giza, Egypt.

ABSTRACT

Clover as green forage and maize, broken bean and wheat bran as concentrated forage and wheat straw as filling material were used in producing silage ration. Silage mixture was inoculated with 2 ml of *Lactobacillus* culture *(Lactobacillus casei as activator bacteria grown on whey)* to obtain *Lactobacillus* silage as first experiment. Whereas, the second experiment, silage was inoculated with 2 ml effective microorganisms culture (EM activator grown on 5% molasses) to obtain EM silage. After incubation the pH values decreased to reach 4.1 and 4.0 in *Lactobacillus* and EM silages compared with that of the control silage. Volatile acids (butyric and acetic) were lower than lactic acid, which increased in *all experiments during ensiling* processes. Moreover, the silage chemical compositions before and after ensiling showed that total inverted sugar at the end was traces. Silage microorganisms counts were (1020 and 990 x 10^6 CFU g^{-1}) and (410 and 320 x 10^6 CFU g^{-1}) for *Lactoacillus* and EM silage on basal media and SANM after four weeks, respectively. However, these results clearly indicate that the synthetic medium (SANM) had limited the microorganisms' colonies size and densities that helps in producing silage of low acidity and high quality silage during the fermentation processes.

Keywords: Silage, *Lactobacillus*, effective microorganisms (EM), whey, clover, *maize, broken bean, wheat bran and wheat straw*

INTRODUCTION

The preservation of forage crops as silage depends on the production of sufficient desirable acid to inhibit activities of undesirable microorganisms. The epiphytic lactic acid bacteria present on forage crops convert sugar to lactic acid during the ensiling process. As a result, the pH is reduced and the forage is protected (Weinberg *et al.*, 1993). Moreover, silage inoculates are used to enhance fermentation by adding lactic acid bacteria at ensiling. When the bacteria inoculants dominate fermentation, the resulting silage has less acetic acid and ethanol, more lactic acid and a lower pH than may occur *if epiphytic bacteria populations drive the fermentation* process (Kent *et al.*, 1989). Several studies have examined the effects of microbial inoculants to improve preservation of chopped alfalfa stored in bunker, tower or laboratory silos. The effect of inoculants on silage *fermentation characteristics and on animal performance have produce* a range of results depending on factors such as epiphytic micro-flora populations, forage moisture and water soluble carbohydrate content and ensiling management (Bolsen *et al.*, 1992).

Idler *et al.* (1998) postulated that the mixtures of *Lactobacillus casei* and *L. rhamnosus* or *L. casei* alone, *Enterococcus faecium* and *L. delbrueckii*

improve silage quality by the fermentation of butyric and the decomposition of protein. Also, Cai *et al.* (1999) treated the three silages with strains of *Lactobacillus casei* or *L. plantarum* and were well preserved, had significantly lower pH values, butyric acid, propionic acid and ammonia nitrogen concentrations, gas production and dry matter losses and had significantly higher contents of residual water soluble carbohydrates and lactic acid than did the respective control silage. Singh *et al.* (1996) ensiled the *Lucerne alfalfa*, and fodder with and without inoculum (*Enterococcus faecalis* or *Lactobacillus plantarum* or both) and 5% molasses. Samples were analyzed at 0, 15, 30 and 60 days of ensiling. A decrease in dry matter content and pH was observed during the ensiling period; addition of molasses decreased pH and ammonia-nitrogen and increased dry matter content. Ammonia-nitrogen, lactic and acetic acids content increased through out ensiling. Whereas, Kim *et al.* (1999) made the alfalfa silage without additive or using formic acid, molasses, or one of both bacterial inoculants including *Lactobacillus plantarum*, additives increased contents of crude protein and nitrogen free extract and decreased crude ash and crude fiber contents.

The aim of this investigation is to produce silage from agricultural wastes using *Lactobacillus casei* and effective microorganisms (EM) as a natural microorganisms inoculation to produce high quality silage with fermentation time reduction.

MATERIALS AND METHODS

Maize, broken bean and wheat bran as concentrated forage and wheat straw as filling material were provided through the Field Crops Research Institute, Agric Res. Center, Giza, Egypt. While, clover as green forage, sugar cane molasses and whey were from the local market. Effective microorganisms (EM) are a solution used as bio-fertilizer in Japan and contains about seventy types of microorganisms belonging to lactic acid bacteria, photosynthetic bacteria, yeast's, fungi and *Actionmycetes* (Higa and Parr, 1994). Whey is sterilized at 1.5-inch pound-1 for 20 min then inoculated with *Lactobacillus casei* bacteria and incubated for 3 days at room temperature to have a bacteria culture grown on whey. A molasses solution (5% W/V) was sterilized then inoculated with EM solution and incubated for 7 days at room temperature to obtain EM culture.

Silage mixture was made from clover, maize, broken bean, wheat bran and wheat straw. One litter bottle with airtight lid were filled with 400 g silage mixture and used as a fermentor to evaluate the fermentation characteristics of the tested silages.

The moisture content of the mixture ranged from 60 to 65 %. Bottles were then, divided into two groups, each group represents an experiment. In the first experiment, silage was inoculated with 2 ml of *Lactobacillus casei* culture, while, in the second experiment, silage was inoculated with 2 ml EM culture. The treatments in both experiments were arranged in complete randomized design with three replicates, each experiment contained bottles with silage only to represent the control treatment. The fermented silages in

each experiment were sampled at different periods of 0, 2, 4, 8 and 12 weeks. After each sampling period, samples were taken to measure pH value, free organic acid contents (Ghoneim *et al.*, 1952) and total microorganisms count. The chemical composition (A.O.A.C., 1990) of the fermented bottles for both experiments was determined after 0 and 12 weeks only. The fermented silage samples at all periods were reviewed for total count. The count was executed using two growing media *viz.*, basal medium (BM) is sterilized at 1.5 inch pound-1 for 15 minutes described by A.P.H.A., (1976) as standard medium and comparable with a new synthesized medium of sodium acetate normal medium (SANM) without sterilized and tap water is used, that developed by (Marcos, 1992). The chemical constituents of both media are shown in Table (1). The total count procedure was done following the serial dilution technique on solid agar.

Table (1): Composition of basal medium and sodium acetate normal medium (SANM)

Materials	Basal medium	SANM
Beef extract	39.0	39.5
Yeast extract	39.0	5.0
Peptone	5.0	5.0
Tryptone	5.0	10.0
Glucose	2.5	10.0
Lactose	2.5	10.0
Tomato juice	100 ml	-
Sodium acetate	-	25.0
Ammonium citrate	-	25.0
Agar	15.2	20.0

RESULTS AND DISCUSSION

Data in Table (2) indicate the decline in pH values with increasing the incubation periods up to 12 week in all tested treatments for both experiments as well as for control. However, at eight weeks a favorite decline in the pH value (4.1) was noticed with EM silage as compared with 4.2 and 4.8 for the silage samples inoculated with *Lactobacillus casei* bacteria and the control treatment, respectively. These findings are in harmony with those obtained by Cai *et al.* (1999).

Table (2): Changes in pH values during ensiling process.

Time in weeks	Control	*Lactobacillus* silage	EM silage
Zero	6.7	6.6	6.6
1	6.1	5.2	5.8
2	5.8	5.0	5.2
4	5.2	4.6	4.4
8	4.8	4.2	4.1
12	4.3	4.1	4.0

The percentage of free volatile acids (as acetic and butyric) and non-volatile acid (as lactic) are recorded in Table (3). A rapid increase in lactic acid production was noticed in the first two week being 1.35 and 1.40% for both *Lactobacillus* and EM silages to reach 2.25 and 2.30% in the eighth week period. However, little amounts of volatile acids (acetic and butyric) were produced during ensiling process in all experiments. The percentage proportion of lactic acid to the total acids amount was over 90% in all treatments during ensiling process. These results are in agreement with Cai *et al.* (1999).

Table (3): Free acids percentage status during the ensiling process.

Free acids	Incubation period (weeks)	Control	*Lactobacillus* silage	EM silage
Volatile acids				
Acetic acid	0	-	-	-
	2	0.10	0.13	0.09
	4	0.11	0.11	0.12
	8	0.13	0.15	0.14
Butyric acid	0	-	-	-
	2	0.03	0.02	0.01
	4	0.04	0.03	0.04
	8	0.08	0.09	0.07
Non volatile acids	0	-	-	-
Lactic acid	2	1.26	1.35	1.40
	4	1.65	1.83	1.97
	8	1.98	2.25	2.30
Lactic / Total acids %	0	-	-	-
	2	90.64	91.21	93.33
	4	91.67	91.96	92.25
	8	90.41	90.36	91.63

Meeske *et al.* (1999) found that the inoculation of silage with lactic acid bacteria resulted in more rapid drop in pH, a higher level of lactic acid and lactic acid bacteria, less protein breakdown and lower numbers of *Enterobacteria*, yeast and mould in comparison with control silage. Moreover, bacteria combined with sugar decreased silage pH, increased lactic acid content and decreased ethanol and ammonia content (Ostrowski, 1999) and gave the lowest content of butyric acid (Kim *et al.*, 1999) and increased the amount of lactic acid to total acids (the ratio of lactic acid to total acid content) Tagawa *et al.* (2001).

The chemical composition (Table 4) before and after ensiling process of the *Lactobacillus* and EM silages didn't change greatly in their nutrient content. It was observed that the dry matter decreased after ensiling with increasing moisture content. Also, the microorganisms utilized total inverted sugar during fermentation and consumed these fractions (Kung *et al.*, 1991). Also, glucose concentration starter reduced the time and increased the amount of lactic acid produced. The fermentation pattern changed during ensiling from hetero and homofermentative (Shiria *et al.*, 2001).

Table (4): Chemical composition of silage at start and after ensiling (on dry weight basis).

Chemical composition	Control		*Lactobacillus* silage		EM silage	
	Start	After ensiling	Start	After ensiling	Start	After ensiling
Moisture	62.80	63.3	63.50	67.43	63.97	69.06
Dry matter	37.20	31.70	36.50	32.57	36.03	30.94
Ash	12.48	12.69	13.85	14.09	13.07	13.17
Crude protein	13.07	12.80	12.77	12.56	13.77	14.77
Crude fibers	22.45	22.76	22.85	23.30	21.27	22.33
Ether extract	1.27	1.96	1.20	1.92	1.35	2.78
Total soluble carbohydrates	50.76	49.79	50.53	49.13	50.54	47.32
Total invert Sugar as glucose	0.87	*Tr.	0.98	Tr.	2.75	Tr.

Tr : Traces

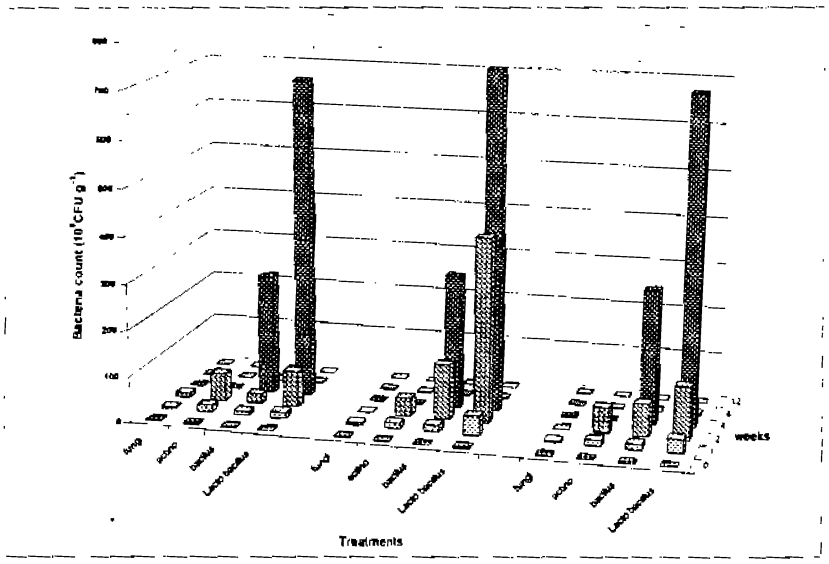
Figures (1 and 2) show growth of fungi, acteinomycetes, *Bacillus* and *Lactobacillus* on basal medium (BM) and sodium acetate normal medium (SANM) after 0, 2, 4, 8 and 12 week during the ensiling process. Results exhibited that up two-week inoculation period both fungi and *Actinomycetes* grown on BM and SANM media did not appear because they ensure a neutral pH values. While, both *Bacillus and Lactobacillus bacteria appeared* on BM and SANM after four week. However, the growth of *Lactobacillus* bacteria on BM was better than it was SANM medium. Also, the growth of *Bacillus* bacteria was less than that of *Lactobacillus* bacteria.

The count of silage microorganisms in all silage treatments on both media is shown in Fig (3). An increase in total counts was noticed at fourth week. However, the total counts in both treatments (*Lactobacillius* and EM *silages) on basal medium (1020 and* 990 $\times 10^6$ CFU g^{-1}) were higher than SANM (410 and 320 $\times$ 10^5 CFU g^{-1}) after four weeks, respectively.

Because, the SANM proved to be of choice for growing different groups of microorganisms that have role in silage preparation, this medium clearly had limited the microorganisms' colonies size and densities.

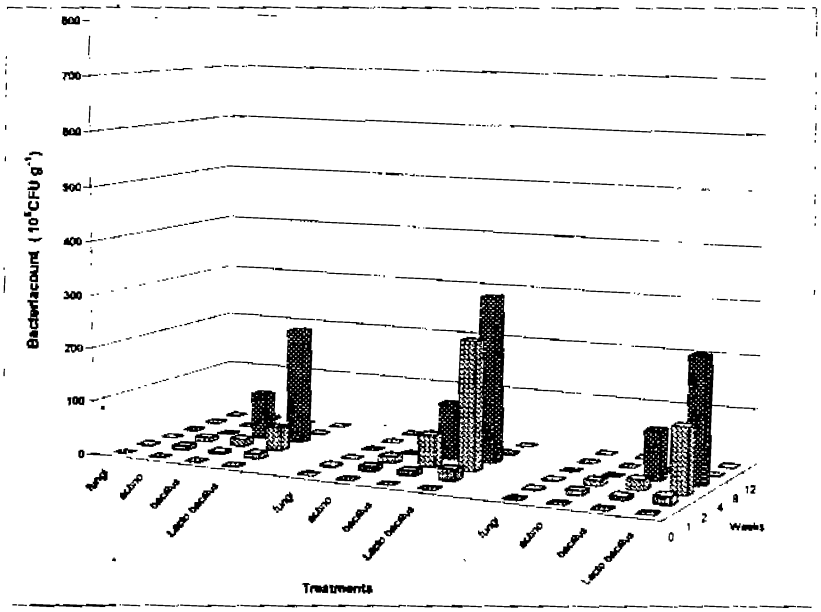
The total counts found with *Lactobacillus* and EM silages inoculated on basal medium were higher than those found on SANM.

These results specified the positive effect of inoculation of silage with *Lactobacillus casei*. Such results are in harmony with Tanaka *et al.* (2000) who declared that the *Lactobacillus* strains effectively improved fermentation quality of the silage. Also, Cai *et al.* (1997) reported that lactobacilli are the *dominant microbial population on forage crops and contribute* to silage fermentation. The lactobacilli play more important role in fermentation processes and efficiently promote lactic acid fermentation for a longer time than do lactic acid producing cocci.



Control silage *Lactobacillus* silage EM silage

Fig. (1): Fractionation and count of different microorganisms in silage grown on basal medium.



Control silage *Lactobacillus* silage EM silage

Fig. (2): Fractionation and count of different microorganisms in silage grown on sodium acetate medium (S.A.N.M).

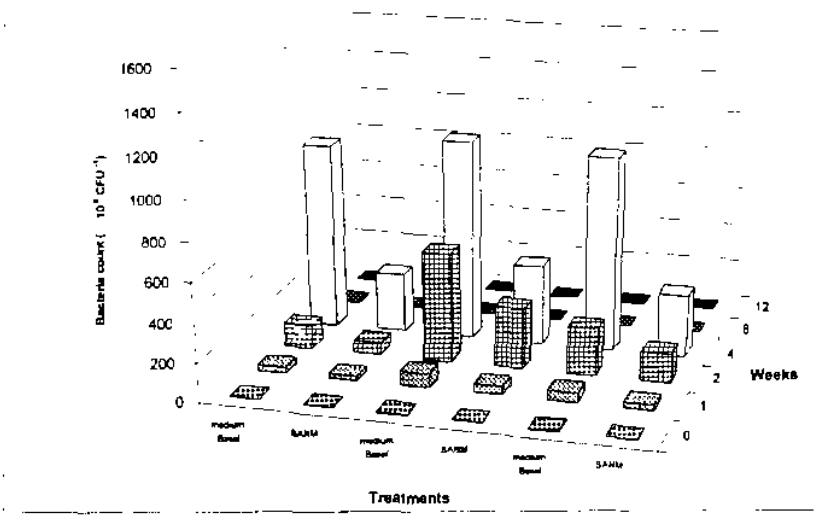


Fig. (3): Total count of different microorganisms in basal medium (BM) and sodium acetate normal medium (S.A.N.M).

Generally, it could be concluded that *Lactobacillus casei* and effective microorganisms (EM) act as activators for whey and molasses which led to obtain *Lactobacillus* culture and EM culture. They were then added to silage for producing high quality silage during fermentation process. Concerning, the microorganisms count grown on basal medium (BM) and sodium acetate standard medium (SASM), it was found that fungi and actinomycetes existed at the beginning had declined after two weeks of ensiling. Also, counts of *Bacillus and Lactobacillus* bacteria continued to increase to reach a peak after four weeks.

It could be recommended that *Lactobacillus* culture and EM culture are good inocula to produce high quality silage during ensiling processes. Moreover, SANM medium, has high content of solids and included few ingredients, therefore it was relatively cheap. Also it was easily prepared with minimum need for microbiological equipment than basal medium.

REFERENCES

A.O.A.C. (1990). Association of Official Analysis Chemists. Official Methods. 15th Ed. Washington, D.C.U.S.A.

A.P.H.A. (1976). American Public Health Association. Compendium of Methods for the Microbiology Examination of Food. Amer. Pub. Health Assoc. Inc. Washington, D.C. USA.

Bolsen, K.K.; C. Lin; B.E. Brent; A.M. Feyerherm; J.E. Urban and W.R. Aimutis (1992). Effect of silage additives on the microbial succession and fermentation process of alfalfa and corn silages J. Dairy Sci., 75: 3066- 3083.

Cai, Y. S.; M.O. Ogawa and K. Kumai (1997). Effect of NaCl – tolerant lactic acid bacteria NaCl and fermentation characteristics and aerobic stability of silage. J. Appl. Microbiol., 83: 307 – 313

Cai, Y.S.; Y. Benno; M. Ogawa; S. Kumai and Y. M. Cai (1999). Effect of lactic acid bacteria isolated from forage crops on fermentation characteristics and aerobic deterioration of silage. J. Dairy Sci., 82(3): 520 – 526.

Ghoneim, A.; M. T. El-Khateb and M. A. Raafat (1952). Quantitative chemical analysis of animal feeds, milk and dairy production. Anglo Egyptian library, Cairo ARE. (in Arabic).

Higa, T. and J.F. Parr (1994). Beneficial and effective microorganisms for a Sustainable Agriculture and Environment. International Nature Farming Research Center, Atami, Japan.

Idler, C.; K . Richter; F. Idler and J. Straksiene (1998). The use of lactic acid bacteria as starter cultures for the conservation for green forages. Field technologies and environment. Proceeding International Conference, Raudonvaris, Lithuania, 24-25- September,

Kent, B. A.; M. J. Arambel; M. D. Winsryg and J.L. Walters (1989). Microbial inoculation of alfalfa haulage : Ensiling characteristics and milk production response when fed to early lactation dairy cows . J. Dairy. Sci., 72: 2325- 2330.

Kim, J.G.; E.S. Chung; W.S. Kang; J.S. Ham; J.D. Kim; S. Seo and J.K. Lee (1999). Effect of the additives on the quality of alfalfa silage. J. of the Korean Society of Grassland Sci., 19(2): 115 – 120

Kung, L.; R.S. Tung and K. Maciorowski (1991). Effect of a microbial inoculant (Ecesyl TM) and / or a glycopeptide antibiotic (vancomycin) on fermentation and aerobic stability of wilted alfalfa silage. Animal Feed Sci. and Technol., 35: 37- 48.

Meeske, R., H.M. Basson and C. W. Cruyagen (1999). The effect of lactic acid bacterial inoculant with enzymes on the fermentation dynamics, intake and digestibility of Digitariaeriantha silage. Animal Feed Sci. and Technol., 81(3-4): 237- 248.

Marcos, Mona M. (1992). Biochemical studies on some agricultural and agroindustrial wastes. M. Sc. Thesis, Cairo Univ. Fac. of Agric.

Ostrowski, R. (1999). Ensiling fresh lucerne supplemented with lactic acid, sugar and mass containing lactic fermentation bacteria. Roczniki Naukowa Zoo Techniki, 26(1): 199- 207.

Shirai, K.; I. Guerrero; S. Huerta; G. Saucedo; A Castillo; R.O. Gonzalez and R.O. Hall G.M. (2001). Effect of initial glucose concentration and inoculation level of lactic acid bacteria in shrimp waste ensilation. Enzyme and Microl. Technol., 48(4-5): 446 – 452

Singh, A.; J.C. Edward; S. Mor and K. Singh (1996). Chemical and microbiological changes during ensiling of lucerne with lactic acid bacteria and molasses. Indian J. Dairy Sci., 49(9): 593- 598.

Tagawa, S.; T. Okajima and M. Ito (2001). Effects of cellulase, lactic acid bacteria and wilting on the fermentation quality of reed canary grass silages. Grassland Sci., 47(2): 157- 162.

Tanaka,O.; K. Mori and S.Ohmomo (2000). Effect of inoculation with *lactobacillus curvatus* on ensiling. Grassland Sci., 46(2): 148- 152.

Weinberg, Z. G.; G. Ashbell; Y. Hen and Azrieli, A. (1993). The effect of applying lactic acid bacteria ensiling on the aerobic stability of silages. J. Appl. Bacteriol., 75: 512- 518.

تأثير الشرش ومركب الميكروبات الفعالة على الصفات الكيماوية والميكروبية للسيلاج

منى ميخائيل مرقص – خالد عبد الفتاح عبد القوى– جمال جورج أنطون

قسم بحوث الميكروبيولوجى – معهد بحوث الأراضى والمياه والبيئة – مركز البحوث الزراعية – جيزة – مصر.

أجريت تجربتين معمليتين لإنتاج خلطة سيلاج كعليقة متزنة متكاملة للحيوان الزراعى وذلك من حيث الرطوبة والبروتين ، وتتكون هذه العليقة من (البرسيم كعلف أخضر – الذرة الشامية وكسر الفول وردة القمح كعلف مركز – تبن القمح كمادة مالئة. وقد لقحت العليقة فى التجربة الأولى بـ ٢ % من بكتريا اللاكتوباسيلس كيزى المنماة على شرش اللبن وفى التجربة الثانية لقحت العليقة بـ ٢ % من مركب الميكروبات الفعالة (EM) المنشطة بـ ٥ % مولاس وذلك لإنتاج حمض اللاكتيك الذى يعمل على خفض الـ pH بسرعة ويساعد على تنشيط الأحياء الدقيقة والمؤثرة فى إنتاج وإنضاج السيلاج فى سبيل الاستفادة من خامات زراعية عديمة القيمة الاقتصادية ، ويمكن تلخيص النتائج فيما يلى ..

١- كانت نسبة كل من حمض البيوتريك والاستيك ضئيلة بالنسبة لحامض اللاكتيك التى تصل نسبته إلى ٩٢ % من نسبة الأحماض العضوية الطيارة الكلية فى كلتا التجربتين .

٢- عند مقارنة التركيب الكيميائى للعليقة المتكاملة فى بداية التجربة ونهايتها أوضحت النتائج عدم تواجد للسكريات فى النهاية و يرجع ذلك إلى أن ميكروبات السيلاج قد استهلكت السكريات أثناء عملية السيلجة .

٣- إن إجراء عملية العد الكلى لكل من الفطر والاكتينومسيتس والباسيلس واللاكتوباسيلس (ميكروبات السيلاج) النامية على البيئة الأساسية الخاصة بالسيلاج Basal أوضح ظهور هذه الميكروبات والتى سرعان ما تنتشر بكتريا الباسيلس والفطريات و يغطى نمو الفطريات معظم مساحة الطبق النامية فيه الميكروبات بعد أربع أسابيع عادة حيث تحجب الرؤية فلا يمكن تحديد مجموعة الأحياء الدقيقة وكثافتها و التى عن طريقها وبمقارنتها بالاحماض ينم معرفة درجة نضج السيلاج .

٤- ولذلك تم استخدام البيئة الجديدة والتى سميت ببيئة خلات الصوديوم العادية (SANM) حيث أن هذه البيئة لا تعقم وتنمى عليها مجموعة الأحياء الدقيقة الأساسية والتى تسود فى السيلاج فوجد أنها تحد من نمو وانتشار بكتريا الباسيلس والفطريات حيث تكون نمواتها ضعيفة بالدرجة التى تمكنا من الاستدلال على تواجد باقى الأحياء الدقيقة المرغوبة فى السيلاج وهى اللاكتوباسيلس .

٥- يمكن استخدام البيئة المستنبطة فى المعامل الغير مجهزة ميكربيولوجيا حيث أنها لا تعقم بل يتم تجانس مكوناتها بطريقة سهلة مثل التسخين والغلى على حمام مائى وذلك خلاف البيئة القياسية والتى يستلزم تعقيمها قبل استخدامها .