ENSILING SWEET SORGHUM AND MAIZE STALKS AS FEEDSTOCK FOR RENEWABLE ENERGY PRODUCTION

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Abstract: *Laboratory scale experiments were conducted consisting of silages of chopped sweet sorghum, sweet sorghum bagasse, combinations of sweet sorghum with maize stalks, treated and untreated with LAB inoculant and ethanol as preservation additives. Twelve types of silages were obtained by combination of types of biomass and additives. The effect of these additives and biomass combinations on the qualities and composition of the resulted silage was examined. Silages were analyzed to assess the preservation of nutritive value and quality of the ensiled biomass. The lowest pH values (3.3) where in silages of sorghum bagasse and sorghum bagasse with ethanol. The highest pH values (6-6.3) where in silages of maize stalks, where the highest number of contaminants where found as well. Soluble sugars where preserved in high concentration in ensiled biomass treated with ethanol: over 91% of the water soluble sugars was found in sorghum ensiled with ethanol, and less than 57% in sorghum ensiled with lactic bacteria. The ethanol additive inhibited as well the breakdown of silage protein and the multiplication of contaminants (1.9 log_{10} cfu g^{-1} in ethanol treated sorghum comparing with 3.2 log_{10} cfu g^{-1} in control sorghum silage without additives). Preservation parameters of maize stalks are improved by adding fresh sweet sorghum to dry maize stalks. Lactic bacteria inoculants ameliorate the preservation parameters in sweet sorghum and mixed biomass, but leads to consumption of sugars. Using ethanol as silage additive the biomass is well preserved, the growth of contaminants, soluble sugars and protein losses are inhibited. The loss in dry matter, crud fats, crude cellulose was not significant in all silages. A small portion of ethanol produced by fermenting sugar from sweet sorghum can be used to preserve the feedstock biomass. Sweet sorghum can be used as well to improve preservation qualities of corn stalks to be used as biogas feedstock.*

Key words*: sweet sorghum, silage, ethanol, inoculant.*

INTRODUCTION

Agricultural biomass is more and more regarded as an important feedstock for renewable energy production. Researches pointed out several types of plants as having great potential as energy crops. For example, the majority of farm-based biogas plants in Europe are operated with energy crops as feedstock, especially corn silage (Weiland, 2010). The ethanol industry relies now mostly on grains, but research dedicated to the development of second generation ethanol production technologies, indicate C4 families of plants as future source of biomass for biofuels production. Sweet sorghum is a C4 plant with photosynthetic activity and drought tolerance therefore it can be cultivated in almost all temperate and tropical climate areas (Gnansounou, 2005). This crop can be used as forage or the sweet juice is extracted and used as feedstock in industrial processes such as sweetener production or fermentations. Production of ethanol as biofuel from sorghum juice has great perspective, even gaseous biofuels as hydrogen has been reported (Antonopoulou, 2011). Remaining solids after juice extraction – bagasse – can have a wide range of applications, such as paper industry (Xilin et al, 1997), composting (Bernal et al,

1996), combustion for energy production, or anaerobic fermentation for biogas production (Picco et al, 2012). Due to high sugar content, sweet sorghum tends to be degraded by microorganisms after harvesting therefore several preservation techniques have been developed. One technique consists in extracting sweet juice from the freshly harvested biomass, evaporating the juice to around 60% sugar containing syrup and drying or ensiling by lactic fermentation of resulting bagasse. Other techniques consist of ensiling the freshly harvested sweet sorghum in presence of formic acid, propionic acid (Rohowsky, 2011, Mills, 2002), or enzyme-assisted ensiling (Schmidt, et al 1997). In case of using sorghum as feedstock for biogas production, ensiling of the fresh biomass based on lactic acid fermentation is the most appropriate preservation technique. It was shown that ensiled biomass by lactic fermentation produce more biogas then freshly harvested biomass (Weiland 2010, Herrman et al., 2011) and the use of silage additives affect the methanogenesis, since methane production depends on chemical composition of organic material (Amon, 2007). In case of using sorghum as feedstock for ethanol production, lactic acid bacteria (LAB) will consume glucose during ensiling by lactic fermentation of the biomass, leading to very low ethanol yields. Ethanol, the final product of the industrial technology in this case can be used as preservative to conserve glucose in the biomass until processing. During processing, the ethanol can be recovered, so only a small part of the final product will be used to preserve feedstock year-around. However, at present the effect of ethanol addition on silage fermentation is not clear.

In the study presented below, lab-scale experiments were conducted consisting of silages of chopped sweet sorghum, sweet sorghum bagasse, combinations of sweet sorghum with maize stalks, treated and untreated with LAB inoculant and ethanol as preservation additives. The effect of these additives and biomass combinations on the qualities and composition of the resulted silage was examined.

MATERIALS AND METHODS

Sorghum (*Sorghum bicolor*) and maize (*Zea mays*) obtained from two different experimental sites in Timiș county (western Romania) were used. Maize whole cobs with grains were harvested separately and the remaining stems and leaves were chopped to 1 cm theoretical length of cut using a forage harvester and used in this experiment for ensiling. Sweet sorghum was harvested as whole crop at the early milk stage of maturity, chopped to 1 cm theoretical length of cut using a forage harvester. Sweet juice was extracted from sorghum using a *Pellet Mills* oil press of 500 kg raw material/hour capacity, the resulted bagasse was ensiled in this study.

Silages were prepared in 1-l plastic bags, air was removed using a vacuum pump and the bags were air-tight sealed. Silages were preserved at 20^{o}C until opening. The biomass was treated with additives before ensiling, except untreated controls.

Treatments with LAB inoculant (*Lactobacillus plantarum* and *Lactobacillus acidophilus)* were applied to achieve a final concentration of 2 - 5 x 10^5 CFU (colony forming units) g^{-1}fresh material. Lactic bacteria strains from our own culture collection were used. The strains were tested before in lab-scale and farm-scale silages (Vintila et al, 2006, 2010).

Treatment with ethanol (8 g 95% ethanol solution/ 100 g^{-1} fresh material) was applied only for sorghum whole plant and sorghum bagasse silages, as this type of biomass can be used as feedstock in ethanol industry (juice for first generation ethanol and bagasse for second generation, lignocellulosic, ethanol production).

Twelve types of silages were obtained by combination of types of biomass and additives: (1) sorghum without additives; (2) sorghum with LAB, (3) sorghum with ethanol, (4) sorghum bagasse without additives, (5) sorghum bagasse with LAB, (6) sorghum bagasse with ethanol, (7) sorghum and maize stalks without additives, (8) sorghum and maize stalks with LAB, (9) sorghum bagasse and maize stalks without additives, (10) sorghum bagasse and maize stalks with LAB, (11) maize stalks without additives, (12) maize stalks with LAB.

To determine the nutritive value of the sorghum used in our experiments, we applied the standard methods according to the WEENDE scheme, respectively:

-***Dry matter (DM %)*** – by oven-drying at 105^{o}C;

-***Crude protein (CP %)*** – by Kjeldahl method;

-***Crude fat (CF %)*** – by Soxhlet method;

-***Crude cellulose (CC %)*** – by Van Soest method;

-***Ash (%)*** – in muffle furnace at 600^{o}C

Further analyses combined with the nutritive indicators mentioned above were performed for the fresh and ensiled biomass. The following analyses were performed on fresh and ensiled biomass:

pH was measured by using a Consort C932 pH meter. The silage samples were prepared by shaking a small amount of silage (5 g silage) with 45 ml of distilled water for 30 minutes.

Colony forming units (CFU), to determine the total number of microorganisms present in the silage samples. From the dilution series, nutrient agar plates were prepared in triplicates and incubated for 2 days at 37^{o}C.

Titratable acidity is defined as the milligrams equivalent (meq/g) of base (0.1 M NaOH) necessary to titrate the pH of a silage sample to 6.5. It measures the total of all hydrogen ions neutralized in order to bring pH to 6.5 and would account for the strength of the acids present. Titratable acidity is highly correlated with total acid levels in sorghum silages because of their low buffering capacity but not as highly correlated with total acid levels in silages with high buffering capacity (as legumes or other high-protein containing biomass). A high number indicates a more extensive fermentation, more acid production, and more stable silage for storage and during feed-out. Too high a level is a concern due to extensive conversion of organic matter to acids (reduced energy) and the challenges of keeping the pH value from dropping too low in case of using the ensiled biomass in biogas fermentation.

Soluble sugars (SS): were determined by suspending the biomass samples in distilled water (10 g / 100 ml) and agitating them for 30 min. The suspensions were centrifuged and the supernatant was analyzed to determine the concentration of reducing sugars dissolved in water from silage samples. To determine the concentration of reducing sugars in water extracts we applied the DNS method [14].

RESULTS AND DISCUSSIONS

Characteristics of the biomass before ensiling are presented in table 1. After 100 days of storage, the silages were opened and analyzed regarding the nutritive and hygienic qualities. Sensorial characteristics are important indicators of silage quality. Immediately after opening, we analyzed the main characteristics which indicate the quality of silage: texture, smell and color.

Table 1

Characteristics of biomass before ensilage

Biomass	Nutritional and quality indicators							
	pH	log_{10} cfu g^{-1}	SS $mg*g^{-1}$	DM %	CP %	CF %	CC %	Ash %
Sorghum	6.9	1.35	65.5	23.5	2.58	2.78	9.05	3.35
Sorghum bagasse	6.2	1.45	50.3	27.7	3.54	2.38	10.48	2.52
Corn stalks	6.4	1.60	38.2	81.7	3.10	1.89	42.65	5.54

All batches of silages maintained their texture, the plant particles maintained their shape and none of the silages has softened (softening is the result of biomass hydrolysis by undesirable microorganisms). Regarding the smell of the batches, there were differences, for example: in sorghum without additives and in sorghum with LAB, the smell was sour (of pickled cucumbers), not very intense; as in sorghum with ethanol, the smell is more pleasant, slightly sour – aromatic, sweet, pomace-like, distilled grains. Batches containing sorghum bagasse – with or without additives – had the most pleasant scent, of freshly leavened bread, tasty, without the flavor of pickled cucumbers, as in whole sorghum silages. Good smell characteristics were found in combinations of sorghum and maize stalks. In maize stalks without additives, the smell of pickles was very weak, while in the maize stalks with LAB silage we found moldy smell. This last batch was infected with fungi – we found a thin layer of white mycelium and rare dark-brown spots. Regarding the color of silages, the fresh green of sorghum and sorghum bagasse has passed to dark olive green in silages without additives and in silages with LAB and to light olive green in silages with ethanol. The maize stalks maintained their original yellow color.

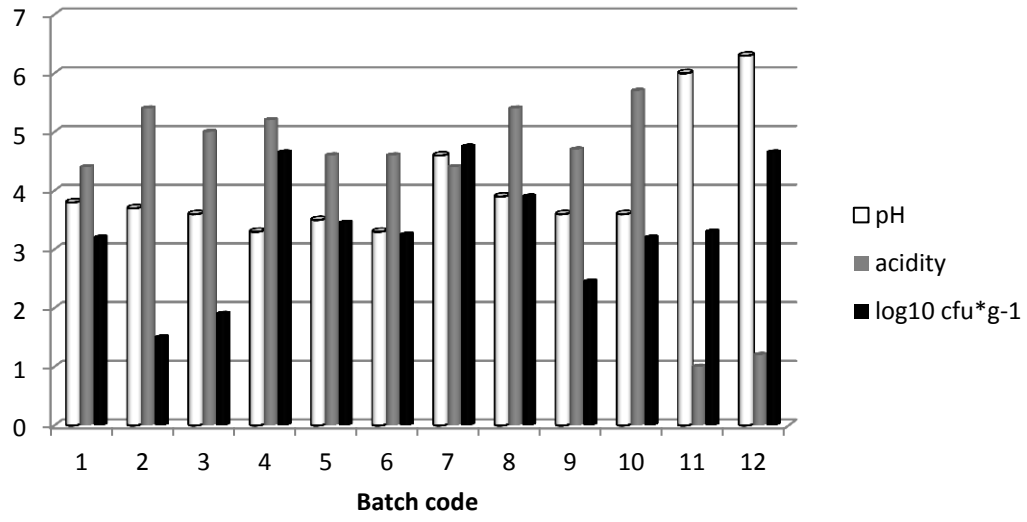

Figure 1: Preservation features of ensiled biomass

Parameters such as pH, acidity and number of microorganisms in silage are the main features that indicate the preservation qualities of the ensiled biomass. High pH values found in maize stalk (batches 11 and 12) indicates that the fermentation did not occur in these silages, even when lactic bacteria were added to dry maize stalks (batch 12). On the contrary, this practice leads to proliferation of contaminating microorganisms in maize stalks (probably due to additional moisture and nutrients added together with the LAB liquid cultures). Addition of LAB to sorghum and sorghum bagasse (batches 2 and 5) improves the preservation qualities of the ensiled biomass (lower pH, higher acidity and less contaminating microorganisms). Adding ethanol as preservation additive (batches 3 and 6), the effect is similar in terms of pH, acidity and contaminants with batches 2 and 5. The difference in these cases consists in preservation of sugar content (see tables 1 and 2 – *S.S.*, soluble sugars). The initial concentration of water soluble sugars in sorghum is 65.5 $mg*g^{-1}$. In sorghum preserved in presence of ethanol, 91.25% from initial content in soluble sugars was found after 100 days of storage. In case of sorghum inoculated with LAB and in sorghum silages without additives, only 56.64% and 65.02% respectively of the initial sugar content was preserved. In sorghum bagasse, our data indicate similar effects: 97.15% from initial content in soluble sugars was found after 100 days of storage in presence of ethanol, while in sorghum bagasse with LAB, only 78.42%, and in silages of sorghum bagasse without additives, 87.79% of initial sugar content was found.

Table 2

Characteristics of silages after 100 days of storage

Batch no.	Content	pH	acidity	log_{10} cfu g^{-1}	SS $mg*g^{-1}$	DM %	CP %	CF %	CC %	Ash %
1	Sorghum	3.8	4,4	3.20	42.59	23.1	2.05	2.62	11.56	3.54
2	Sorghum + LAB	3.7	5,4	1.5	37.10	22.5	2.54	2.93	10.91	3.41
3	Sorghum + ethanol	3.6	5	1.9	59.86	23.8	2.60	2.05	9.20	3.47
4	Sorghum bagasse	3.3	5,2	4.65	44.16	27.5	3.06	2.32	10.82	2.55
5	Sorghum bagasse + LAB	3.5	4,6	3.45	39.45	28.4	2.90	2.47	14.14	3.54
6	Sorghum bagasse + ethanol	3.3	4,6	3.25	48.87	26.8	3.53	2.30	13.64	2.77
7	Sorghum + corn stalks	4.6	4,4	4.75	46.52	39.6	3.13	2.30	15.00	6.10
8	Sorghum + corn stalks + LAB	3.9	5,4	3.9	32.39	38.4	3.51	2.44	15.33	3.91
9	Sorghum bagasse + corn stalks	3.6	4,7	2.45	33.18	35.9	2.80	1.92	19.58	4.21
10	Sorghum bagasse + corn stalks + LAB	3.5	5,7	3.20	34.75	38.1	3.05	2.11	15.72	4.05
11	Corn stalks	6	1	3.30	36.22	82.5	3.08	1.82	44.81	6.10
12	Corn stalks + LAB	6.3	1,2	3.65	33.39	76.8	3.49	1.97	40.75	5.54

Due to the low moisture content in corn stalks, ensiling this type of dry biomass is difficult. This residual lignocellulosic biomass can be used to produce biofuels (the biogas production potential of corn stalks is around 380-460 liters of biogas / kg organic dry mater [15]).

Consequently, in this work we searched to increase the moisture and fermentable sugars contents in silages containing corn stalks by adding whole fresh sorghum biomass and sorghum bagasse (batches 7, 8, 9, 10). Data in table 2 indicates that 1:1 mixtures of corn stalks and the two types of sorghum biomass lead to lactic fermentation and ensiling of the biomass. Acidity, pH and CFU values in all four batches indicate occurrence of fermentation and good preservation of the biomass. Addition of LAB leads to higher acidity and lower pH, especially in silage containing mixture of whole sorghum biomass and corn stalks. These results recommend addition of lactic bacteria inoculants in this type of silages.

Analyzing the other indicators followed in our research (crude protein, crude fat, crude cellulose and ash), data obtained and presented in table 2, show very low variations comparatively with the values found in the biomass before ensilage. This demonstrates that in all silages the nutritive indicators have been preserved, even in dry corn stalks (batches 11 and 12). In these last silages, the preservation occurred later due to the low moisture and lack of oxygen by vacuum removal of air. But, these conditions can't be realized in large scale for preservation of corn stalks. However, an important finding in this work we consider the inhibition of protein losses in sorghum silages treated with ethanol (batches 3 and 6) as compared to untreated silages (batches 1 and 4) and silages inoculated with LAB (batches 2 and 5). Although the differences are not high, it is observed that in both silages treated with ethanol, the protein concentration is higher than in silages without ethanol.

CONCLUSIONS

Addition of lactic bacteria in sorghum silages and mixed biomass ameliorate the preservation parameters, but leads to higher losses of soluble sugars.

Soluble sugars where preserved in high concentration in ensiled biomass treated with ethanol. The ethanol additive inhibited the breakdown of silage protein as well as the multiplication of contaminants. Using ethanol as silage additive preserves the biomass, reduces the growth of contaminants and inhibits soluble sugars and protein losses.

The loss in dry matter, crud fats, crud cellulose was not significant in any of the silages take into study.

A small portion of ethanol produced by fermenting sugar from sweet sorghum in biorefineries can be used to preserve the feedstock biomass to be used in that biorefinery.

Preservation parameters of maize stalks are improved by adding fresh sweet sorghum or sorghum bagasse to dry maize stalks. Sweet sorghum can be used as well to improve preservation qualities of corn stalks to be used as biogas feedstock.

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