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THE EFFECT OF MICROBIAL SILAGE ADDITIVES ON BIOGAS PRODUCTION FROM PERENNIAL ENERGY GRASSES

Summary

The effect of two different microbial additives on perennial energy grasses – switchgrass and big bluestem - was tested regarding the general silage quality and the biogas production from ensiled biomass. Biomass was harvested at the end of June 2013 and ensilaged with or without silage additives comprising different lactic acid bacteria strains. Methane fermentation of experimental silages was carried out at 39°C for at least 30 days. During ensiling process the content of structural polisaccharides was reduced. The effect of additives on the chemical composition of perennial grass silages was varied depending on the species of grass. Regardless of the additives, in all silages higher amount of acetic acid (methane precursor) than lactic acid was detected. The highest biogas production was obtained from switchgrass silages treated with 11CH4 additive and big bluestem silages treated with Lactosil additive. The increase was compared to the lowest lignine content in these silages.
***Key words**: perennial grasses, biogas, silages, switchgrass, big bluestem, silage additive*

WPŁYW MIKROBIOLOGICZNYCH DODATKÓW KISZONKARSKICH NA PRODUKCJĘ BIOGAZU Z TRAW WIELOLETNICH

Streszczenie

Badano wpływ mikrobiologicznych dodatków kiszonkarskich na jakość kiszonek i produkcję biogazu z traw wieloletnich – prosa rózgowatego i palczatki Gerarda. Biomasa zebrana została pod koniec czerwca 2013 r. i zakiszona bez oraz z dodatkiem dwóch preparatów zawierających różne szczepy bakterii fermentacji mlekowej. Fermentację metanową kiszonek przeprowadzono w temperaturze 39°C przez co najmniej 30 dni. W wyniku kiszenia zmniejszyła się w biomasie zawartość polisacharydów strukturalnych. Wpływ dodatków na skład chemiczny kiszonek był zróżnicowany w zależności od gatunku trawy. Niezależnie od dodatków we wszystkich kiszonkach większa była zawartość kwasu octowego (prekursora metanu) niż mlekowego. Istotnie więcej biogazu otrzymano z kiszonek z prosa z dodatkiem preparatu 11CH4 oraz z kiszonek z palczatki sporządzonych z dodatkiem preparatu Lactosil, czyli z kiszonek, w których oznaczono mniejszą zawartość ligniny w stosunku do kontroli.
***Słowa kluczowe**: trawy wieloletnie, biogaz, kiszonki, proso rózgowate, palczatka Gerarda, dodatki kiszonkarskie*

1. Introduction

Biogas is a versatile renewable energy source produced through anaerobic digestion of organic substances of plant and animal origin. It consists of methane and carbon dioxide and can be used for replacement of fossil fuels in both heat and power generation.

It is foreseen that in energy production the second generation raw materials (non-edible crops) will play the most important role, because of the fact, that such materials do not compete for food and feed production [9]. In this context, biogas from lignocellulosic raw materials, such as introduced perennial energy grasses, will be of vital importance in the future. However, this kind of materials have to be pretreated in order to increase the degradation of lignocellulosic complex [12]. Chemical (alkaline or enzymatic hydrolysis, oxidation, ozonation) or physical (thermal, mechanical shredding, microwaves, ultrasounds etc.) methods are the most commonly used for biomass pretreatment as well as biological methods [4, 13, 15]. Biological methods of biomass pretreatment are associated with the use of microorganisms, mainly fungi, to break down the lignocellulosic fraction [1]. However, these kinds of methods have a large limitation in industrial application because the hydrolysis of lignocellulose complex by fungi lasts too long and is less efficient than chemical or physical methods of biomass degradation [4].

After harvesting biomass has to be preserved. Ensiling method is the most commonly used for biomass preservation. Ensiled plants can be stored all year round which provides the possibility of continuous delivery of ensiled substrate to the fermentation chamber in agricultural biogas plant [5]. The quality of ensiled plants is of great importance for methane fermentation. The use of spoiled silages reduces biogas production [8]. The quality of silages could be improved by silage additives, such as microbiological inoculants containing lactic acid bacteria (LAB) strains [16]. Ensiling is a complex process which can also be used as the method of biomass pretreatment, where different processes occur, different products of lactic fermentation are formed. However, the relationship between ensilage procedures, as well as silage additives and biogas production during anaerobic digestion is not well known.

2. The aim of the study

The effect of different microbial silage additives on perennial energy grasses – switchgrass and big bluestem - was tested regarding the general silage quality and the biogas production from ensiled biomass.

3. Materials and methods

3.1. Substrate

Perennial grasses: switchgrass (*Panicum virgatum L.*, var. Dacotah) and big bluestem (*Andropogon gerardii L.*, var. Bison*)* were obtained from the experimental field located in the central Poland (Skierniewice) belonging to Faculty of Agriculture and Biology Warsaw University of Life Sciences and harvested at the end of June 2013. Grasses were freshly chopped to a 2 cm-sized particles.

3.2. Silage preparation

Silages were prepared in 30 L plastic barrels. Each barrel contained around 10 kg of chopped material. Two different biological additives were used to enhance the ensilation: Lactosil containing homo- and heterofermentative lactic acid bacteria strains (*Lactobacillus plantarum* KKP 593/p, *Lactobacillus plantarum* C KKP 788/p, *Lactobacillus buchneri* KKP 907/p) and 11CH4 (Pioneer) containing *Lactobacillus buchneri* LN 40177. The appropriate amounts of additives were dissolved in sterile tap water and applied into the biomass using a hand sprayer. The number of bacteria added was around 2,0 x 10^8 cfu·kg^{-1} biomass. Barrels were filled completely and tightly closed, thus, no headspace was left. Barrels were stored at room temperature for 3 months. Control silages were also prepared without bacteria addition.

3.3. Anaerobic batch tests

Biogas production and quality from both freshly harvested and ensiled plant material were analyzed in batch anaerobic digestion tests. Tests were performed in 1,3 L glass bottles using 5 g of substrate and 100 ml of inoculum (content of secondary fermenter from agricultural biogas plant). Bottles were closed with measuring heads of OxiTop® Control (WTW) pressure monitoring system. Assays with inoculum but without substrate were incubated as controls. Finally, bottles were flushed with N_2 to remove O_2 from the headspace. Anaerobic digestion was conducted at 39°C for at least 30 days. All tests were performed in triplets. During methane fermentation increasing pressure of biogas produced was measured every day. The data were wirelessly transmitted (infrared) to the OxiTop® OC 110 controller and then transferred to a PC and processed in Excel program. Value of the gas pressure was converted into the amount of biogas (in moles) using the ideal gas equation:

$$pV = nRT, \qquad (1)$$

p – pressure [Pa]; V – reactor capacity [m^3]; T – temperature [K]; R – universal gas constant 8,31 [J $(mol\ K)^{-1}$]; n – number of moles.

The amount of biogas was then converted into the volume of biogas expressed in cubic meters and referring to normal conditions: the pressure 1013.25 hPa and temperature 0°C (Nm^3).

Biogas composition was analyzed by gas analyzer (COMBIMASS®GA-m).

3.4. Analytical methods

Dry matter content (DM) was determined by drying the material at 105°C to constant weight of the sample. Organic dry matter (ODM) was measured by determining the ash content of dry samples in a muffle furnace at 550°C. The sward and silages samples were grounded and then chemical components were analyzed (NIRS method with a NIRFlex N-500 using appropriate presets created by INGOT®). pH of silages was determined by potentiometric method. The content of organic acids was determined by enzymatic assays using UV tests (r-Biopharm).

The results were subject to statistical analysis using Statistica 12.0 software (Statsoft, Poland). Statistical analyses of repeated measurements were performed with one-way ANOVA followed by Tukey's test. P-values of $p \leq 0.05$ were considered to be statistically significant.

4. Results and discussion

Grasses were analyzed for several parameters to characterize the biomass (tab. 1).

Energy grasses, such as switchgrass or big bluestem, originated from North America, characterized by high content of cellulose and hemicellulose (tab. 1). In Europe these grasses are intended for combustion and usually harvested at the end of vegetation period [10]. In this study grasses were harvested in the middle of vegetation period in order to obtain dry matter content appropriate for ensiling.

Table 1. Characterisation of fresh and ensiled biomass
Tab. 1. Charakterystyka świeżej i zakiszonej biomasy

Species	Material	DM [%]	ODM [% DM]	Protein [% DM]	Mono sugars [% DM]	Cellulose[1] [% DM]	Hemi-cellulose[2] [% DM]	Lignine[3] [% DM]	Dry matter digestibility[4] [%]
Switch-grass	Fresh	35.2	93.4	9.3	5.1	35.3	23.2	5.1	57.4
	Control	27.6^a	92.5	10.5	4.9^a	26.2^a	19.8^a	4.5^a	65.0
	Lactosil	27.6^a	92.9	10.1	4.0^{ab}	26.5^a	13.2^b	4.6^a	64.7
	11CH4	26.9^a	92.6	10.0	7.1^c	26.4^a	6.4^c	3.1^b	62.0
Big bluestem	Fresh	28.7	93.5	7.5	4.9	37.0	23.1	5.4	55.8
	Control	20.6^a	90.9	9.7	4.8^a	26.1^a	10.1^a	4.6^a	65.0
	Lactosil	19.9^a	91.6	10.2	4.0^{ab}	26.3^a	11.0^b	3.6^b	64.1
	11CH4	20.0^a	91.8	10.4	6.7^c	26.9^a	12.9^{ab}	4.8^c	64,0

[1] calculated as difference between the content of ADF and ADL fibres; [2] calculated as difference between the content of NDF and ADF fibres; [3] as the content of ADL fibres; [4] calculated from the formula 88.9-0.779 x ADF
a, b etc. – statistically different groups

Source: own work / Źródło: opracowanie własne

As it can be seen, during ensiling dry matter content decreased but between silages dry matter content was comparable. The most important fact was that ensiling process increased dry matter digestibility which is connected with decreasing in concentration of lignocellulose complex. In case of silages from switchgrass the lowest content of hemicellulose and lignin was found in silages treated with 11CH4 additive. In case of big bluestem the lowest content of lignin was found in silages treated with Lactosil additive. In silages treated with 11CH4 additive the content of mono sugars was the highest. Lactosil and 11CH4 additives did not influence on cellulose content compared to control silages (tab. 1).

Silages for biogas production should exhibit quality and digestibility similar to silages intended for feeding [8]. Anaerobic digestibility of lignocellulosic biomass is hard to achieve because of its structural characteristics, specially protection cellulose by lignin, which contributes to the recalcitrance of lignocellulosic biomass to hydrolysis (the first step of methane fermentation) [12]. Lactic acid bacteria have the ability to hydrolyze structural polysaccharides [11]. For that reason, the use of microbial silage additives is intended not only to support ensiling process by stimulating lactic acid production, but also to increase the degree of structural polysaccharides hydrolysis.

To determine the activity of lactic acid bacteria, the samples were analyzed for organic acids concentration (tab. 2).

Table 2. Content of organic acids in silages
Tab. 2. Zawartość kwasów organicznych w kiszonkach

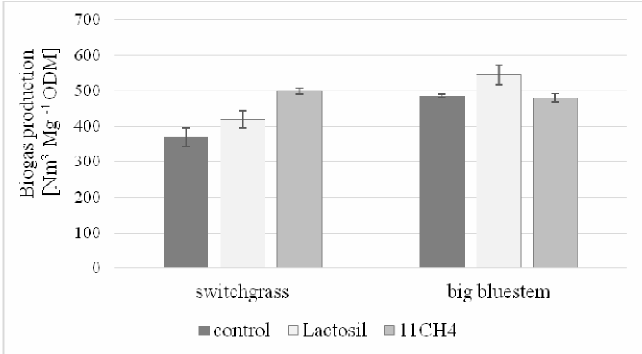
Species	Silage	pH	Organic acids [g kg^{-1} DM]	
			Lactic acid	Acetic acid
Switchgrass	Control	5.4	4.0	23.0
	Lactosil	5.3	6.1	64.7
	11CH4	5.5	10.9	19.6
Big bluestem	Control	4.9	56.0	69.2
	Lactosil	4.9	32.3	31.2
	11CH4	4.9	2.5	24.2

Source: own work / Źródło: opracowanie własne

Almost all silages are characterized by higher concentration of acetic acid than lactic acid. Butyric acid was not detected (data not shown) that means spoilage processes caused by *Clostridium* bacteria did not occur during ensiling. In case of switchgrass silages Lactosil additive influenced on increasing concentration of lactic and acetic compared to control silages. 11CH4 additive increased only lactic acid concentration compared to control silages. In case of big bluestem additives did not influence on increasing concentration of organic acids in silages. What is more, content of acids was lower in treated silages compared to control silages (tab. 2).

Acetic acid is a substrate from which methane is synthesized by methanogen bacteria [7]. High concentration of acetic acid in silages intended for biogas production is claimed to be a positive effect of ensiling process, while it might even enhance methane formation [8]. Higher concentration of acetic than lactic acid in experimental silages indicates that heterolactic fermentation prevailed. It might have been a result of activity of heterofermentative LAB strains included in silage additives. Heterofermentative lactic acid bacteria, especially *Lactobacillus buchneri*, ferment pentose (xylose, arabinose) released from hemicellulose (e.g. xylan) and then acetic acid is produced [2]. Nevertheless, in silages prepared with 11CH4 additive (*Lactobacillus buchneri* LN 40177) the concentration of acetic acid was lower than in control silages. As in [6] studies, the lack of the effect of the use of microbiological silage additives was also observed. This was explained that the bacteria added to biomass probably did not dominate the epiphytic microflora found on the ensiling plants.

The results of anaerobic digestion of experimental silages are presented in fig. 1.



Source: own work / Źródło: opracowanie własne

Fig. 1. Biogas production from grass silages depending on silage additive
Rys. 1. Produkcja biogazu z kiszonek z traw w zależności od dodatku kiszonkarskiego

In case of switchgrass biogas production from silages treated with 11CH4 additive was significantly the highest compared to the other silages ($p \leq 0.05$). Biogas production from control silages and treated with Lactosil additive did not differ significantly (p□0.05) (fig. 1). Methane content in biogas from switchgrass silages varied between 54.9-55.7%.

In case of big bluestem the highest biogas production was obtained from silages treated with Lactosil additive ($p \leq 0.05$) (fig. 1). Methane content in biogas from big bluestem silages varied from 56.2-56.7%.

The highest biogas production from switchgrass silages treated with 11CH4 additive and big bluestem silages treated with Lactosil additive seems not to be connected with acetic acid concentration (tab. 2). These silages had the lowest content of lignin compared to the other experimental silages (tab. 1). Cellulose and hemicellulose are potential source of fermentable sugars necessary for biochemical reactions [14]. The presence of lignin, which incrusted cellulose, impedes enzymatic hydrolysis of the carbohydrates [12]. The lower concentration of lignin, the higher biogas production from ensiled plants. As in the studies cited by [3], addition of *Lactobacillus buchneri* PTA 6138 strain to ensiled grasses and maize increased methane production comparing with control silages. This strain had an ability to ferulic esterase production, the enzyme involved in lignin decomposing.

5. Conclusion

Ensiling was an appropriate method of perennial grasses pretreatment because during this process the content of structural polisaccharides was reduced. The effect of microbiological additives on the chemical composition of perennial grass silages was varied depending on the species of grass. Increase in biogas production from inoculated silages compared to controls was achieved in case of silages where the content of lignin was reduced.

6. References

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