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IMPACT OF BACTERIAL PREPARATION ON IMPROVEMENT OF AEROBIC STABILITY AND BIOGAS YIELD FROM MEADOW SWARD SILAGE

Summary

The aim of the study was to evaluate the impact of bacterial preparation, containing strains of Lactobacillus genera that stimulate the synthesis of 1,2-propanediol and carboxylic acids of low molecular weight, on aerobic stability of meadow sward silages and biogas yield by means of heterofermentation. Lactobacillus buchneri, L. diolivorans and L. reuteri, which are synergistically active in the synthesis of acetic acid and products of its metabolism: 1,2-propanediol and propionic acid, have been selected in the earlier studies and their co-fermentation conditions have been determined. The composition of the Lactosil Biogas starter culture was designed to conserve renewable raw materials, including meadow grass, for the production of biogas. The preparation was used for ensiling on the production scale. Based on the results of the silage analysis it was found that the action of starter culture of the new bacterial preparation resulted in an increase in acetic acid, 1,2-propanediol and propionic acid content in experimental silages and prolongation of their aerobic stability against control silages during three months of ensiling. The application of the Lactosil Biogas to the meadow grass resulted in an increase in biogas yield of the silage, processed in methane fermentation of the plant material, by 39.9 NI kg dry organic matter^{-1}, while increasing the content of methane by 3.7% and reducing the biogas contaminants.
***Keywords**: bacterial preparation, silage, aerobic stability, biogas yield and purity*

WPŁYW PREPARATU BAKTERYJNEGO NA POPRAWĘ STABILNOŚCI TLENOWEJ I UZYSK BIOGAZU Z KISZONEJ RUNI ŁĄKOWEJ

Streszczenie

Celem badań było określenie wpływu preparatu bakteryjnego, zawierającego szczepy bakterii z rodzaju Lactobacillus, które stymulują syntezę 1,2-propanodiolu i niskocząsteczkowych kwasów karboksylowych, na poprawę stabilności tlenowej kiszonek z runi łąkowej i uzysku biogazu na drodze heterofermentacji. W wyniku wcześniejszych badań wyselekcjonowano szczepy bakterii z gatunków: Lactobacillus buchneri, L. diolivorans i L. reuteri, synergicznie działające w kierunku syntezy kwasu octowego i produktów jego metabolizmu: 1,2-propanodiolu oraz kwasu propionowego, i określono warunki ich kofermentacji. Opracowano skład kultury starterowej preparatu Lactosil Biogaz, przeznaczonego do konserwowania surowców odnawialnych, w tym runi łąkowej, w celu produkcji biogazu,. Preparat zastosowano do sporządzenia w skali produkcyjnej kiszonek z runi łąkowej. Na podstawie wyników analizy kiszonek stwierdzono, że działanie kultury starterowej nowego preparatu bakteryjnego w procesie trzymiesięcznego kiszenia spowodowało wzrost zawartości kwasu octowego, 1,2-propanodiolu i kwasu propionowego w kiszonkach doświadczalnych oraz przedłużyło czas ich stabilności tlenowej w stosunku do kiszonek kontrolnych. Zastosowanie preparatu Lactosil Biogas do kiszenia runi łąkowej, w efekcie poddania tego surowca fermentacji metanowej, spowodowało wzrost wydajności biogazu z kiszonki o 39,9 jednostek NI kg smo^{1}, przy jednoczesnym wzroście o 3,7 % zawartości metanu oraz obniżeniu zawartości zanieczyszczeń biogazu.
***Słowa kluczowe**: preparat bakteryjny, kiszonka, stabilność tlenowa, wydajność i czystość biogazu*

1. Introduction

In the present time there is a growing need for preserved plant materials that will be converted to methane with high yield because of the intense developement of renewable energy technologies [4, 10]. According to Directives of the European Parliament and the Council of Europe, renewable energy is being promoted and the criteria for sustainable development of agriculture in terms of increasing the use of biofuels, assume that by 2020 greenhouse gases emissions to the environment should be reduced by 45% relative to past years. The prerequisite for the development of the biogas market in Poland is the application of solutions that will minimize the cost of raw material while achieving high biogas yields [13].

The contemporary research directions aiming at the development of bacterial preparations improving the biogas profitability from renewable raw materials concern the co-fermentation of different species of lactic acid bacteria, including the recognition of metabolic pathways of synthesis and catabolism of organic acids, as well as the characteristics of the metabolites and their industrial uses [13, 14].

The high content of acetic acid in silages are of particular importance for the proper methane fermentation of the plant material. In the phase of acetogenesis about 70% of methane is formed from acetic acid, whereas the high content of lactic acid is negligible. The determination of conditions of acetic acid synthesis and its conversion to propionic acid in the co-fermentation of *Lactobacillus* species allows to deepen the knowledge of possibilities to stimulate the synthesis of desired metabolites by these bacteria [15].

Due to the demand of farmers for improving aerobic stability of silages for both feed and biogas production and improving biogas yield from the raw material The Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology has provided experiments on bacterial preparations for preserving plant renewable raw materials by ensiling process. The experiments included the synergistic effect of bacterial strains derived from heterofermentative species of the genus *Lactobacillus*: *L. buchneri*, *L. diolivorans* and *L. reuteri*, to improve aerobic stability and increase biogas yields from ensiled plants [13].

It is well known from the literature that the ability to synthesize 1,2-propanediol is characterized by the strains of *L. buchneri*. Quide Elferink et al. [8] have shown that *L. buchneri*, like *L. parabuchneri*, is capable of synthesizing 1,2-propanediol under anaerobic conditions by converting 1 mole of lactic acid to 0.5 moles of acetic acid and 0.5 mole of 1,2-propanediol. Up to the year 2014 it had been known that pure cultures of *L. buchneri* were not capable of further metabolizing 1,2-propanediol to propionic acid until a new strain *L. buchneri* of both abilities: the synthesis and metabolism of 1,2-propanediol was found [12]. The ability to metabolize 1,2-propanediol to 1-propanol and propionic acid is characterized by strains derived from the species *L. diolivorans* [3, 6]. Whereas *L. reuteri* bacteria are capable of synthesizing cobalamin, which is a coenzyme for diol dehydratase - enzyme catalyzing the conversion of 1,2-propanediol to 1-propanol and propionic acid [1, 9, 11].

In the process of co-fermentation of bacterial strains from these species a stimulation of synthesis of the desired metabolites: acetic acid, 1,2-propanediol and propionic acid occurs. Their presence in renewable feed silage can improve their aerobic stability and increase the biogas yield. Based on the research results of the authors, the composition of bacterial preparation Lactosil Biogas for stimulating ensiling process of renewable raw materials has been developed [12, 14, 15].

2. Aim of the study

The aim of the study was to evaluate the impact of bacterial preparation, containing strains of *Lactobacillus* genera that stimulate the synthesis of 1,2-propanediol and carboxylic acids of low molecular weight, on aerobic stability of meadow sward silages and biogas yield by means of heterofermentation.

3. Materials and methods

3.1. Bacterial preparation

Lactosil Biogas preparation was used as a starter culture in ensiling process. The preparation consisted of following bacterial strains: *Lactobacillus buchneri* A KKP 2047p, *L. diolivorans* K KKP 2056p, *L. reuteri* M KKP 2048p.

3.2. Methods of ensiling meadow sward

Silage experiments were carried out in production conditions at the experimental station of the Institute of Technology and Life Sciences in Falenty. The meadow sward field was cut at the initial flowering in times (2 cut). The preparation was applied to the plant material, before it was formed into round bales weighing about 780 kg. A preparation in a dose of 5 grams per ton of plants was applied after dissolving in water, in the form of a spray. 1 g of the preparation contained 5 x 10^9 CFU g^{-1}. Control and experimental silages were ensiled for 90 days when were taken for the determination of: pH value, dry matter content, lactic, acetic, butyric and propionic acids, 1-propanol and 1,2-propanediol content. The aerobic stability of silages was also determined. Silages were subject to methane fermentation to determine biogas yield and purity of biogas.

3.3. Chemical analyses of silages

- Lactic acid bacteria were enumerated by pour plating of MRS medium according to standard PN-EN 15787:2009;
- Dry matter content was determined using the gravimetric method in line with the PN-ISO 6496:2002 standard;
- Determination of pH by potentiometry;
- Determination of L- and D-lactic acids, acetic acid and D-3-hydroxybutyric acid concentrations using enzymatic methods (Boehringer Mannheim, Germany) (measurement error 0,15-0,03 g/dm^3);
- Propionic acid, 1-propanol and 1,2-propanediol were determined by gas chromatography, which used an Agilent Technologies 7890A gas chromatograph with a flame ionisation detector (FID) and a capillary column with a diameter of 0.53 mm and a length of 30 m with phase DB-FFAP (J & W Scientific Columns). Helium was used as a carrier gas with a flow rate of 85 mL min^{-1} and the following temperature program: 35°C (0.5 min), with an increase of 20°C min^{-1} to 90°C, and an increase of 10°C min^{-1} to 200°C (0.5 min);
- Basic analysis of silages with NIRS method using NIRFlex N-500 apparatus;
- Aerobic stability was performed according to a temperature method described by Honig [5];
- Biogas yield of ensiled renewable material subject to methane fermentation was determined according to analytical procedure of Institute of Technology and Life Sciences [7];
- Biogas composition was determined by GA 2000 Geotechnical Instruments and Multi Gas gauge [7].

3.4. Statistical analyses

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

4. Results and discussion

Effects of the addition of bacterial preparation in ensiling of meadow grass were assessed in experiments carried out on a production scale in the experimental farm. After 90 days of ensiling the quality of the silages, their aerobic stability, and the biogas yield and its purity were assessed. The results of the meadow sward silages analyses are presented in Tables 1 and 2 and on Figures 1 and 2.

Silages made with the addition of bacterial starter culture consisting of three heterofermentative bacterial species were characterized by high quality, lack of butyric acid, appropriate ratio of lactic acid to acetic acid (2:1). Higher content of acetic acid was detected in silages prepared with Lactosil Biogas what is particularly beneficial in the case of the use of ensiled raw material for biogas production [15].

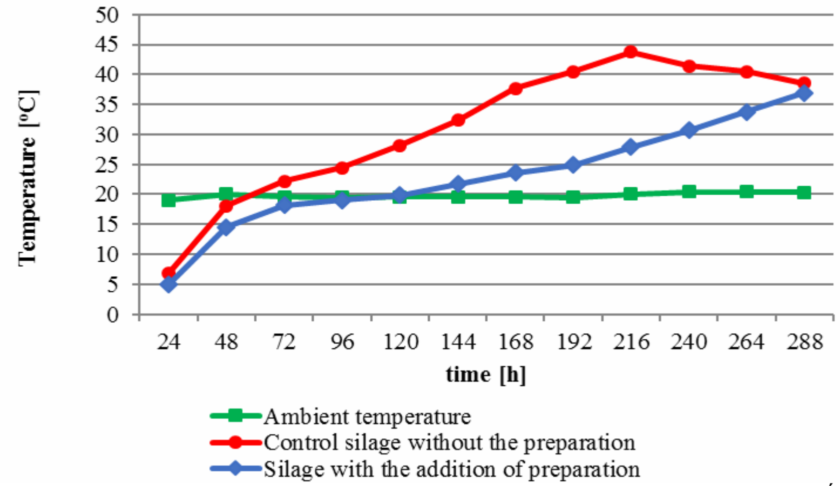
Due to the synergistic effect of the bacteria present in the starter culture of the preparation with the ability to synthesize and metabolize 1,2-propanediol, the content of this compound (103.7 mg 100 g^{-1}) and the presence of 1-propanol – metabolite of 1,2-propanediol (70.8 mg 100 g^{-1} silage) were measured. In addition, the propionic acid content was 10-times higher (135.1 mg 100 g^{-1}) in the silage in comparison to control silages prepared without the preparation.

The aerobic stability of control and experimental silages was determined using the temperature method described by Honig [5]. According to this method, the silages are stable until the si-

Table 1. The chemical analysis of meadow grass silages made with and without bacterial preparation Lactosil Biogas
Tab. 1. Analiza chemiczna kiszonek z runi łąkowej, bez i z dodatkiem preparatu bakteryjnego Lactosil Biogaz

Meadow sward silages with 44% dry matter content	pH	Concentration of carboxylic acids, g 100 g^{-1} of raw silage			Concentration of bacterial metabolites, mg 100 g^{-1} of raw silage		
		L- and D-lactic acid	Acetic acid	D-3-hydroxybutyric acid	1,2-propanediol	1-propanol	Propionic acid
Silage without preparation	4,94 ± 0,1	1,27 ± 0,2	0,35 ± 0,1	0,15 ± 0,15	not detected	not detected	12,23 ± 3,4
Silage treated with preparation	4,70 ± 0,1	1,51 ± 0,3	0,75, ± 0,15	brak	103,7 ± 12,3	70,8 ± 15,4	135,1 ±7,0

The results are the mean of three randomly selected silages made on a production scale. There was shown a standard deviation for each measurement (after the symbol "±") / Wyniki są średnimi z trzech losowo wybranych bel kiszonek wytworzonych w skali produkcyjnej. Podano odchylenia standardowe dla każdego wyniku (po symbolu "±") *Source: own work / Źródło: opracowanie własne*



Source: own work / Źródło: opracowanie własne

Fig. 1. Impact of Lactosil Biogas preparation on aerobic stability of meadow grass silages
Rys. 1. Wpływ preparatu Lactosil Biogaz na stabilność tlenową kiszonek z runi łąkowej

lage temperature will increase by 3°C in relation to the room temperature of 20°C, in which the stability test is performed.

The results obtained after a 12 day stability test showed that the experimental silages underwent a significantly extended period of stability apart from control silages.

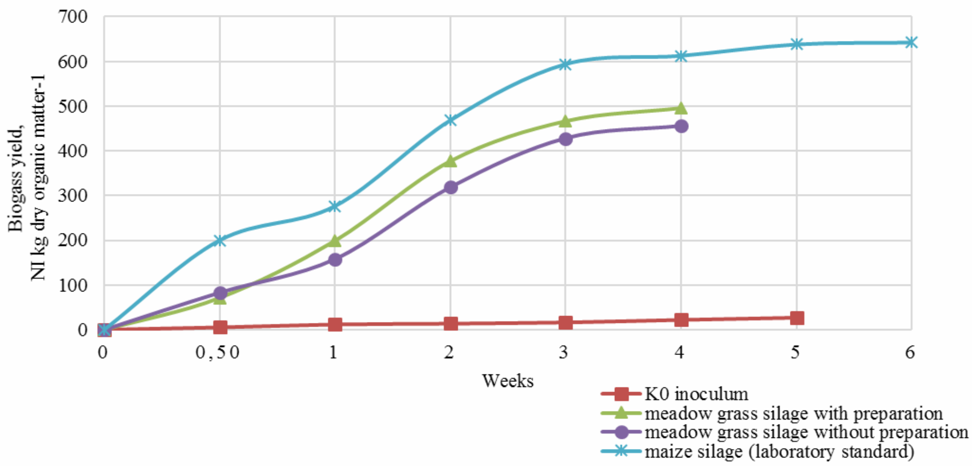
Experimental silages with Lactosil Biogas addition were characterized by 168-hour aerobic stability, compared to control silages, prepared without the addition of preparation characterized by 96 hours. The results are shown on Fig. 1.

At the next stage of the study, biogas profitability of methane fermentation of both, control and experimental silages was evaluated, including biogas yield and assessment of its purity. Results from these studies are presented in Table 2. As a result a higher by 39.0 NI kg dry organic matter^{-1} biogas yield was obtained for silage prepared with Lactosil Biogas preparation. At the same time, it was observed an increase in methane content by 3.7 percentage points compared to the silage made without the addition of bacterial starter culture. Biogas produced from silage with Lactosil Biogas was characterized by a 36% reduction in ammonia content and a 45% reduction in the amount of hydrogen sulphide compared to control silage, what is beneficial due to the costs incurred in subsequent processes of biogas purification (Table 2).

Table 2. Impact of the bacterial preparation Lactosil Biogas on the improvement of biogas yield and gas purity made of meadow sward silages
Tab. 2. Wpływ preparatu bakteryjnego Lactosil Biogaz na poprawę uzysku i czystości biogazu uzyskanego z kiszonek z runi łąkowej

Silages	Silage content, % in dry matter			Biogas yield determined by spfm method			
				Biogas yield	Biogas content		
	Dry matter, %	Organic mass, % in dry matter	Ash, % in dry matter	NI kg dry organic matter^{-1}*	Methane, CH_4 %	Ammonia, NH_3, mg/m^3	Hydrogen sulfide, H_2S, ppm
Silage without preparation	43,6 ± 0,9	89,5 ±2,7	10,5 ± 0,4	455,0 ±24,7	66,5	23,8	642
Silage treated with preparation	44,4 ± 0,9	88,1 ± 2,6	11,9 ± 0,4	494,9 ±22,8	70,2	15,3	357

** The results are the average of 3 samples. Unit of biogas yield NI kg dry organic matter^{-1} is a standardized netto liter of biogas obtained from the 1 kg of dry organic material of a given sample and refers to the stated dry matter content and organic dry matter content.*
Wyniki są średnimi z 3 próbek. Jednostka uzysku NI kg smo.$^{-1}$ jest znormalizowanym litrem biogazu netto uzyskanym z kg suchej masy organicznej danej próbki i odnosi się do podanej zawartości suchej masy i suchej masy organicznej.
Source: own work based on analyzes Myczko et al., 2017 [7] / Źródło: badania własne na podstawie analiz Myczko i in., 2017 [7]



Source: own work based on analyzes Myczko et al., 2017 [7] / Źródło: badania własne na podstawie analiz Myczko i in., 2017 [7]

Fig. 2. Biogas yield measured by spfm method of substrates - meadow sward silage prepared without and with the addition of the bacterial preparation with respect to laboratory standard: maize silage. Values for the inoculum are provided for information
Rys. 2. Uzysk biogazu mierzonego metodą spfm badanych substratów - kiszonek z runi łąkowej bez i z dodatkiem preparatu bakteryjnego w odniesieniu do standardu laboratoryjnego: kiszonki z kukurydzy. Wartości dla inokulum przedstawione zostały w celach informacyjnych

Biogas desulphurisation is one of the basic steps of biogas purification, in the case of high biogas burden with hydrogen sulphide. The desulphurisation is carried out in multistage reactors, which significantly increases methane production costs [2].

Comparison of dynamics and final biogas yield during methane fermentation of meadow sward silages made without or with the addition of the preparation is shown in Fig. 2. The results are set together with the standard of maize silage.

After 4 weeks of methane fermentation of meadow sward silages prepared without Lactosil Biogas 455.90 NI kg dry organic matter^{-1} of biogas were obtained. While 494.90 NI kg dry organic matter^{-1} of biogas were obtained from experimental silage. For comparison, the biogas yield obtained from standard maize silage after 6 weeks of fermentation was 642.31 NI kg dry organic matter^{-1}.

5. Conclusions

1. Experimental silages made with Lactosil Biogas were characterized by a high quality and extended aerobic stability by 72 hours in comparison to control ones; moreover, the experimental silages contained 2-times more acetic acid and more than 10-times higher propionic acid content than silages prepared without the preparation.

2. On the basis of the evaluation of biogas profitability it was concluded that the bacterial preparation addition and its action during the meadow sward ensiling process resulted in a higher biogas yield from the raw material by 39.9 NI kg dry organic matter^{-1}, with higher methane content in the biogas by 3.7%, while lowering the content of biogas pollution with ammonia and hydrogen sulphide.

3. A positive effects of the experiments presented in the study resulted in the commercial production at the prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology of bacterial preparation Lactosil Biogas for renewable raw plants ensiling and its further use in biogas production.

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