Krystyna J. ZIELIŃSKA[1], Agata U. FABISZEWSKA[2], Barbara WRÓBEL[3]
[1] Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology, Poland
[2] Warsaw University of Life Sciences, Poland
[3] Institute of Technology and Life Sciences Warszawa, Poland
e-mail: krystyna.zielinska@ibprs.pl
e-mail: agata_fabiszewska@sggw.pl
e-mail: b.wrobel@itep.edu.pl

Received: 2018-06-14 ; Accepted: 2018-07-10

INCREASE IN BIOGAS YIELD FROM WHOLE CROP MAIZE SILAGE PREPARED WITH THE ADDITION OF INOCULANT LACTOSIL BIOGAZ PLUS

Summary

The aim of the study was to evaluate the biogas profitability of silages made of whole crop maize ensiled with the addition of inoculant, containing strains of Lactobacillus genus from species that stimulate the synthesis and/or metabolism of 1,2-propanediol to propionic acid. As a result of the experiments on the synergistic action of bacterial strains an inoculant composition Lactosil Biogas Plus has been developed, which contains the following bacterial strains: L. buchneri A KKP 2047 p, L. diolivorans K KKP 2057 p and L. reuteri M KKP 2048 p. Silages made of whole crop maize with the addition of the inoculant Lactosil Biogas Plus were prepared under production conditions at the experimental station. Based on the results of the silage analyses, it was found that the effect of inoculant in the ensiling process resulted in the improvement of their microbiological purity expressed by pathogenic bacteria elimination such as bacteria of the genus Salmonella and Escherichia coli species and by fivefold reduction of molds. In the experimental silages increased content of acetic acid, 1,2-propanediol and propionic acid was observed and their aerobic stability was 192 hours, while the aerobic stability of silages prepared without the addition of inoculant was only 96 hours. Thus the experimental silages subject to methane fermentation by laboratory method allowed to achieve the biogas yield higher by 60.2 Nl kg dry organic matter^{-1}, the methane content of the biogas higher by 6.4 percent relative to the standard silage without the addition of the inoculant.
***Key words**: biogas yield, inoculant, silages from whole crop maize*

WZROST WYDAJNOŚCI BIOGAZU Z CAŁYCH ROŚLIN KUKURYDZY KISZONYCH Z DODATKIEM INOKULANTA LACTOSIL BIOGAZ PLUS

Streszczenie

Celem badań była ocena biogazodochodowości kiszonek z całych roślin kukurydzy konserwowanych z dodatkiem inokulanta, zawierającego szczepy bakterii z rodzaju Lactobacillus z gatunków, które stymulują syntezę i/lub metabolizm 1,2-propanodiolu do kwasu propionowego. W wyniku badań dotyczących synergizmu działania szczepów bakterii opracowano skład inokulanta Lactosil Biogaz Plus, który zawiera następujące szczepy bakterii: L. buchneri A KKP 2047 p, L. diolivorans K KKP 2057 p i L. reuteri M KKP 2048 p. Kiszonki z całych roślin kukurydzy z dodatkiem inokulanta Lactosil Biogaz Plus sporządzono w warunkach produkcyjnych w gospodarstwie doświadczalnym. Na podstawie wyników analizy kiszonek, stwierdzono, że działanie inokulanta w procesie kiszenia roślin spowodowało poprawę ich czystości mikrobiologicznej wyrażającej się wyeliminowaniem bakterii patogennych z rodzaju Salmonella i gatunku Escherichia coli i obniżoną pięciokrotnie liczbą pleśni. W kiszonkach doświadczalnych nastąpił wzrost zawartości kwasu octowego, 1,2-propanodiolu i kwasu propionowego, a ich stabilność tlenowa wynosiła 192 godziny, podczas gdy stabilność tlenowa kiszonek bez dodatku inokulanta tylko 96 godzin. W efekcie poddania kiszonek doświadczalnych laboratoryjnej fermentacji metanowej metodą spfm, uzyskano wzrost wydajności biogazu o 60,2 jednostek Nl kg smo., wzrost zawartości metanu w biogazie o 6,4 procent w stosunku do kiszonki standardowej, bez dodatku inokulanta.
***Słowa kluczowe**: inokulant, kiszonki z całych roślin kukurydzy, wydajność biogazu*

1. Introduction

Directions of research aimed at developing technical bacterial preparations that improve the biogas profitability of renewable raw materials refer to the cofermentation of various species and strains of lactic acid bacteria and include the understanding of metabolic pathways in the synthesis and transformation of organic acids, as well as characterization of metabolites formed in this process and their industrial use [1, 11]. The high content of acetic acid in silages is particularly important for the proper course of methane fermentation, because in the acetate-genesis phase, about 70% of methane is formed from acetic acid, whereas high lactic acid content adversely affects the activity of methane fermentation bacteria, although it inhibits development of aerobic microorganisms during ensiling [16].

There is a commercial need for bacterial preparations that improve the aerobic stability of silages intended for feed as well as for the production of biogas from renewable raw materials, which take into account the efficiency of methane production from these materials [4, 5, 11]. Bacterial preparations added to the ensiled raw plants intended for animal feeding and inoculants intended for the production of biogas should have a slightly different action. The biochemical processes occurring in the process of ensiling renewable raw materials for biogas production should be aimed at limiting the synthesis of lactic acid, while increasing the synthesis of acetic and propionic acids, thus ensuring durability and aerobic stability and increasing the efficiency of biogas from these silages [14]. In all cases, it is necessary to use silages characterized by high microbiological quality and durability of functional features, and for these reasons it is necessary to use inoculants for their production, which include effectively acting in this direction strains of *L. buchneri* [2, 9, 12, 18]. In order to enhance the effect of inoculants used for ensiling whole crop maize for biogas production, in addition to strains of *L. buchneri* bacteria, the participation of strains from *L. diolivorans* [8] and *L. reuteri* species is important [13, 19]. Strains of bacteria from *L. buchneri* species are characterized by the ability to synthesize 1,2-propanediol. Strains of L. diolivorans are characterized by the ability to metabolize 1,2-propanediol to 1-propanol and propionic acid. Bacteria of *L. reuteri* species are capable of synthesizing the form of cobalamine, which is a coenzyme of diol dehydratase - the enzyme catalysing the reaction of conversion of 1,2-propanediol to propionic acid [13, 17].

As a result of model experiments, it was found that the maximum B_{12} uptake of *L. reuteri* occurs during cultivation in MRS medium enriched with glycerol and 1,2-propanediol [13, 15]. At prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology in the earlier studies of the authors on the cofermentation of selected strains of bacterial species *L. buchneri*, *L. reuteri* and *L. diolivorans*, it was found that there is the possibility to stimulate the synthesis of desirable metabolites of acetic acid as 1,2 -propanediol and propionic acid, whose presence in silage from renewable raw materials improves their aerobic stability and increases biogas yield. Based on isolated from the natural environment characterized genetically and biochemically strains of these species, the bacterial composition of the Lactosil Biogas series was developed, intended for ensiling renewable raw plant materials such as whole plants of maize, grass and meadow sward [19, 20].

In the previous publication [21] the effect of the starter culture of Lactosil Biogas series with the following bacterial composition: *L. buchneri* A KKP 2047 p, *L. diolivorans* KKP 2057 p and *L. reuteri* M KKP 2048 p, on biogas yield from silage made of meadow sward from second cut was presented. Experimental silages with the addition of a newly developed preparation were characterized by high quality and extended by 72 hours aerobic stability, more than twice higher acetic acid content and more than ten times higher propionic acid content, in comparison to control silages prepared without addition of the preparation. On the basis of the biogas profitability of these silages it was found that the preparation's action in the ensiling process resulted in a higher biogas yield from the raw material by 39.9 NI kg dry organic matter^{-1} and a higher methane content in biogas by 3.7%, with simultaneous reduction of biogas contamination content with ammonia and hydrogen sulphide [21].

2. Aim of the study

The aim of the study was to use the bacterial preparation Lactosil Biogas Plus for ensiling whole crop maize silages and to show its impact on increased purity and biogas yield, which can be produced from this material during methane fermentation.

3. Materials and methods

Experiments were carried out with the usage of Lactosil Biogaz Plus preparation, which contained three bacterial strains:

- *Lactobacillus buchneri* A KKP 2047p,
- *L. diolivorans* K KKP 2056p,
- *L. reuteri* M KKP 2048p,

deposited in collection of cultures of industrial microbes in Institute of Agricultural and Food Biotechnology.

Silage experiments were carried out in production conditions at the experimental station of the Institute of Technology and Life Sciences in Falenty. Experimental silages from whole crop maize, with the addition of the developed preparation and without its addition, were made from plants with a dry matter content of approximately 34%. The preparation, at a dose of 5 grams per ton of plant masses, was given after dissolving in water, in the form of spraying, using an applicator, before loading the plant material into the silo.

After 60 days of ensiling the probes were taken for analyses: pH value, lactic, acetic, butyric and propionic acids, 1-propanol and 1,2-propanediol content and for microbiological measurements: number of molds, number of bacteria of the genus or species as following: *Salmonella* sp., *Escherichia coli*, coliform bacteria and *Clostridium perfringens.*

Methods of silage analyses:

- lactic acid bacteria were measured by pour plating of MRS medium according to ISO standard (PN-EN 15787:2009),
- the number of moulds was determined by the plate method according to the ISO standard (PN-ISO 21527-2: 2009, part 2) using agar medium with dichloran and 18% glycerol,
- determination of *Escherichia coli* and coliform bacteria was done using a plate method with the use of technical medium – 3M™ Petrifilm™ *E. coli*/Coliform Count Plates (Noack, USA),
- *Salmonella* sp. were measured by a plate method with the use of Rambach Agar (Merck, USA)
- *Clostridium perfringens* number was measured by a plate method on selective medium TSC with D-cycloserine according to the ISO standard (PN-EN ISO 7937:2005),
- determination of pH by potentiometry,
- dry matter content with the PN-ISO 6496:2002 standard,
- 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 $g \cdot dm^{-3}$),
- 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); SPE C-18 columns for cleaning the probes,
- aerobic stability was performed according to a temperature method described by Honig [7], biogas yield was determined by methane fermentation method according to analytical procedure of Institute of Technology and Life Sciences no. PB 01/LBMPZ 2010/FM [10],
- biogas composition was determined by GA 2000 Geotechnical Instruments and Multi Gas gauge.

Statistical analyses of the results were performed using following software: Microsoft Excel 2003 and STATISTICA 8.0 (Statsoft, Poland). Standard deviation was calculated.

4. Results and discussion

The effect of the preparation was evaluated in experiments in which the time of ensiling whole crop maize was 60 days. After this time, the microbiological and physicochemical quality of silages, their aerobic stability and biogas profitability were evaluated. The results of the analysis of silage from whole crop maize are presented in Tables 1 and 2 and in Fig. 1 and 2.

Experimental silages which were under the influence of the starter culture of the preparation, composed of strains from three heterofermentative bacterial species, were characterized by high quality and microbiological purity; over three times higher content of acetic acid was observed in relation to control silages, which is particularly advantageous when using a raw material for biogas production (Table 1, Fig. 1).

In the process of ensiling whole crop maize the bacteria of the genus *Salmonella* sp. and from the *E. coli* species were completely eliminated under the influence of the bacterial preparation (Fig. 1). The number of coliform bacteria decreased from 3.3 log CFU g^{-1} to 1.0 log CFU g^{-1}, while the number of *C. perfringens* decreased from 2.3 log CFU g^{-1} to the value of 0.5 log CFU g^{-1}. Silage prepared with the addition of the Lactosil Biogas Plus inoculant was also characterized by a fivefold lower number of mold expressed in log CFU g^{-1}.

As a result of subjecting the experimental silages prepared with the addition of Lactosil Biogaz Plus to laboratory methane fermentation, a biogas yield was obtained from whole maize plants with a value of 686.20 NI kg dry organic $matter^{-1}$, with an increase in methane content in biogas by 6.4%. In this experiment, the biogas yield was higher by 60.2 NI kg dry organic $matter^{-1}$ in relation to the result obtained from standard silage prepared without added inoculant [10]. The results are presented in Table 2 and graphically in Fig. 2.

The dynamics of the methane fermentation process was accelerated and overtaking in relation to the standard maize silage. In addition, there were no factors that threatened the usage of the substrate obtained in the ensilage process with the addition of the preparation in biogas plant (Table 2, Fig. 2).

Biogas produced from silage prepared with the addition of the preparation was characterized by a reduced by 32% content of ammonia, in relation to the standard silage, what is particularly advantageous due to the costs incurred in further purification processes (Table 2).

Table 1. The content of organic acids, 1,2-propanediol and 1-propanol in silages from whole crop maize, prepared with and without the addition of the preparation Lactosil Biogaz Plus

Tab. 1. Zawartość kwasów organicznych, 1,2-propanodiolu i 1-propanolu w kiszonkach z całych roślin kukurydzy, bez i z dodatkiem preparatu Lactosil Biogaz Plus

Whole crop maize silages with 38% of dry matter content	pH	Acid content, g/100 g of silage			Metabolite content, mg/100 g of silage		
		lactic	acetic	butyric	1,2-propanediol	1-propanol	propionic acid
Silage without the addition of preparation	4,56 ± 0,1	1,18 ± 0,2	0,45 ± 0,1	0,15 ± 0,05	not detected	not detected	19,3 ± 3,4
Silage with the addition of preparation	3,70 ± 0,1	1,82 ± 0,3	1,58 ± 0,15	not detected	154,7 ± 12,3	70,3 ± 15,4	204,8 ± 17,0

The results are the average of 3 chosen silos, ±standard deviation / Wyniki są średnimi z trzech wybranych balotów, ±odchylenie standardowe

Source: own work / Źródło: opracowanie własne

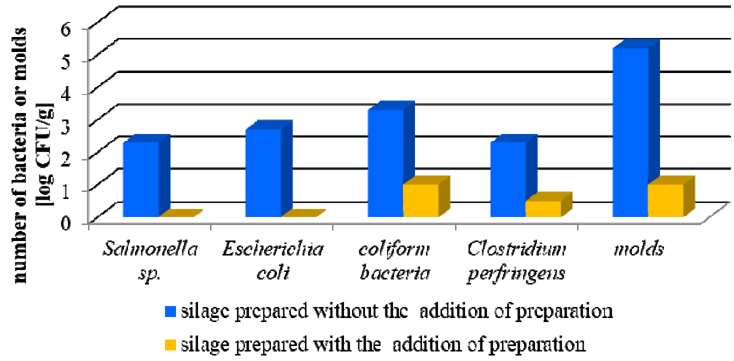
Table 2. Yield and purity of biogas produced from whole crop maize silage: standard - prepared without the preparation and treated with the preparation Lactosil Biogaz Plus

Tab. 2. Wydajność i czystość biogazu wyprodukowanego z kiszonek z całych roślin kukurydzy: standardowej i z dodatkiem preparatu Lactosil Biogaz Plus

Whole crop maize silage	pH	Silage content, % in dry matter			Biogas characteristic after 5 weeks of spfm fermentation		
					Biogas yield NI kg dry organic $matter^{-1}$*	Biogas content	
		Dry matter, %	Organic mass, % in dry matter	Ash, % in dry matter		methane, %	NH_3, mg/m^3
Silage without preparation (standard silage)	4,56 ± 0,1	33,96 ± 2,1	95,42 ± 0,08	3,51 ± 0,2	626,00 ± 2,03	47,89 ± 1,08	4,7 ± 0,5
Silage treated with preparation	3,70 ± 0,1	34,10 ± 2,3	96,50 ± 0,1	3,70 ± 0,2	686,20 ± 4,99	54,30 ± 1,20	3,2 ± 0,5

** 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.

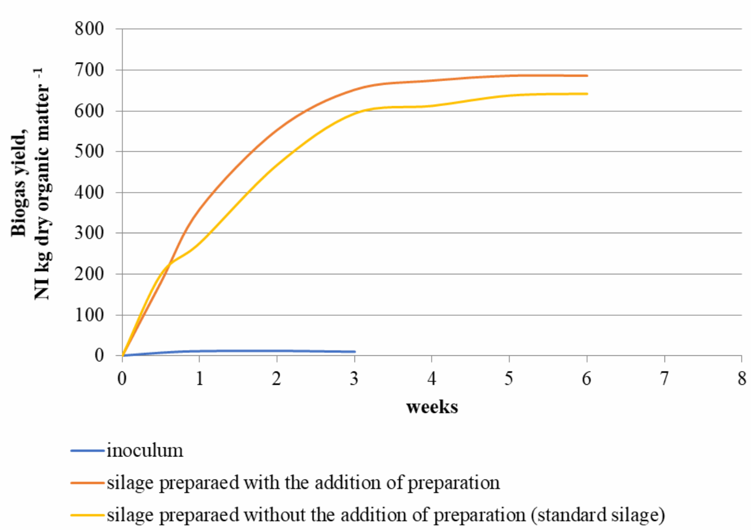
Source: own work on the basis of [10] / Źródło: opracowanie własne na podstawie [10]



** The results are the average of 3 samples / Wyniki są średnimi z trzech wybranych balotów kiszonek*

Source: own work / Źródło: opracowanie własne

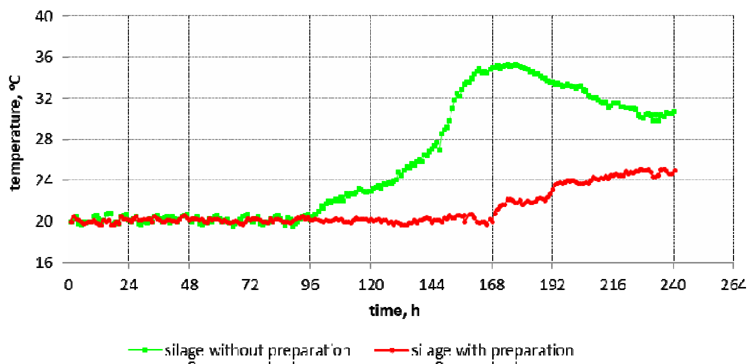
Fig. 1. The effect of Lactosil Biogas Plus inoculant on the microbiological purity of silages from whole crop maize
Rys. 1. Wpływ inokulanta Lactosil Biogaz Plus na czystość mikrobiologiczną kiszonki z całych roślin kukurydzy



** 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 on the basis of [10] / Źródło: opracowanie własne na podstawie [10]

Fig. 2. Accumulated plot of biogas yield obtained by methane fermentation from silages from whole crop maize
Rys. 2. Wykres kumulowany uzysku biogazu metodą fermentacji metanowej z kiszonek z całych roślin kukurydzy



Source: own work / Źródło: opracowanie własne

Fig. 3. The effect of the inoculant on the results of the aerobic stability test of silages from whole crop maize
Rys. 3. Wpływ inokulanta na wyniki testu stabilności tlenowej kiszonek z całych roślin kukurydzy

The results of the research presented in this paper have confirmed the positive effect of inoculants intended for ensiling renewable raw materials on the biochemical processes which occur in the ensilage process: limiting the synthesis of lactic acid, and increasing the synthesis of acetic and propionic acids, which consequently improve the durability, microbiological purity and aerobic stability of silage. As a result of methane fermentation of renewable raw materials prepared with the addition of special inoculants an increase in biogas yield from organic matter of dry plant is obtained [5].

The aerobic stability test was performed according to Honig [7]. The stability was evaluated on the basis of changes in the temperature of experimental and control silages observed at room temperature for 10 days. The experimental silages with the addition of inoculant did not show elevated temperatures above 23°C for 192 hours, while the control silage without added inoculant was stable only for 96 hours, after which time its temperature began to rise, reaching the highest temperature of 35°C at 168 hour of incubation (Fig. 3).

5. Summary

Under the influence of the inoculant Lactosil Biogas Plus, composed of *L. buchneri* A KKP 2047 p, *L. diolivorans* K KKP 2057 p, *L. reuteri* M KKP 2048 p, experimental silages from whole crop maize, relative to control silages prepared without preparation, were characterized by more than ten times higher propionic acid content, absence of potentially pathogenic bacteria *(Salmonella* sp. and *E. coli*) and five times lower number of mold expressed in log CFU g^{-1}. In addition, the aerobic stability time of experimental silage was twice as long as the aerobic stability of control silages and was 192 hours.

As a result of subjecting experimental silages to laboratory methane fermentation an increase in biogas yield by 60.2 NI kg dry organic matter^{-1} was obtained, with a methane content in biogas higher by 6.4%, compared to the standard silage, which was prepared without any addition of the inoculant.

The obtained results confirm the influence of the Lactosil Biogas Plus inoculant in the process of ensiling of renewable raw materials, intended for production of biogas, such as maize, grass and meadow sward for improvement of their quality, microbiological purity and increase in synthesis of compounds that prolong aerobic stability [10, 21]. Favourable changes in the chemical properties of silages transfer into an increase in the biogas yield from the dry matter of the organic feedstock subject to methane fermentation, and therefore the use of appropriate inoculants for preserving renewable raw materials improves economics of biogas production.

6. References

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Acknowledgements
This Research was financed by the Ministry of Science and Higher Education of the Republic of Poland.
Badania zostały sfinansowane z dotacji przyznanej przez MNiSW na działalność statutową.