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The effect of silage additive on the kinetics of biogas production from lignocellulosic perennial crops

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Abstract: The aim of the study was to assess the effect of silage additive containing heterofermentative lactic acid bacteria (LAB) strain of *Lactobacillus buchneri* species on ensiling quality, as well as methane yield and the kinetics of biogas production from ensiled perennial energy grasses: *Miscanthus* × *giganteus* (miscanthus), *Spartina pectinata* (cordgrass), *Panicum virgatum* (switchgrass) and *Andropogon gerardii* (big bluestem). The listed plants are not commonly used for biogas production, their susceptibility to ensiling is also little known, hence the need to investigate their suitability for these processes. Effective methods for increasing the biogas yield from biomass are still demand, hence the research on the use of LAB for this purpose.

After harvesting the grasses were cut and ensiled in barrels with and without (controls) the usage of commercial silage inoculant containing *Lactobacillus buchneri* LN40177. After 90 days of ensiling obtained silages were analysed in order to compare their chemical composition: organic acids content, the loss of dry matter, the differences in particular fibres composition. The silages were then subjected to methane fermentation using OxiTop® sensors and exposed to air in order to check their aerobic stability.

The silages prepared with LAB additive had higher concentration of acetic acid than the control silages prepared without LAB addition, which contributed to increased aerobic stability but had no effect on the methane yield of miscanthus, switchgrass and big bluestem. Using the microbial inoculant during ensiling had beneficial effect in terms of reducing the duration of biogas production process from obtained silages: lag phase was shortened, daily biogas production rate was increased and 90% of biogas was produced in a shorter period of time compared to the control silages from investigated grasses. The modified Gompertz model well reflected the kinetics of biogas production process.

Keywords: anaerobic digestion, biogas production, Gompertz model, lactic acid bacteria, methane yield, silage

INTRODUCTION

The current direction of research in the area of counteracting climate changes, is the use of widely available lignocellulosic biomass as the second generation material for biofuels production. Lignocellulosic biomass can be acquired from a wide range of plants, including perennial grasses, which are high yielded with low energy cumulative expenditure [Piątek *et al.* 2016]. Nowadays, lignocellulose biomass is used mainly in the combustion process [Bilandzija *et al.* 2017]. Anaerobic digestion (AD) is one of the method of biomass processing, as a result of which biogas (mixture of methane and carbon dioxide) is produced.

Installations where biogas is produced (biogas plants) use from a few dozen to a few hundred megagrams of biomass per day. Thus, biomass, which is harvested and supplied to the biogas plant in large amount once a year, must be conserved, with ensiling being the most common way. Ensiling has become a widely used method of pretreatment of biomass prior to AD [Sun *et al.* 2021; Texeira Franco *et al.* 2016].

Ensiling is a dynamic, four phases, well known biochemical process in which various species of anaerobic microorganisms are

involved with lactic acid bacteria (LAB) as the main group [Texeira Franco *et al.* 2016]. The role of LAB in ensiling process is leading fermentation of water soluble carbohydrates (WSC) under anaerobic conditions, which results in lactic acid production as the main end product of hexoses biotransformation. Lactic acid lowers the pH of ensiled biomass to around 4.0, inhibiting the growth of harmful microbes [Herrmann *et al.* 2011]. The higher the WSC level in the biomass, the more lactic acid may be produced by LAB. Lignocellulose biomass is known to be difficult to ensile because of high cellulose and low WSC content [Zhao *et al.* 2017]. However, there are a few ways to make the ensiling process more effective.

In order to improve the course of ensiling process, silage additives are commonly used [Adesogan *et al.* 2002; Jankea *et al.* 2019]. Silage additives are divided into chemical (enzymes) and microbial inoculants which are added into biomass before biomass compaction [Zhao *et al.* 2017]. Microbial inoculants, which stimulate fermentation process, are widely used for poorly ensilable crops, such as unwilted alfalfa clovers or some grasses [Kalać 2011]. Biomass chemical composition changes during ensiling, which directly or indirectly affects AD [Prochnov *et al.* 2009]. Heterofermentative LAB ferment pentoses as a result of which acetic acid is produced as the main end product. Acetic acid is an intermediate for later methanogenesis, so that its high concentration in ensiled biomass intended for biogas production is required [Yadvika *et al.* 2004]. However, there are some contradictory reports about an impact of microbial inoculants on the methane yield obtained from ensiled biomass.

Feng *et al.* [2018] found that biological additives containing both hetero- and homo-fermentative LAB and enzymes improved both organic dry matter preservation and biogas production from *Festuca arundinacea* silages. The addition of *Lactobacillus plantarum* and *Pediococcus acidilactici* to grasses, despite increasing acetic acid concentration in low and medium solid crops, did not boost biogas production from acquired silages, according to another study [Pakarinen *et al.* 2008].

Undoubtedly, the addition of microbiological inoculants during biomass ensiling affects the quality of obtained silages. High quality and long stability of silages have an impact on the course of methane fermentation [Prochnow *et al.* 2012]. Some authors claimed that ensiling could be beneficial in terms of reducing the duration of biogas production [Kafle, Kim 2013].

The modified Gompertz equation is frequently used to assess the progress of methane fermentation [Budiyono *et al.* 2010; Lo *et al.* 2010]. Latinwo and Agarry [2015] found that a modified Gompertz plot had a higher connection with cumulative biogas output than an exponential increase to maximum plot. Another study [Piątek *et al.* 2016] found that the Gompertz distribution provides for a fairly accurate prediction of methane yield for specific plants.

Studies on the impact of microbial inoculants on kinetics of biogas production from lignocellulose biomass have so far been limited. There are also few research on the ensiling of perennial grasses, such as switchgrass, miscanthus, cordgrass and big bluestem [Kupryś-Caruk *et al.* 2019, 2021; Whittaker *et al.* 2016]. To assess the effect of microbial additive on quality of obtained silages from perennial energy grasses, as well as on methane production and the kinetics of biogas production was the purpose of this work.

MATERIALS AND METHODS

STUDY MATERIALS

Miscanthus (*Miscanthus* × *giganteus* J.M. Greef & M. Deuter), prairie cordgrass (*Spartina pectinata* Bosc ex Link), switchgrass (*Panicum virgatum*, var. Dacotah), and big bluestem (*Andropogon gerardii*, var. Bison) were obtained from the Warsaw University of Life Sciences, located in the Experimental Station in Skierniewice (51°57' N, 20°09' E). Grasses were cultivated on soil class IVa, a good rye complex and fertilised in early spring (N-P-K: 90-40-150 kg ha^{-1}). The biomass (in the 8th year of cultivation) were picked in early July, cut into 1 cm pieces, and stored in plastic barrels without drying. In a barrel, 10 kg of each chopped grass was tightly compacted up to the lid (in three repetitions). Following grass compression, the barrels were hermetically sealed with lids equipped with gas release valves. For three months, the barrels were kept at ambient temperature. After 90 days the barrels were opened for silage analysis and anaerobic digestion.

During compaction, the biomass was inoculated with *Lactobacillus buchneri* LN40177 strain from the silage supplement 11CH4 (Pioneer, USA). In 200 cm^3 of demineralised water, 0.02 g of the preparation was dissolved and sprayed onto 10 kg of biomass. A total of $2.0 \cdot 10^8$ CFU·kg^{-1} of bacteria were introduced to the ensiled biomass. The control silages were made without the silage ingredient but with the addition of 200 cm^3 of water.

STUDY METHODS

Dry matter (*DM*) of fresh (before ensiling) and ensiled materials was determined by drying a 25 g sample of plant material at 105° C to a constant weight according to ASABE standard S358.2. Sun *et al.* [2021] described how *DM* was adjusted for the loss of volatiles. The dry materials were burned at 550°C to determine the organic dry matter (*ODM*).

After homogenising 10 g of a sample with 100 cm^3 of distilled water for 25 min, the pH value of silages was determined using the potentiometric method. All extracts were deproteinised using Carrez solutions before being filtered through a 0.45 m PVDF (polyvinyl difluoride) syringe filter for HPLC analysis (high performance liquid chromatography). The HPLC method includes photometric detection at 210 nm, a separation temperature of 35°C, a mobile phase of 4 mM sulphuric acid, and a flow rate of 0.6 $cm^3 \cdot min^{-1}$.

The Luff–Schoorl method was used to determine water soluble carbohydrates (*WSC*) according to the Polish standard PN-R-64784:1994. Air dry subsamples were crushed and sieved with a 1-mm sieve for further analysis. Total protein, neutral-detergent fiber (*NDF*), acid-detergent fiber (*ADF*), and acid-detergent lignin (*ADL*) were determined using the PN-EN 13342, PN-EN ISO 16472:2007P, and PN-EN ISO 13906:2009P methods respectively. The FibertecTM 8000 technology was used to identify the specific fibers (Foss, Denmark). Hemicellulose was estimated as the difference between *NDF* and *ADF* fibre, and cellulose was computed as the difference between *ADF* and *ADL* fibre. The *ADL* content was assessed to be lignin.

The following formula was used to determine the digestibility of dry matter (*DDM*) [Kim *et al.* 2005]:

$$DDM = 88.9 - 0.779ADF \quad (1)$$

Aerobic stability test

After 90 days of ensiling, barrels were opened and aerobic stability tests were performed. About 3 kg of each silage was sampled from the inside of the barrel and mixed thoroughly, thus aerating the silage and then placed in perforated plastic bags. The bags were placed in a room, where the temperature was maintained at 21°C and the humidity was 40%. Twice a day, the temperature of silage inside each bag was monitored using a laboratory mercury thermometer. The measurements were carried out for 30 days or until the temperature of the silage increased by at least 2°C relative to the ambient temperature.

Anaerobic digestion

Glass fermenters with a capacity of 1.3 dm^3 were used to test biogas production from silages (substrates), with 5 g of silage and 100 cm^3 of inoculum introduced. Inoculum was taken from a digester at one of the agricultural biogas plants in central Poland as a source of methanogen bacteria. To reduce biogas production from the inoculum, it was pre-incubated for seven days at 39°C. Manometry sensors (OxiTop® Control, WTW, Germany) were used to uncap fermenters. Fermenters were flushed with N_2 before beginning anaerobic digestion. As control experiments, fermenters with only inoculum were used. All of the assays were done five times. At 39°C, fermenters were set up on mixing platforms (WTW, Germany). Anaerobic digestion was carried out until a plateau was reached. During this time, OxiTop® sensors monitored and recorded the pressure of the biogas produced, and the data was through infrared to the OxiTop® OC 110 controller and subsequently to a PC for further processing. A gas analyser (COMBIMASS®GA-m, Germany) was used to examine the biogas composition.

The following calculations were used in the quantitative evaluation of the results:

1) the number of moles of obtained biogas using the ideal gas equation:

$$pV = nRT \tag{2}$$

where: p = the pressure (Pa), V = the reactor capacity (m^3), n = the number of moles, R = the universal gas constant 8.31 J·(mol K)$^{-1}$, T = the temperature (K);

2) the volume of biogas taking into account that in normal conditions (p = 1013.25 hPa, T = 273.15 K) one mole of gas occupies 22.4 dm^3;
3) subtraction from the volume of biogas produced from the silage the volume of biogas produced only from the inoculum;
4) reduction of the volume of biogas by 6.5%, which refers to the part of the water vapour that is in biogas under normal conditions.

The cumulative volume of biogas produced from organic dry matter (*ODM*) of silages was calculated as biogas or methane yields.

Lo *et al.* [2010] utilised a modified Gompertz model to match experimental data characterising the anaerobic digestion process:

$$P_c = P_{\max} \cdot \exp\left[-\exp\frac{R_{\max} \cdot \mathrm{e}(\lambda - t)}{P_{\max} + 1}\right] \tag{3}$$

where: P_c = the cumulative biogas yield produced during AD period (m^3·Mg^{-1} *ODM*); $P_{\max}$ = the maximum potential of biogas production (m^3·Mg^{-1} *ODM*), $R_{\max}$ = the maximum biogas production rate (m^3·Mg^{-1} *ODM*·d^{-1}), λ = the lag phase (d); t = time of AD period (d); e = Euler's number (2.7183).

The graph of the Gompertz function is a sigmoidal curve, which is why the results were adjusted using non-linear regression. Estimation of the parameters of the regression model was carried out using the Levenberg–Marquardt non-linear least-squares method (LM) at Statistics v.8 software.

The evaluation of the fit of the model was based on the determination coefficient:

$$R^2 = \frac{\sum_{i=1}^{N}(y_i - y_{pi})\ \sum_{i=1}^{N}(y_i - y_{ei})}{\sqrt{\left[\sum_{i=1}^{N}(y_i - y_{pi})^2\right]\left[\sum_{i=1}^{N}(y_i - y_{ei})^2\right]}} \tag{4}$$

and the global error:

$$\delta_g = \frac{\sqrt{\sum_{i=1}^{N}(y_{ei} - y_{pi})^2}}{\sqrt{\sum_{i=1}^{N}(y_{ei})^2}} 100 \tag{5}$$

where: y_i = the value of the dependent variable, y_{ei} = the experimental value, y_{pi} = the predictive value, N = the number of measurements.

Data analysis

After ensuring that the data met the ANOVA assumptions of normality of distribution and equality of variance, repeated-measures (ANOVA) were performed. A post hoc analysis was performed when substantial discrepancies between certain mean values were found (Tukey test). The significance level for all of the results was set at 0.05. Statistica 8.0 was used to conduct the analysis. On the basis of Kolmogorov–Smirnov test, it was found that the distribution of methane yield obtained from each type of investigated silage was a normal distribution ($P > 0.05$; $d > 0.13$). Based on Levene's test it was also found that the methane yields obtained from each type of investigated silage obtained displayed homogeneity ($P > 0.05$; F-value within the range of 0.0021–0.2227).

RESULTS AND DISCUSSION

THE QUALITY OF THE SILAGES

Results of the chemical analyses of the fresh and ensiled biomass are shown in Tables 1–2. Ensiling process resulted in *DM* losses at the range of 0.3–12.3%, wherein the silages from cordgrass, switchgrass and big bluestem prepared with the inoculant addition showed significantly higher dry matter losses compared to the control silages. The highest *DM* losses (9.6–12.3%) were observed in the case of switchgrass silage (Tab. 1).

In relation to miscanthus, ensiling, regardless of the silage additive, did not have an effect on the changes in the content of particular cell walls components, such as cellulose, hemicellulose and lignin compared to the fresh biomass. In miscanthus silages the content of crude protein decreased as compared to the fresh biomass, no water soluble carbohydrates were detected (Tab. 2).

In relation to cordgrass, switchgrass and big bluestem the ensiling process had very different influence on cellulose or

Table 1. Basic parameters of the fresh and ensilaged grasses after 90 days of ensiling

Grass	Sample	*DM* (%)	*ODM* (% *DM*)	*DDM*	*DM* loss rate
				%	
Miscanthus	F	17.2[a]	92.4[a]	55.8[a]	–
	C	16.8[a]	90.0[a]	54.3[a]	2.3[a]
	I	16.8[a]	92.2[a]	55.1[a]	2.3[a]
Cordgrass	F	30.7[a]	95.1[a]	55.4[a]	–
	C	30.6[a]	94.5[a]	56.3[a]	0.3[a]
	I	29.9[a]	94.6[a]	55.0[a]	2.6[b]
Switchgrass	F	29.2[a]	94.3[a]	58.0[a]	–
	C	26.4[a]	93.3[a]	57.3[a]	9.6[a]
	I	25.6[a]	93.5[a]	58.9[a]	12.3[b]
Big bluestem	F	21.5[a]	94.3[a]	58.4[a]	–
	C	21.3[a]	93.6[a]	58.7[a]	0.9[a]
	I	21.1[a]	93.8[a]	58.9[a]	1.9[b]

Explanations: F = fresh, C = control, I = inoculant, a, b = different letters (in columns for the same grass) indicate significant differences between mean values ($P < 0.05$).
Source: own study.

Table 2. Chemical composition of the fresh and ensilaged grasses after 90 days of ensiling (% *DM*)

Grass	Sample	Content (%)				
		crude protein	cellulose	hemicellulose	lignin	*WSC*
Miscanthus	F	9.3[b]	37.1[a]	23.7[a]	6.3[a]	3.5
	C	6.4[a]	37.2[a]	22.9[a]	6.2[a]	n.d.
	I	6.8[a]	37.2[a]	23.5[a]	6.2[a]	n.d.
Cordgrass	F	7.1[b]	36.7[a]	22.5[b]	6.3[a]	2.5
	C	6.6[a]	36.3[a]	22.6[b]	6.6[a]	n.d.
	I	6.7[a]	36.9[a]	21.8[a]	6.6[a]	n.d.
Switchgrass	F	10.2[b]	34.5[b]	23.1[b]	5.1[a]	5.4[c]
	C	8.2[a]	34.9[b]	23.6[b]	5.6[a]	0.04[a]
	I	7.8[a]	32.5[a]	22.8[a]	5.0[a]	1.0[b]
Big bluestem	F	9.2[c]	34.0[c]	25.3[b]	5.2[a]	7.6[c]
	C	8.1[b]	33.2[b]	23.7[ab]	5.6[a]	0.6[a]
	I	7.5[ab]	32.4[a]	22.7[a]	5.5[a]	1.2[b]

Explanations: *WSC* = water soluble carbohydrates, n.d. – not detected (below the detection limit of the method), the other as in Tab. 1.
Source: own study.

hemicellulose content compared to the content of these molecules in the fresh material. The addition of the inoculant resulted in significantly lower hemicellulose content in the silages of switchgrass and cordgrass compared to the control silages. The cellulose content was also significantly lower in the silages prepared from switchgrass and big bluestem with the inoculant addition. The silage additive did not have any impact on the lignin content in the silages, which remained on the same level as in the fresh biomass of all investigated grasses. The crude protein content decreased in cordgrass, switchgrass and big bluestem silages compared to the fresh biomass, regardless from the silage inoculant addition. Water soluble carbohydrates were not found in the silages made from miscanthus and cordgrass (Tab. 2).

At opening, the control silages prepared from miscanthus, switchgrass and big bluestem showed visible signs of molding on the surface. The control silages of miscanthus had a noticeable smell of butyric acid. All silages revealed generally high pH values (4.9–5.4). The content of lactic acid was relatively low with the highest value (69.5 g·kg^{-1} *DM*) in the silages from cordgrass prepared with the inoculant addition. In all obtained silages much higher content of acetic acid than lactic acid was detected. The content of acetic acid was significantly higher in the inoculated silages than in the control silages and amounted to 109.3–299.1 g·kg^{-1} *DM*. Butyric acid, as the indicator of *Clostridium* activity, was not detected in cordgrass silages and in the inoculated silages made from miscanthus. In the silages made from switchgrass the inoculant addition influenced on the significantly lower content of butyric acid compared to the control silages (Tab. 3).

Table 3. Volatile compounds of the silages after 90 days of ensiling, prepared with or without the inoculant addition

Grass	Silage	pH	Organic acids (g·kg^{-1} *DM*)		
			lactic	acetic	butyric
Miscanthus	C	5.0	10.3[a]	103.1[a]	1.0
	I	5.1	12.1[a]	299.1[b]	n.d.
Cordgrass	C	5.2	26.8[a]	42.5[a]	n.d.
	I	5.1	69.5[b]	109.3[b]	n.d.
Switchgrass	C	5.4	13.1[b]	70.0[a]	0.8[b]
	I	5.3	9.3[b]	157.0[b]	0.2[a]
Big bluestem	C	4.9	16.1[a]	119.6[a]	0.1
	I	4.9	16.3[a]	169.0[b]	0.1

Explanations: C, I, a, b, n.d. as in Tab. 1.
Source: own study.

AEROBIC STABILITY OF THE SILAGES

Higher acetic acid level in silages is linked to heterofermentative sugar fermentation by lactic acid bacteria, which improves aerobic stability once the silage is opened [Nascimento Agarusi *et al.* 2022]. Figure 1 depicts the temperature change in silages during aerobic storage.

The addition of microbial inoculant into miscanthus biomass improved the aerobic stability of silages obtained. An increase of temperature over ambient temperature in the control silages occurred on the 14th day of exposure and after 26 days in the inoculated silages.

Silages from cordgrass were characterised by very long aerobic stability which lasted 27 days in relation to the control silages and 28 days in relation to the inoculated silages. In the control silages, secondary fermentation processes started to develop earlier (after 17 days of exposure) than in the inoculated

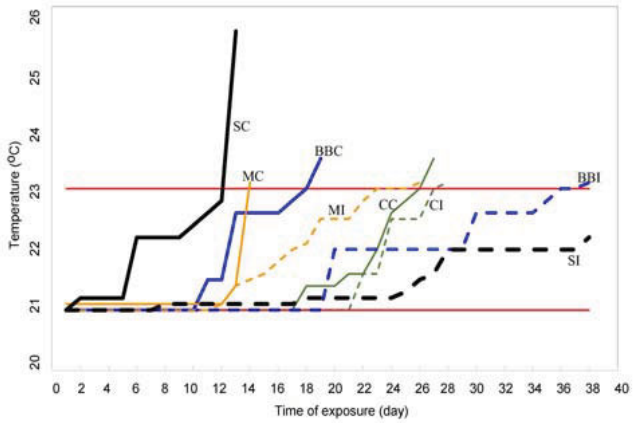

Fig. 1. Temperature changes in the silages during exposure to aerobic condition; CC = control cordgrass, CI = inoculated cordgrass, BBC = control big bluestem, BBI = inoculated big bluestem, MC = control miscanthus, MI = inoculated miscanthus, SC = control switchgrass, SI = inoculated switchgrass; source: own study

silages (after 21 days of exposure), which was manifested by a gradual increase in silages temperature.

Similar effect of silage additive on aerobic stability was observed in relation to switchgrass silages. Aerobic stability of the silages made with the inoculant addition was improved compared with aerobic stability of the control silages. An increase in temperature of the control silages above ambient temperature occurred after 12 days of exposure to aerobic conditions. On the first day of exposure to aerobic conditions the secondary fermentation began, manifesting as a gradual increase in the temperature of the control silages above the ambient temperature. Aerobic stability of the inoculated silages was observed throughout the duration of the experiment, i.e. for 37 days.

Aerobic stability of the control silages made from big bluestem lasted 18 days, while aerobic stability of the inoculated silages was observed during the entire period of experiment, i.e. for 37 days. Second fermentation processes started in the inoculated silages after 19 days, and in the control silages much earlier, i.e. after 10 days of exposure to aerobic conditions.

EFFECT OF ENSILING ON METHANE YIELD

The control silages had methane yields of 267.7–390.0 $Nm^3\ Mg^{-1}$ *ODM*, while inoculated silages had yields of 331.4–399.9 $Nm^3\ Mg^{-1}$ *ODM*. Only cordgrass silages showed a significant effect of silage additive on methane yield. When compared to the control silages, inoculated cordgrass silages produced about 23% higher methane ($P \leq 0.05$) – Figure 2.

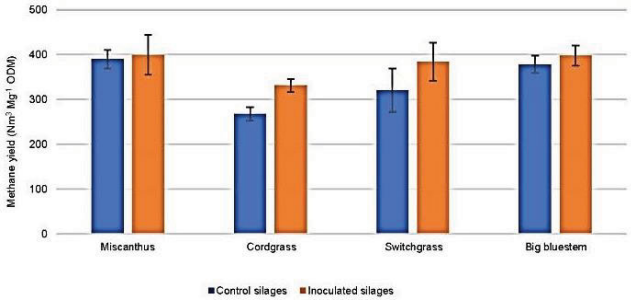

Fig. 2. Methane yield from perennial grasses silages prepared with or without silage additive (± standard deviation); source: own study

The content of methane in the biogas obtained from investigated grasses was at the range of 55–56% regardless of the method of silages preparation.

EFFECT OF SILAGE ADDITIVE ON THE KINETICS OF BIOGAS PRODUCTION

The parameters of the modified Gompertz model depending on the silage used for anaerobic digestion and the assessment of the model's fit to the data using determination coefficient (R^2) and the global error (δ_g) are shown in Table 4. Determination coefficients in the range of 97.4–99.6% and global errors not greater than 8.04% indicated a good adjustment of the modified Gompertz model to the experimental data.

The highest daily biogas production rate was obtained in the case of miscanthus silage prepared with the inoculant addition: 61.5 $Nm^3 \cdot Mg^{-1}$ *ODM*, and the lowest from the control switch-

Table 4. Kinetic parameters of biogas production from ensilaged grasses with or without silage additive based on the modified Gompertz model

Grass	Silage	Regression coefficient			Time after which 90% of biogas was produced (day)	R^2	δ_g
		P_{max}	R_{max}	λ (day)			
		$m^3 \cdot Mg^{-1}$ *ODM*				%	
Miscanthus	C	878.7	38.3	2.25	20.51	98.9	5.20
	I	733.2	61.5	0.20	12.50	99.4	3.28
Cordgrass	C	619.9	34.4	0.01	13.60	99.1	4.20
	I	777.3	39.0	1.55	16.43	97.4	8.04
Switchgrass	C	846.9	32.5	1.56	22.13	99.1	4.61
	I	599.5	42.6	0.01	15.20	99.6	2.63
Big bluestem	C	807.8	39.6	1.12	17.71	99.6	3.15
	I	806.3	53.1	0.83	14.59	99.2	4.22

Explanations: C = control silage, I = inoculated silage, P_{max} = the maximum potential of biogas production, R_{max} = the maximum biogas production rate, λ = the lag phase, R^2 = determination coefficient, δ_g = global error.
Source: own study.

grass silages: 32.5 $Nm^3 \cdot Mg^{-1}$ *ODM* . Based on the determined regression coefficients it was found that the addition of the inoculant during ensiling had beneficial effect on the kinetic of biogas production from miscanthus, switchgrass and big bluestem grasses. The lag phase (the period from the setting of anaerobic digestion to the minimum, measurable amount of biogas) of AD process of the inoculated silages was shorter compared to the lag phase of AD process of the control silages. It was also observed that 90% of total volume of biogas from miscanthus, switchgrass and big bluestem silages prepared with the inoculant addition was obtained within a shorter period of time compared to the control silages (in relation to the silages from miscanthus prepared with the inoculant addition the time of 90% biogas production has been shortened by almost half compared with the control silage). The maximum daily biogas production from all silages inoculated with the silage additive was higher than the control silages (Tab. 4).

The curves of cumulative biogas production from miscanthus, switchgrass or big bluestem as shown in Figure 3 indicated that biogas production from the inoculated silages achieved a *plateau* after shorter period of time compared to the control silages. However, the increase in daily biogas production was not related to the increase in cumulative biogas yield obtained from miscanthus, switchgrass and big bluestem. In relation to cordgrass higher methane yield was obtained from the inoculated silages that the controls, but the biogas production has been slow down, which was indicated by i.a. much longer lag phage. The reason of these observations could be connected with the effect of inhibitory compounds released from lignocellulose complex as a results of silage additive activity. But this issue needs further investigations. The results of the kinetics study using the modified Gompertz model presented above confirmed its high usefulness in describing the parameters of biogas production because of its simplicity and well-fitting to batch data, as it was also confirmed by many other authors [Deepanraj *et al.* 2015].

Ensiling as a popular method of biomass preservation used in feed production. High quality of silage is very important not only in animal nutrition, but also in the use of silages as a substrate for biogas production. Silage additives (enzymes, LAB inoculant) are usually recommended as ensiling process stimulants in grassland biomass [Herrmann *et al.* 2011; Texeira Franco *et al.* 2016]. However, another author suggested, that silage additives are more recommended for poorly ensilable crops with e.g. low *WSC* content [Kalać 2011].

The fresh biomass of investigated grasses, such as species used in this study, were characterised by low *WSC* content (2.5–7.6% *DM*) thus the use of silage additive in this case was justified. Sufficient *WSC* content is necessary for proper acidification of ensiled plant material. Nevertheless, the investigated grasses were susceptible to ensiling, although the ensiling process was not intense.

The lack of differences between *DM* digestibility of the fresh and ensilaged biomass was also observed (Tab. 1). Moreover, pH of obtained silages was typical for silages prepared from the biomass with high *DM* [Kalać 2011], however *DM* of investigated grasses before ensiling was low, especially in relation to miscanthus and big bluestem (17.2 and 21.5%, respectively). A moisture of plant material may increase the amount of energy needed in the ensiling process [Lisowski *et al.* 2017], as well as the

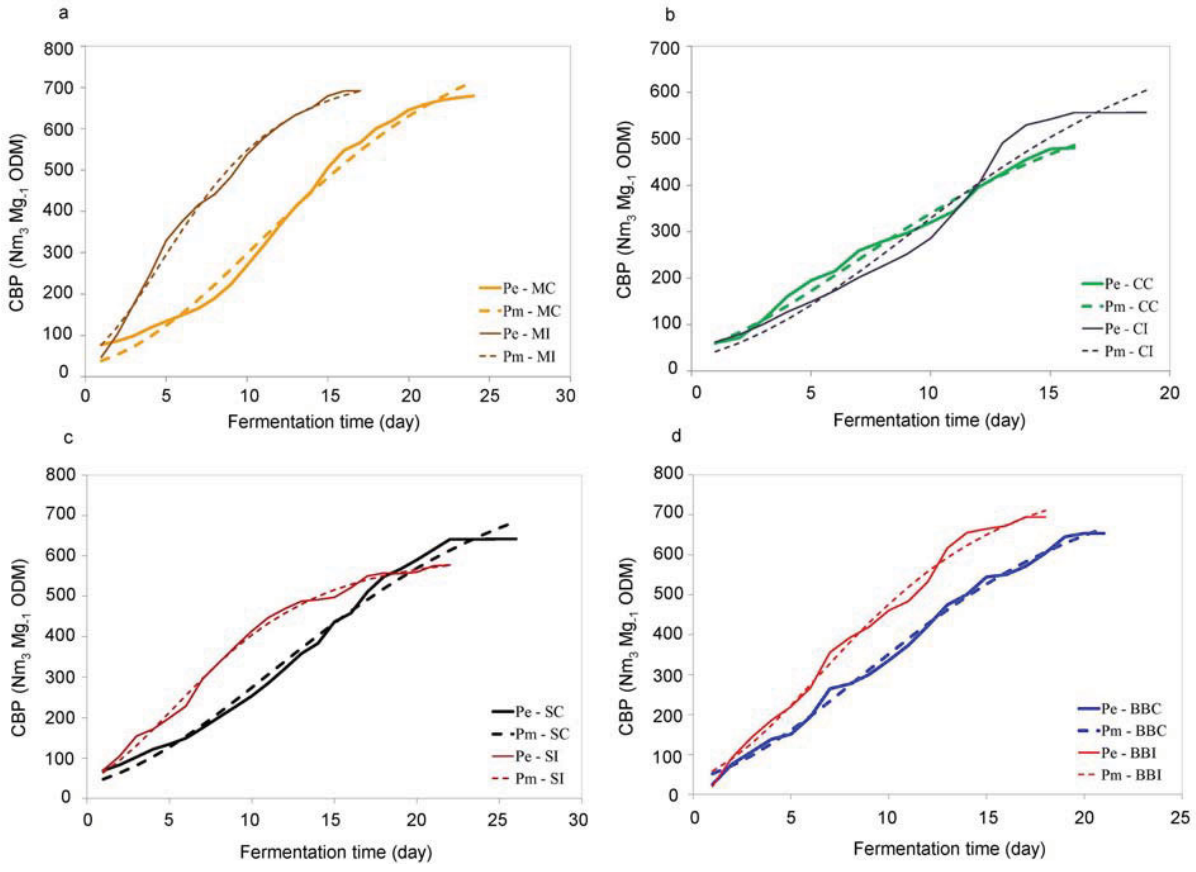

Fig. 3. Cumulative biogas production from the control (C) and inoculated (I) silages: a) miscanthus (M), b) cordgrass (C), c) switchgrass (S), d) big bluestem (BB); Pe = experimental plots, Pm = modified Gompertz plots; source: own study

risk of leaking cell juice during silage storage [Texeira Franco *et al.* 2016]. In this study higher *DM* loss was observed in the case of cordgrass, switchgrass and big bluestem silages prepared with the inoculant compared to the control silages, however *DM* loss rate was low and in the range of *DM* losses described in the literature [Emery *et al.* 2014; Whittaker *et al.* 2016], that is why the effect of the inoculant on the dry matter loss can be of minor importance.

In the presented study the addition of LAB to biomass had no effect on lactic acid content in the obtained silages (except from cordgrass silages), but the content of acetic acid was increased compared to the control silages. It was shown that the activity of the lactic acid bacteria strain utilised in the added inoculant resulted in high level of acetic acid [Muck *et al.* 2018]. *Lactobacillus buchneri* species may convert moderate levels of lactic acid to acetic acid and 1,2-propanediol [Oude Elferink *et al.* 2001]. *Lactobacillus buchneri* is a heterofermentative species that can convert hexoses and pentoses, resulting in lactic and acetic acid production [Texeira Franco *et al.* 2016]. Pentoses like xylose and arabinose are monomers that make up hemicellulose [Emery *et al.* 2014]. In the absence of hexoses, heterofermentative LAB strains can ferment pentoses via the pentosophosphate pathway, which results in the formation of acetic acid [Khalid 2011]. This statement could be the explanation why the silages prepared with the bacterial inoculant addition had lower hemicellulose content than the controls (in relation to cordgrass and switchgrass), as it was observed in presented study. While high amounts of acetic acid in silages intended for ruminants are undesirable, large quantities of this acid are necessary for the AD process because acetic acid is a precursor of methane [Vervaeren *et al.* 2010].

Using heterofermentative LAB strains as inoculants for silage preparation intended for biogas production seems to be very desirable. However, it was proved that heterofermentative pathway of sugars fermentation causes increased dry matter losses compared to homofermentative pathway, which leads to primarily lactic acid formation [Borreani *et al.* 2018]. For economical and sustainable use of crops for biogas production it is important to obtain low-loss preservation of plant material. Some authors observed acetic acid increase in silages from maize, sorghum hybrid, forage rye and triticale treated with both homo- and heterofermentative LAB and considerably higher *ODM* losses compared with silages without microbial additive [Herrmann *et al.* 2011]. In another study, addition of heterofermentative strain of *Lactobacillus brevis* onto switchgrass biomass during ensiling resulted to more than twice lower *DM* loss compared to untreated silage [Zhao *et al.* 2017]. In yet another study, *DM* loss was reduced during ensiling of *Miscanthus* × *giganteus* biomass treated with heterofermentative LAB strains compared to silages not treated with LAB [Whittaker *et al.* 2016].

Silage additives, such as *Lactobacillus buchneri* species, have an excellent impact on aerobic stability [Filya 2003] and are likely to reduce BMP losses after feed-out [Texeira Franco *et al.* 2016]. Furthermore, because acetic acid is a precursor of methane, silages with high acetic acid concentrations are said to be a promising substrate for biogas production [Vervaeren *et al.* 2010]. Higher biogas yields from silages treated with the silage supplement and with higher acetic content were expected as a result of this. However, despite the increased acetic acid concentration in the silages prepared with the inoculant, higher methane output was not obtained from all treated silages compared to the specific controls in the presented study. Another group of researchers also found no difference in biogas production rates between treated and untreated *Miscanthus* × *giganteus* silages, despite the fact that silages treated with homo- or heterofermentative lactic acid bacteria produced more lactic and acetic acid than "poor" control silages [Whittaker *et al.* 2016].

In the literature both positive and negative impact of plant species as well as silage additives on methane yield from ensiled biomass have been described. For example in the study of Zhao *et al.* [2017] more biogas was obtained from ensiled switchgrass compared to the amount of biogas obtained from not ensiled biomass. The main reason of this effect the authors explained by the conversion of cell wall structures during ensiling process, which accelerates the breakdown of these molecules by methanogens during AD process. Moreover, the methane potential rate of switchgrass ensiled with heterofermentative LAB was higher than of the untreated silages or raw material [Zhao *et al.* 2017].

Similar results were obtained by Kupryś-Caruk *et al.* [2021], who prepared silages from *Spartina pectinata* with *L. buchneri* M B/00077 addition. The addition of LAB increased the content of acetic and propionic acid in silages and 20% more biogas was obtained from inoculated silages compared with those treated with commercial enzymes.

On the other hand, the study of Pakarinen *et al.* [2008] showed that the addition of *Lactobacillus plantarum* and *Pediococcus acidilactici* to grasses did not increase the biogas production from the silages despite increased acetic acid concentration in low and medium solid crops. In another study the influence of different chemical and biological inoculants, added to maize, sorghum and rye, on methane productivity was investigated. Addition of bacterial inoculants had a positive effect on methane production compared to untreated silages. But after taking into account higher dry matter loss as a result of ensiling process in silages prepared with bacteria addition compared to control silages, it was concluded that LAB did not contribute to a significantly higher methane productivity [Herrmann *et al.* 2011].

High content of acetic acid in silages prepared with the inoculant addition may explain higher daily biogas production rate compared to AD of the control silages, which was observed in the present study. Similar results were obtained by other authors, who observed an increase of R_{max}, when using silages from switchgrass prepared with LAB, enzyme or the mixture of LAB and enzyme compared to the control silages or non-ensiled raw material. The authors claimed that accumulation of different volatile compounds during ensiling, which provided abundant nutrients for methanogens, contributed to rapid methane fermentation [Zhao *et al.* 2017]. But on the other hand, results of another study showed that lactic, acetic acid and ethanol content did not show a significant correlation coefficient with biogas potential rate [Whittaker *et al.* 2016]. This could be an explanation of the lack of differences between methane yield obtained in this study from the treated and untreated silages despite the differences in organic acids concentrations.

CONCLUSIONS

In the research the biomass of perennial energy grasses was used, which are still little known in terms of their suitability for biogas production after preservation by ensiling. The influence of

bacterial inoculants on the production of biogas from the obtained perennial grass silages is also little known in the literature.

The silage inoculant consisted of heterofermentative lactic acid bacteria strain of *Lactobacillus buchneri* species added to biomass of *Miscanthus* × *giganteus*, *Spartina pectinata*, *Panicum virgatum* and *Andropogon gerardii* influenced on the quality of all silages obtained: the content of acetic acid increased, aerobic stability was improved compared to untreated silages. Using the bacterial inoculant during ensiling was beneficial in terms of reducing the duration of biogas production from the silages prepared from miscanthus, switchgrass and big bluestem, but it had no effect on the methane yield. Addition of silage inoculant influenced on higher methane yield from cordgrass silages, but had no effect on the rate of biogas production. It was concluded that in terms of increasing methane yield from a given ensiled feedstock, silage additives, which decompose structural polysaccharides effectively, would be useful but the inhibitory effect of the compounds released from lignocellulose complex on AD process should be considered and further investigated. The modified Gompertz model well reflects the kinetics of biogas production and can be used to predict and study AD process in the field of investigated plant materials under process conditions.

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