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Methanogenic potential of biomass from roadside verges preserved with various additives*

Cezary Purwin, Barbara Pysera, Maja Fijałkowska, Krzysztof Lipiński

Department of Animal Nutrition and Feed Science, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-718 Olsztyn, Poland

Corresponding author: Cezary Purwin, Department of Animal Nutrition and Feed Science, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-718 Olsztyn, Poland; Phone (+48) 89 523 3232; E-mail: purwin@uwm.edu.pl

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ABSTRACT

The aim of the present research was to evaluate the chemical composition and storage capacities, as well as the efficiency and composition of biogas from biomass collected from roadside verges. The biomass was collected in July and October and then preserved in microsilos (10L) with and without formic acid, bacterial inoculant, bacterial-enzymatic preparation, enzymatic preparation. After 180 days of storage, biomass samples were analyzed for chemical composition, organic dry matter (ODM) losses and biogas and methane yield (Oxi-Top Control). Biomass from the summer period had a higher ($p<0.01$) content of dry matter, neutral detergent fiber, hemicellulose and cellulose and a lower ($p<0.01$) content of ether extract and acid detergent fiber. Loss of organic matter during preservation and biomass storage without additives was higher in the material from the summer period. However, when compared with the autumn period, summer biomass stored without additives had a higher methane production potential (288 vs. 215 L_N $CH_4 \cdot kg^{-1}$ ODM). The additive which most effectively reduced the loss of organic matter was formic acid. However, the most beneficial for biogas efficiency and methane were the bacterial-enzymatic preparation (summer harvest) and addition of formic acid (autumn harvest). Methane efficiency equaled 314 and 299 $L_N \cdot kg^{-1}$ ODM, and its concentration in biogas amounted to 60.4 and 59.4% for summer and autumn biomass, respectively. The results indicated the possibility of storing and using biomass from roadside verges as a source of biogas. The primary aim of using added preservatives was to reduce the loss of organic matter during biomass storage as well as to improve the efficiency of methanogenesis.

INTRODUCTION

Energy production from renewable sources is a key issue in environmental protection and balanced development of manufacturing and services (Stelmach et al. 2010). The recent development of small agricultural biogas stations has resulted in plant biomass becoming a precious raw material.

Cultivated plants which are most frequently used as substrates in biogas production include corn, rye, triticale and sugar beet (Mikołajczak et al. 2009). However, biomass production from these plants for energy purposes is connected with excluding significant areas of arable land from food production (Gołaszewski 2011). Potential sources of substrates for agricultural biogas stations can be waste produced during maintenance of green areas, including biomass from roadside verges that comprise grass, weeds and leaves falling from roadside trees (Pieńkowski 2010). Production of biogas from this biomass facilitates utilization of this waste and solves the problem of its management.

The aim of the present research was to evaluate the chemical composition and storage capacities, as well as the

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potential for biogas production from biomass collected from roadside verges in summer and autumn.

MATERIALS AND METHODS

Biomass from roadside verges was collected with a mower in summer (July) and autumn (October). It was then ground into strand with a theoretical length of 12mm. This biomass was then placed in plastic microsilos (10L), sealed with silicone and equipped with a valve to release fermentation gases. Every trial was repeated three times. After compacting, microsilos were weighed so as to gain an identical degree of densification of a given biomass material. Organic dry matter (ODM) losses were estimated on the basis of the mass of microsilos' content and ODM concentration before sealing and after 180 days of storage.

Biomass was stored:

- 0 – without additives;
- A – with the addition of 96% formic acid, $5g \cdot kg^{-1}$ of fresh material;
- B – with fermentation stimulator in a dose of $5x10^7$CFU including:
  - *Lactobacillus plantarum* KKP/593/P,
  - *L. plantarum* KKP/788/P,
  - *L. brevis* KKP 839,
  - *L. buchneri* KKP 907;
- C – with $0.005g \cdot kg^{-1}$ endo-1,4-beta-glucanase 100 JCMC, endo-xylanase 100 JX and fermentation stimulator in a dose of $5x10^7$CFU including:
  - *L. plantarum* KKP/593/P,
  - *L. plantarum* KKP/788/P,
  - *L. brevis* KKP 839,
  - *L. buchneri* KKP 907;
- D1 – with $0.004g \cdot kg^{-1}$ beta-glucanase 300 JCMC, endo-xylanase 300 JX, glucoamylase 1500 JGA;
- D2 – with $0.004g \cdot kg^{-1}$ hemicellulose and cellulose with the activity of 94 I.U.

The chemical analysis of biomass included: basic chemical composition according to standard methods (AOAC 2005), the content of water soluble carbohydrates (WSC) with Anthrone Method (Thomas 1977), fractions of structural carbohydrates: neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were identified with ANKOM 220, the content of hemicellulose was estimated as the difference of NDF-ADF, whereas the content of cellulose as the difference of ADF-ADL (Van Soest et al. 1991).

The research of biogas potential of stored biomass was conducted in the Department of Environmental Protection Engineering, University of Warmia and Mazury in Olsztyn (Poland). The biomass was ground mechanically with a cutting mill ROBO 3000 for medium-size 2–3mm particles. Respirometric measurements were conducted with Oxi–Top Control sets.

The amount of the batch material of a particular biomass used for respirometric measurements was estimated on the basis of its dry matter (DM) content as well as organic dry matter (ODM). The measurements were conducted at 35°C, the time span was 40 days. The sets were implanted with a batch from the anaerobic reactor designed to decompose plant substrate. The batch was „starved" before conducting respirometric measurements. The content of methane in biogas was provided in volume percentage (%v/v). The amount of produced biogas and methane was provided in normalized values, i.e. in normal liters (L_N) per kilogram (kg) of organic dry matter (taking a normalized unit of gas volume L_N or m^3 in normal conditions, i.e. pressure=1013.25mbar, temperature=0°C, humidity=0%.

RESULTS

The chemical composition of fresh biomass collected in summer and autumn varied significantly (Table 1). Green forage collected in July had a higher content of dry

Table 1. Chemical composition of fresh biomass from roadside verges.

Item	Summer harvest	Autumn harvest	SEM
Dry matter (DM, %)	39.1^{A}	32.2^{B}	1.6
Organic dry matter (ODM, % DM)	91.0	90.1	0.5
Crude protein (% DM)	8.7^{B}	12.0^{A}	0.8
Ether extract (% DM)	1.6^{B}	5.2^{A}	0.8
Water soluble carbohydrates (WSC, % DM)	8.4^{a}	7.1^{a}	0.3
Lignin (% DM)	6.1^{A}	12.2^{B}	1.3
Hemicellulose (% DM)	24.0^{A}	9.6^{B}	4.4
Cellulose (% DM)	36.3^{A}	23.4^{B}	2.9

SEM – standard error of the mean

Means in the same line with different superscripts differed significantly at ^{AB} (P<0.01), ^{ab} (P<0.05) matter, hemicellulose, cellulose ($p<0.01$) and water soluble carbohydrates (WSC) ($p<0.05$). The biomass collected in October had a greater amount of general protein, ether extract and lignin ($p<0.01$). Despite these differences in the chemical composition of organic substances, the overall content of ODM in both types of biomass did not differ. The content of dry matter and organic substance in the silaged biomass was close to the concentration of these components before ensilaging (Table 2). However, a lower content of WSC in both types of biomass was observed. A higher amount of lignin during storage was observed only in the autumn biomass. In the course of fermentation, the degree of ensilaging of both types of biomass did not exceed 4.99, which does not guarantee stable biomass storage. Neither of the used preserving additives had an influence on the amount of DM and ODM in the biomass collected in autumn. The highest amount of DM in preserved biomass from summer was observed in the combination with formic acid, whereas the lowest with the addition of hemicellulose and cellulose ($p<0.01$).

Enzymatic additives (D_1, D_2) as well as the bacterial-enzymatic additive (C) lowered the content of hemicellulose and cellulose in the biomass from autumn period and simultaneously increased the concentration of water soluble carbohydrates (D_1, D_2). This effect was not observed in

Table 2. Chemical composition of biomass from roadside verges after 180 days of storage. ODM – organic dry matter, WSC – water soluble carbohydrates.

Item	Additive						SEM
	0	A	B	C	D_1	D_2	
Summer harvest							
pH	5.41	5.04	5.72	4.99	5.27	5.31	0.1
Dry matter (DM, %)	38.8^{a}	39.3^{A}	38.7^{a}	38.0	38.0	36.3^{Bb}	0.4
ODM (% DM)	89.7^{b}	91.3	91.1	91.4	92.7^{a}	92.0	0.7
WSC (% DM)	0.3^{B}	7.8^{A}	1.4^{B}	6.9^{A}	1.1^{B}	0.7^{B}	0.1
Lignin (% DM)	5.9	4.7^{B}	5.4	5.9	6.6^{A}	6.3^{A}	0.1
Hemicellulose (% DM)	26.4^{A}	17.6^{C}	22.7^{B}	23.2^{B}	18.8^{C}	19.2^{C}	0.7
Cellulose (% DM)	37.5^{b}	36.4^{c}	38.2^{b}	39.6^{a}	39.5^{a}	36.5^{b}	0.7
ODM losses (% DM)	8.89^{A}	3.89^{B}	6.19^{b}	7.72	9.38^{A}	12.9^{Aa}	0.5
Autumn harvest							
pH	5.56	5.11	5.88	5.51	5.31	5.26	0.1
Dry matter (DM, %)	32.8	33.8	33.2	33.1	32.3	32.2	0.4
ODM (% DM)	90.3	91.3	91.2	90.5	91.8	91.3	0.6
WSC(% DM)	1.5^{D}	5.4^{A}	3.3^{C}	3.4^{C}	4.5^{B}	4.7^{B}	0.1
Lignin (% DM)	17.9^{A}	15.6^{Ba}	16.0^{B}	12.3^{D}	14.2^{Cb}	16.3^{B}	0.4
Hemicellulose (% DM)	9.2^{A}	9.4^{A}	8.0^{B}	1.8^{D}	4.1^{C}	2.1^{D}	0.1
Cellulose (% DM)	26.1^{ac}	25.1^{a}	27.9^{c}	23.0^{b}	21.3^{b}	23.5^{b}	0.5
ODM losses (% DM)	7.11	4.95^{B}	5.16	8.17	9.71^{A}	10.8^{A}	0.4

0 – without additives;
A – the addition of 96 % formic acid;
B – fermentation stimulator (*Lactobacillus plantarum, L. plantarum, L. brevis, L. buchneri*);
C – fermentation stimulator (*L. plantarum, L. plantarum, L. brevis, L. buchneri*) and endo-1,4-beta-glucanase, endo-xylanase;
D_1 – beta-glucanase, endo-xylanase, glucoamylase;
D_2 – hemicellulose and cellulose.
SEM – standard error of the mean.
Means in the same line with different superscripts differ significantly at ^{AB} ($P<0.01$), ^{ab} ($P<0.05$).

summer biomass. Addition of formic acid (A) with regard to both types of biomass had an impact on the higher content of DM and WSC. The content of hemicellulose in biomass from summer with this additive was the lowest, but the highest in autumn biomass.

Addition of formic acid reduced the loss of ODM during storage of both biomass types. During storage, the loss of summer biomass with formic acid was almost two times lower than biomass stored without additives and addition of D_1. They were also three times lower than biomass stored with D_2 additive ($p<0.01$). A similar relation was observed in the storage of autumn biomass. Addition of formic acid reduced ODM loss, whereas enzymatic additives D_1 and D_2 increased the loss irrespectively of the period of biomass harvest.

Table 3. Biogas and methane yield from roadside verges.

Item	Additive						SEM
	0	A	B	C	D_1	D_2	
Summer harvest							
Biogas ($L_N \cdot kg^{-1}$ODM)	513^{A}	455^{B}	395^{C}	520^{A}	462^{B}	442^{B}	9.4
Methane (%)	56.1^{A}	54.9^{B}	50.4^{B}	60.4^{A}	53.7^{B}	57.7^{A}	1.1
Methane ($L_N \cdot kg^{-1}$ODM)	288^{B}	250^{B}	199^{C}	314^{A}	248^{B}	255^{B}	7.0
Methane/FM	100^{A}	90^{B}	70^{C}	109^{A}	87^{B}	85^{B}	2.4
Autumn harvest							
Biogas ($L_N \cdot kg^{-1}$ODM)	394^{B}	503^{A}	399^{B}	346^{C}	445^{B}	450^{B}	11.4
Methane (%)	54.5^{a}	59.4^{Ab}	50.5^{Bb}	53.8^{B}	51.5^{Bb}	55.3^{a}	1.1
Methane ($L_N \cdot kg^{-1}$ODM)	215^{Bb}	299^{A}	201^{Bb}	186^{Cb}	229^{B}	249^{Ba}	5.5
Methane/FM	64^{CD}	92^{A}	61^{CD}	56^{D}	68^{Bb}	78^{Ba}	2.5

ODM – organic dry matter; FM – fresh matter.
SEM – standard error of the mean.
Means in the same line with different superscripts differ significantly at ^{AB} ($P<0.01$), ^{ab} ($P<0.05$).

The amount of biogas from summer biomass stored without additives (0) was $513L_N$ per 1kg ODM (Table 3). The content of methane in this biogas was 56.1%. In contrast, autumn biomass stored without additives had a lower biogas potential. The amount of biogas gained from this biomass was $394L_N \cdot kg^{-1}$, and methane $215L_N \cdot kg^{-1}$ ODM. Additives which were used in storage had a varied influence on the amount of produced biogas and methane. In the case of summer biomass, biogas potential was most effectively increased by addition of bacterial-enzymatic preparation (C), while the least effective was the bacterial implant (B). The biogas efficiency of the treatment with the bacterial-enzymatic (C) preparation ($520L_N \cdot kg^{-1}$ ODM), and contribution of methane in this biogas (60.4%) were significantly higher ($p<0.01$) in comparison to other additives. With regard to autumn biomass, the highest biogas potential resulted from the treatment with the addition of formic acid (A). Production of biogas from 1kg ODM of this biomass was higher by 27.7% when compared with control biomass, and methane increased by 39.1% ($p<0.01$). In contrast, bacterial-enzymatic preparation (C) turned out to be highly ineffective. These results were lower ($p<0.01$) than those observed for biomass stored with other additives and for control biomass, reaching 346 and $186L_N \cdot kg^{-1}$ ODM for biogas and methane, respectively.

DISCUSSION

The efficiency of biogas and methane production from biomass collected from green areas is highly varied and depends on the period of harvest and botanical composition, including the amount of weeds (Massė et al. 2011; Mikołajczak et al. 2009). Prochnow et al. (2008) observe that the gain of methane from fresh grass biomass in the phase of earing and at the beginning of flowering amounted to $221\text{-}362L_N \cdot kg^{-1}$ ODM and was reduced to $171\text{-}153L_N \cdot kg^{-1}$ in further development phases. In other research (Massė et al. 2011), the production of methane from switchgrass harvested in mid-summer, late summer and early autumn reached respectively 0.233, 0.217 and $0.185L_N \cdot g^{-1}$ ODM. As previously observed, we found higher potential for methane production in control biomass harvested in summer when compared to autumn (288 vs. 215 L_N methane·kg^{-1} ODM). Our results suggest that this was due to differences in chemical composition, including the content of the lignocellulose fraction. Biodegradation of cellulose is higher than of lignin, and therefore the biomass with a small content of lignin is more useful for the processes of anaerobic methane fermentation (Podlaski et al. 2010). Analyzed biomass from summer harvest was composed of various species of grass and weeds, whereas the biomass from autumn harvest was composed of shoots of these plants after the first harvest and leaves from roadside trees (European maple, lime).

The results of Pakarinen et al. (2008) and of Plöchl et al. (2009) concerning the impact of added preservatives on biogas and methane production have been inconclusive. In the current study the most effective additive was formic acid with regard to biomass from autumn harvest and bacterial-enzymatic preparation with regard to summer harvest of biomass. In both cases higher efficiency of biogas production can be explained by greater content of WSC in the stored biomass. In the case of formic acid, it can be explained by a decrease in fermentation preserving more carbohydrates in the biomass during storage. However, in the case of bacterial-enzymatic preparation there is an increased intake of WSC as a result of hydrolysis of structural carbohydrates (Florek et al. 2004). The results confirmed a significant impact of soluble carbohydrates on the biogas efficiency of substrates (McEniry and O'Kiely 2012; Mikołajczak et al. 2009).

CONCLUSIONS

Biomass from green areas, specifically roadside verges, can become a significant source of raw material for biogas production. Biomass from summer harvest turned out to be a better material. Summer biomass showed considerable advantages in reducing storage losses than autumn biomasses, whilst the latter demonstrated greater efficiency on biogas production. The effect of preserving additives can be varied with regard to the chemical composition of biomass, ODM loss, and methane efficiency.

The main purpose of using preserving additives is justified by the reduction of loss of organic matter during storage but also by the improved efficiency of methanogenesis.

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