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## Comparison of biogas production from wild and cultivated varieties of reed canary grass

Marta Oleszek<sup>a,\*</sup>, Aleksandra Król<sup>a</sup>, Jerzy Tys<sup>a</sup>, Mariusz Matyka<sup>b</sup>, Mariusz Kulik<sup>c</sup><sup>a</sup> Institute of Agrophysics, Polish Academy of Sciences, Doswiadczalna 4, 20-290 Lublin, Poland<sup>b</sup> Institute of Soil Science and Plant Cultivation, State Research Institute, Czartoryskich 8, 24-100 Pulawy, Poland<sup>c</sup> University of Life Sciences, Akademicka 13, 20-950 Lublin, Poland

### HIGHLIGHTS

- Effect of habitat of reed canary grass on biogas yield was investigated.
- Wild variety had greater indigestible lignocellulose content than cultivated one.
- High indigestible fraction of crude fiber reduce biogas quantity and quality.
- Frequent mowing and fertilization helps to keep lignification at a low level.

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### ABSTRACT

The chemical composition and efficiency of biogas production in the methane fermentation process of silages of wild and cultivated varieties of reed canary grass were compared. An attempt was made to answer the question on how the habitat and the way of utilization of plants affect chemical composition and biogas yield. Physicochemical properties such as dry matter, organic dry matter, protein, fat, crude fiber fraction, macro- and microelements content were considered. The anaerobic digestion process and FTIR analysis were also carried out. The results showed that the two varieties differ essentially in their physical and chemical properties. The cultivated variety was characterized by higher biogas yield (406 Ndm<sup>3</sup> kg<sup>-1</sup> VS) than the wild one (120 Ndm<sup>3</sup> kg<sup>-1</sup> VS). This was probably related to the chemical composition of plants, especially the high content of indigestible crude fiber fractions and ash. These components could reduce biogas quantity and quality.

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## 1. Introduction

Reed canary grass (*Phalaris arundinacea* L.) is one of perennial grasses belonging to the Poaceae family. Due to the high yield per hectare, it is cultivated as a potential energy plant, both for combustion and for biogas or bioethanol production (Kandel et al., 2013; Kallioinen et al., 2012).

It occurs also in natural grass stands, most commonly in the vicinity of water basins (Stražil, 2012). It can be cultivated on low value areas, which are not needed for food production. In Poland, it has been largely used as a fodder crop until recently. Currently, following the implementation of agri-environmental schemes, especially those concerning extensive grassland management, the biomass has a low forage value. Furthermore, due to the lack of melioration and low profitability of livestock production,

the demand for pasture forage and hay decreased, which consequently contributed to the increase in the number of unused meadows and pastures. For example, in Lubelskie Province permanent grasslands are estimated in 2012 at 231 134 ha, out of which 25.1% were not used. This makes 58 015 ha (48 096 ha for the whole Poland) that can be used for biogas production (CSO, 2013).

Therefore, a change in the manner of utilization thereof is necessary. One of the possibilities is utilization of this type of biomass for energy purposes, such as biogas production (Prochnow et al., 2009).

Biogas is a mixture of gases (mainly methane and carbon dioxide) formed in the methane fermentation process (Oleszek et al., 2013). It is mostly used to generate electricity and heat in cogeneration (Igliński et al., 2012). In addition, it can be conditioned to natural gas quality and injected into a gas grid. The quantity and quality of biogas depend on the substrate used in the process. The most commonly used substrates are manure, organic waste, and energy crops in the form of silage such as corn, rye, or

\* Corresponding author. Tel.: +48 817445061.

E-mail address: [m.oleszek@ipan.lublin.pl](mailto:m.oleszek@ipan.lublin.pl) (M. Oleszek).

perennial grasses (Budzianowski, 2012). Previous studies have confirmed the high potential of application of reed canary grass growing in the field as an energy crop (Geber, 2000; Lakaniemi et al., 2011; Kacprzak et al., 2012). However, there are no studies concerning the application of wild plant species as a biomass source. Given the low cost of production the wild species as well as its large unused resources may be a good alternative for cultivated varieties.

The aim of this study was comparison of the potential for methane production of wild and cultivated reed canary grasses and assessment of their suitability for biogas production.

## 2. Methods

### 2.1. Research material

Silage of wild and cultivated varieties of reed canary grass was used for testing. The cultivated variety (Swedish Bamse) was obtained from a field experiment located in the Experimental Station of IUNG in Osiny (51°28'N, 21°39'E). The experiment was conducted in a random sub-blocks with 4 replications. The plant was harvested regularly twice a year since 2004. A naturally occurring variety originating from unused meadow communities of *Ch. Ass. P. arundinacea* from the valley of the Wieprz-Krzna Channel in Lublin Province was used as a wild species. The harvested material was chopped and ensiled. Silages were prepared in June 2012 and then they were subjected to physical and chemical analyses and to measurements of the methane fermentation process.

### 2.2. Chemical analysis

Total solids (TS), volatile solids (VS), and ash were determined using the gravimetric method after drying at 105 °C and 550 °C, respectively, according with PN-EN 12880 and PN-EN 12779. Total nitrogen and protein contents were analyzed using the Kjeldahl method, the ammonium nitrogen was measured by spectrophotometry, and macro- and microelements were tested by the Inductive Coupled Plasma-Optical Emission Spectrometer (ICP-OES). The analysis of the total organic carbon (TOC) was performed on the TOC-V CPN analyser with Solid Sample Combustion Unit SSM-5000A, according with manufacturer protocol. Crude fat was determined according to Polish norm PN-76/R-64753. Crude fiber fractions (NDF, ADF, ADL) were evaluated with the van Soest and Wine method (Van Soest and Wine, 1967). Hemicellulose and cellulose contents were calculated based on these parameters by subtracting NDF from ADF and ADL from ADF.

### 2.3. Elemental analysis

In order to determine the content of individual elements, the test samples were subjected to digestion using a microwave mineralizer Berghoff Speedwave Four in Teflon vessels DAP 100. Mixture of 6 ml 65% HNO<sub>3</sub> and 1 ml 30% H<sub>2</sub>O<sub>2</sub> were used for mineralization. The resulting solutions were analyzed by ICP-OES (Thermo Scientific iCAP Series 6500), equipped with a charge injection device (CID) detector and TEVA software. The instrumental parameters are given in Table 1. A multi-element standard solution for ICP-OES containing 6 elements: Cu, Fe, Mg, P, K, and Na (1000 ppm, Analytik-46) and a multi-element standard solution containing 5 elements: B, Ba, Li, S, and Si (40 ppm, Analytik-47) obtained from Inorganic Ventures (US, Virginia) were used for standardization. The wavelengths of 766 nm for K, 186 nm for P, 181 nm for S, 285 nm for Mg, and 589 nm for Na were applied.

**Table 1**

ICP-OES operational parameters for determination of K, P, S, Mg, Na.

Parameter	Value
Auxiliary flow	0.4 L min <sup>-1</sup>
Carrier gas flow rate	0.65 L min <sup>-1</sup>
Coolant gas flow rate	16 L min <sup>-1</sup>
RF power	1150 W
Frequency of RF generator	27.12 MHz
Pump rate	50 rpm
Viewing configuration	Axial

### 2.4. FTIR analysis

For rapid comparison of the two varieties and for determination of lignin and crystallinity of cellulose, an analysis was carried out with the use of a Nicolet 6700 FT-IR spectrometer according to the procedure described by Nawrocka and Cieřła (2013).

### 2.5. Biogas production

Periodic mesophilic fermentation was performed according to the DIN 38414-S8 Protocol (1984). The initial loading of 60 g VS L<sup>-1</sup> and substrate to inoculum ratio (S/I) of 1:1 (based on the VS) was established. The pH was adjusted to 7.0 with sodium bicarbonate. Anaerobic conditions were ensured by blowing nitrogen gas across the reactor volume. Once a day, the composition of the produced gas was checked by an automated analyser. The volume of biogas was determined by the method of liquid displacement (Oleszek and Tys, 2013). The process was performed in three independent replications until the daily yield was lower than 1% of the previous total biogas yield. The values of the biogas volume obtained were converted into standard conditions (1013 mbar, 273 K) (Oslaj et al., 2010).

### 2.6. Statistical analysis

Data of the chemical and elemental composition (Tables 2 and 3) were expressed as the mean ± standard deviation (SD) of three independent replicates. To examine the statistical significance of the differences between the varieties, the Student's *t* test was performed using Statistica 10. The level for accepted statistical significance was *p* < 0.05.

**Table 2**

Chemical composition of two varieties of reed canary grass.

Characteristics	Unit	Mean ± S.D.	
		Wild	Cultivated
TS	wet weight%	35.72 ± 1.02 <sup>a</sup>	28.02 ± 0.86 <sup>b</sup>
VS	TS%	98.46 ± 0.13 <sup>a</sup>	99.46 ± 0.52 <sup>b</sup>
NDF	TS%	63.60 ± 0.56 <sup>a</sup>	70.50 ± 2.36 <sup>b</sup>
ADF	TS%	37.80 ± 0.28 <sup>a</sup>	40.90 ± 0.78 <sup>b</sup>
ADL	TS%	8.04 ± 0.02 <sup>a</sup>	5.04 ± 0.12 <sup>b</sup>
Hemicellulose	TS%	25.80 ± 0.84 <sup>a</sup>	29.60 ± 3.14 <sup>b</sup>
Cellulose	TS%	29.76 ± 0.30 <sup>a</sup>	35.86 ± 0.90 <sup>b</sup>
Lignin	TS%	8.04 ± 0.02 <sup>a</sup>	5.04 ± 0.12 <sup>b</sup>
CP	TS%	9.14 ± 0.26 <sup>a</sup>	8.56 ± 0.44 <sup>a</sup>
CF	TS%	2.05 ± 0.63 <sup>a</sup>	3.07 ± 0.55 <sup>a</sup>
ASH	TS%	1.54 ± 0.13 <sup>a</sup>	0.54 ± 0.06 <sup>b</sup>
pH		5.57 ± 0.20 <sup>a</sup>	4.90 ± 0.25 <sup>b</sup>

FM, fresh matter; TS, total solids; VS, volatile solids; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; CP, crude protein; CF, crude fat.

Mean values with different superscript letters within column differ significantly (*p* < 0.05).

**Table 3**  
Elemental composition of two varieties of reed canary grass.

Element	Unit	Mean $\pm$ S.D.	
		Wild	Cultivated
C	TS%	61.94 $\pm$ 1.25 <sup>a</sup>	55.73 $\pm$ 0.96 <sup>b</sup>
N	TS%	1.46 $\pm$ 0.12 <sup>a</sup>	1.70 $\pm$ 0.05 <sup>b</sup>
K	mg kg <sup>-1</sup>	5834.33 $\pm$ 145.02 <sup>a</sup>	19200.14 $\pm$ 112.03 <sup>b</sup>
P	mg kg <sup>-1</sup>	2525.33 $\pm$ 63.12 <sup>a</sup>	3911.02 $\pm$ 27.33 <sup>b</sup>
S	mg kg <sup>-1</sup>	2497.00 $\pm$ 72.15 <sup>a</sup>	2215.30 $\pm$ 43.65 <sup>b</sup>
Mg	mg kg <sup>-1</sup>	2570.19 $\pm$ 17.22 <sup>a</sup>	1779.01 $\pm$ 22.89 <sup>b</sup>
Na	mg kg <sup>-1</sup>	550.13 $\pm$ 8.91 <sup>a</sup>	177.47 $\pm$ 3.64 <sup>b</sup>
C/N		42 $\pm$ 2.02 <sup>a</sup>	33 $\pm$ 1.08 <sup>b</sup>

Mean values with different superscript letters within column differ significantly ( $p < 0.05$ ).

### 3. Results and discussion

#### 3.1. Chemical composition

The analysis of the chemical composition of both plants showed significant differences ( $p < 0.05$ ) between the wild and cultivated varieties. The greatest differences were found for the TS, NDF, ADL, and ash content (Table 2). The elemental composition analysis revealed significant differences in the content of potassium, phosphorus, and sodium probably caused by fertilization applied in the cultivation (Table 3). The elemental composition can be used as one of the indicators determining usefulness of plant material for fermentation. Especially the C/N ratio is essential and should range between 10 and 30. In case of wild plant forms the C/N ratio has been much higher and that might limit biogas yield (Braun, 1982). Moreover according to literature data the ratio of C:N:P:S should be 600:15:5:1, which is essential for best growth and performance of methanogen bacteria. This ratio is closer to optimal in cultivated species (600:18:4:2) rather than in the wild plants (600:14:2:2) (Weiland, 2001). Additionally determination of elemental composition has an additional meaning for evaluation of the potential usefulness of post-ferment sediments as fertilizer.

The differences in the dry matter content were the result of the varying harvest frequency of both communities. The cultivated variety was mowed twice a year starting from 2004, while the wild one was completely unused. As reported by previous studies, mowing prevents excess contents of total solids at harvest and premature cell wall lignification (Kandel et al., 2013; Geber, 2000). An excess level of maturity and lignification is considered as a factor inhibiting the process of methane production (Massé et al., 2010; Seppälä et al., 2009). The considerably higher ADL fraction confirmed a higher level of lignification in the case of the wild variety. There were no significant differences ( $p > 0.05$ ) in the protein and fat content.

#### 3.2. FTIR analysis

An analysis of the plant material by FTIR (Fourier Transform Infrared Spectroscopy) was carried out for simple and rapid comparison of the two varieties. It was shown that the wild variety had a much higher lignin content than the cultivated one, as evidenced by the absorbance values of 0.0071 and 0.0062 a.u., respectively, at the wave frequency of 1512 cm<sup>-1</sup> (Allison et al., 2009) (Table 4). Moreover, the absorbance ratio of 1420 cm<sup>-1</sup>–898 cm<sup>-1</sup> (A1420/A898) was also investigated. The first peak corresponds to crystalline cellulose and the second one to amorphous cellulose. Their ratio indicates the degree of crystallinity of cellulose contained in plant material and is referred to as the Lateral Order Index (LOI) (Monlau et al., 2012; Teghammar et al., 2012; Jehanipour et al., 2010). The analysis showed that wild reed

**Table 4**  
Results of FTIR analysis.

Variety	LOI A1420/A898	Lignin A1512 [a.u.]
Wild	0.85	0.0071
Cultivated	0.73	0.0062

LOI, lateral order index.

canary grass contained more crystalline cellulose than the cultivated one, as demonstrated by the higher ratio A1420/A898: 0.85 and 0.73, respectively (Table 4). These data confirmed the results of the chemical analyses, namely that the wild reed canary grass included more indigestible components than cultivated one.

#### 3.3. Biogas yield

The methane fermentation results revealed a significantly higher ( $p < 0.05$ ) biogas yield from the cultivated variety of canary grass than from the wild one: 406  $\pm$  21 Ndm<sup>3</sup> kg<sup>-1</sup> VS and 120  $\pm$  16 Ndm<sup>3</sup> kg<sup>-1</sup> VS, respectively. This was in agreement with the research of Triolo et al. (2012), who reported that wild grasses showed a clear trend for lower biodegradability than lawn cuttings. Graphs of the daily biogas yield showed that the silage of cultivated reed canary grass started to ferment faster than that prepared from the wild variety, demonstrating significant biogas yields even in the first days of the trial (Fig. 1). In both cases, there were three peaks of fermentation, wherein the highest yield was

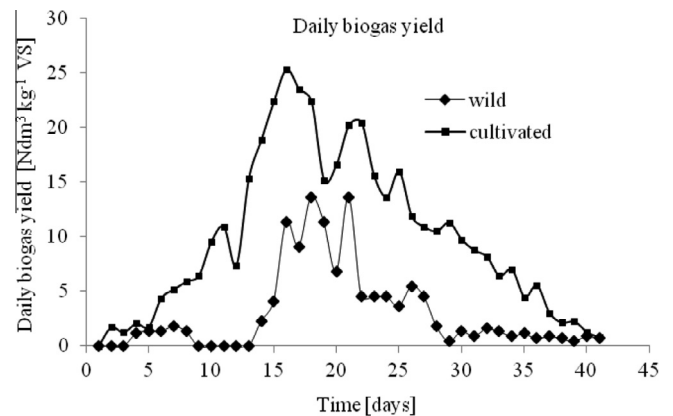


Fig. 1. Daily biogas yield from wild and cultivated reed canary grass.

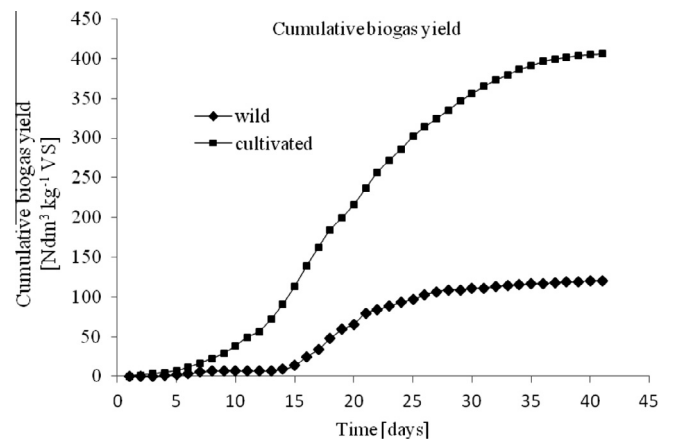


Fig. 2. Cumulative biogas yield from wild and cultivated reed canary grass.

obtained on the 16th day of the process in the case of the cultivated variety and 18th day in the case of the wild one. The significant decrease in the biogas yield between these peaks was most probably caused by the accumulation of volatile fatty acids, particularly propionic acid, which is an inhibitor of the methane fermentation process (Yang et al., 2009). The flattened beginning of the graph of the cumulative biogas yield indicated significant inhibition and delay of the hydrolysis process (DIN 38414-S8, 1984) and, consequently, the whole process of anaerobic digestion (Fig. 2). This inhibition may have been caused by the high content of the indigestible fraction of crude fiber ADL. The research performed by many authors proved that high lignin content significantly reduces the digestibility of plant material and effectively inhibits the process of anaerobic digestion (Triolo et al., 2011; Monlau et al., 2012; Zhong et al., 2011). This fact may explain the very large differences between the tested varieties. In addition to lignin, the degree of cellulose crystallinity also affects digestibility (Jeihanipour et al., 2010; Triolo et al., 2012). It is much higher in the case of wild reed canary grass.

#### 4. Conclusion

The study has shown that both varieties are substantially different in terms of physical and chemical properties. It is believed that high dry matter content and the indigestible fraction of crude fiber have a significant reducing effect on biogas quantity and quality. The FTIR Analysis facilitated rapid comparison of the two varieties. Frequent mowing and fertilization helps to keep lignification at a low level. Nonetheless, both varieties have proved to be noteworthy substrates for biogas production. Given the fact that wild *P. arundinacea* L. grows on unused areas, it may become a cheap source of biomass. The increase in mowing frequency should contribute to improving the quality of plant material as a potential feedstock for biogas production.

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