Nitrogen Fertilization Level and Cutting Affected Lignocellulosic Crops Properties Important for Biogas Production

Marta Oleszek [a,]* and Mariusz Matyka [b]

The influence of the nitrogen fertilization level was investigated relative to the chemical composition of lignocellulosic energy crops and their usefulness as a substrate for the purpose of biogas production. In the case of perennial crops, such as Virginia mallow (VM) and reed canary grass (RCG), the impacts of individual swath and cutting frequency were examined. The results showed that raised nitrogen fertilization improved the biomass quality. This was important for biogas production, primarily through decreased lignin content, and for an increased ratio of structural carbohydrates to lignin. It is believed that this tendency may facilitate the digestion of the tested substrate and increase the methane fermentation efficiency. Likewise, the swath of perennial crops differed significantly in terms of the analyzed properties, which also may have been reflected in the suitability of biomass as a feedstock for biogas plants.

Keywords: Nitrogen fertilization level; Energy crops; Biogas production; Cutting frequency; Reed canary grass; Virginia mallow

*Contact information: a: Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin, Poland; b: Institute of Soil Science and Plant Cultivation, State Research Institute, Czartoryskich 8, 24-100 Pulawy; *Corresponding author: m.oleszek@ipan.lublin.pl*

INTRODUCTION

In Europe biomass is the most widely used renewable energy source (Krzemińska *et al.* 2015). In recent years, a great proportion of the biomass was used for biogas production. Furthermore, energy crops constitute a large part of the applied substrates (Oleszek and Tys 2013; Gansberger *et al.* 2015). It is often emphasized that the cultivation of energy crops should not compete with that of plants that are used for food and feed production in arable land areas (Oszust *et al.* 2017). Therefore, it is important to reach a high yield of energy crops and high biomass quality in relation to the subsequent use of raw material. In the case of energy production from biogas, the demand for the utilization agricultural area (UAA) depends not only on the yield of a specific crop, but also on the methane yield, which is reflected as the final energy productivity per unit area. Thus, in addition to the concern for the highest yield, it is important to ensure that the quality of biomass will meet the substrate requirements for biogas production.

To achieve high yields, elevated nitrogen fertilization is frequently applied. The primary goal of fertilization is to supply plants with an adequate amount of nutrients for obtaining high yields without a negative influence on quality (Gołębiewska and Wróbel 2010). However, it has been shown that nitrogen may affect the chemical composition of plant materials and alter their suitability for different purposes. For example, nitrogen fertilization improves the baking value of flour, determines the suitability of barley for beer production, and modifies the calorific value of biomasses. All of these result from the changes in the chemical composition of plants (Peyraud and Astigarraga 1998; Lewandowski and Kauter 2003; Allison *et al.* 2012). Consistent with the examples listed above, there is frequently a conflict between increasing yields and maintaining the quality of plant material. Therefore, good quality is a relative term and depends directly on the destination and subsequent application of feedstock (Lewandowski and Kauter 2003).

Just as the cultivation of plants for food purposes differs from their cultivation for fodder purposes, another approach must also be applied in the case of their cropping as the substrates for biogas production (Kalač 2011). Currently, there is a lack of detailed information on the influence of nitrogen fertilization levels on the quality of energy crops for biogas production and on the guidelines for the optimal doses. The influence of nitrogen fertilization level on biogas production was the subject of few studies, but primarily in terms of increase in the biomass yield resulting in an increase in productivity of biogas and energy per unit area (Kacprzak et al. 2012). The effect on chemical composition, which could also cause improvement in biogas yield, seems to have been neglected.

It is believed that in the case of energy crops, more focus can be placed on a high biomass yield, rather than on a high nutritional value for these plants. In contrast, it is commonly known that the chemical composition of substrates has an effect on biogas yield. The main components of plant materials are carbohydrates, lipids, and proteins. It should be mentioned that none of the above polymers are completely decomposed under anaerobic conditions. Therefore, the calculations of biogas yield based on their content have to take into account their digestibility as well. The highest biogas yield is typically obtained from lipids, which are also the most digestible of the components. Moreover, the biogas with the highest content of methane was produced in the anaerobic digestion of proteins, even though proteins are the least decomposable (Jacobi *et al.* 2012). The degree of decomposition for proteins was tested on ruminants. This fact can be the reason for an error in biogas yield estimation, as methane fermentation in the rumen, in spite of many similarities, differs in some respects from methane fermentation in biogas plants (Bayané and Guiot 2011).

Many mathematical models describing biogas and methane yield with dependence on the chemical composition of feedstock have been developed. Many of them take lignin into account as an inhibitory factor, which is generally strongly and negatively correlated with biogas yield (Triolo *et al.* 2011; Dandikas *et al.* 2014). Ash and protein have also been found to be components that negatively affect biogas yield (Goliński and Jokś 2007). There is not a single universal model for all of the substrates (Tsavkelova and Netrusov 2012).

The goal of this study is to determine the influence of the nitrogen fertilization level, cut system, and a particular swath on the chemical composition of selected energy crops, important for biogas production.

EXPERIMENTAL

Materials

Field experiment

The field experiment was established at the Experimental Station of the Institute of Soil Science and Plant Cultivation in Osiny, Poland (N: 51 27´ E: 21 39´) and Jelcz-Laskowice, Poland (N: 51 27´ E: 21 39´) during 2012 through 2014 in a randomized complete block design as a "split-plot" system with four replications (Table 1). The area of one plot was 60 m^2, of which 40 m^2 was set for harvest.

The nitrogen fertilization level was the first-order factor, and the species of the energy crops was the second-order factor. Six plant species were examined: maize (*Zea mays* L., var. Ułan), sorghum (*Sorghum bicolor* L., var. Rona 1), sunflower (*Helianthus annuus* L. var. Kornelka), triticale (*x Triticosecale* Wittm. ex A. Camus, var. Leontino), reed canary grass (*Phalaris arundinacea* L. var. Bamse) (RCG), and Virginia mallow (*Sida hermaphrodita* L.) (VM). For each crop, three levels of nitrogen fertilization were established. As the nitrogen fertilizer (Grupa Azoty, Pulawy, Poland), the ammonium nitrate was used in a divided dose (Table 2). Moreover, phosphorus and potassium fertilization was also applied at the dosage of 60 kg P_2O_5•ha^{-1} and 80 kg K_2O•ha^{-1}, respectively. In the case of RCG and VM, differences between swaths were also determined.

Table 1. Locations and Soil Conditions of the Experiments

Location	Voivodship	Soil	Species	Year of Cultivation
Jelcz-Laskowice	Lower Silesian	Light soil (sandy loam)	Virginia mallow (VM)	2014
Osiny	Lublin	Medium soil (loamy sand)	Maize	2012
			Sorghum	2012
			Sunflower	2012
			Reed canary grass (RCG)	2013
			Triticale	2014

Table 2. Nitrogen Fertilization Level and Dosage of Nitrogen Fertilizer

Species	Nitrogen Fertilization Level [kg N ha^{-1}]	Dosing System
Maize	80, 120, 160	50% before sowing 50% at plant height of 30%
Sorghum		
Sunflower	40, 80, 120	50% before sowing 50% at the plant height of 30 cm
Triticale		50% in early spring 50% during flowering
Virginia mallow		50% for first cut, 50% for second cut
Reed canary grass		60% for first cut, 40% for second cut

Maize was harvested in the late-wax maturity stage, sorghum just after the first frost, triticale in the milky-wax maturity stage, sunflower in the so-called "yellow anthodium" stage, and the first swath of reed canary grass was in the earing stage, just before flowering. Furthermore, two ways of harvest were applied to reed canary grass: a two-cut and a three-cut system. In the case of the two-cut system, reed canary grass was harvested in June (first swath (I/II/III)– common with three-cut system) and in October (second swath (II/II)). In the case of the three-cut system, reed canary grass was harvested in June (I/II/III), in August (second swath (II/III)), and in October (third swath (III/III)). Virginia mallow was harvested twice; the first swath (I/II) was collected at the beginning of July and second one (II/II) in the middle of October.

Plant material was collected separately from each of the four plots for the individual plant species and nitrogen fertilization level. Then, the plant material was fragmented and biomass from each of the four plots was combined, and approximately 3 kg of them were ensiled. Silage was prepared in sealed, 5-L plastic barrels with the silage additive consisting of lactic acid bacteria. The barrels were then stored in the dark.

Methods

Chemical analysis

After the ensiling process, the chemical analysis was performed according to methods described by Oleszek and Krzemińska (2017). Total solids (TS), volatile solids (VS), and crude ash (CA) were determined by the weight-drying method at 105 °C for the first and 550 °C for the latter two, respectively. Total nitrogen (N_{tot}) was analyzed by the Kjeldahl method (Kjeldahl 1883), and crude protein (CP) was calculated by multiplying the N_{tot} by a coefficient of 6.25. The organic carbon (C_{org}) was determined by using a TOC-V CPN analyzer with a Solid Sample Combustion Unit SSM-5000A (Shimadzu, Kyoto, Japan). The determination of the fiber fractions (neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL)) was performed according to the Van Soest method (Van Soest *et al.* 1991). Cellulose (CL) and hemicellulose (HCL) were calculated by subtracting the ADF from NDF and ADL from ADF, respectively (Oleszek *et al.* 2016). Crude fat (CF) was determined by the extraction in a Soxhlet apparatus, with petroleum ether (Avantor Performance Materials Poland S.A., Gliwice, Poland) applied as a solvent. The non-fiber carbohydrates (NFC) was calculated according to Eq. 1,

$$NFC = 100\% - (CP + CF + NDF + CA) \quad (1)$$

where NFC is non-fiber carbohydrates (% TS), CP is the crude protein (% TS), NDF is neutral detergent fiber (% TS), CF is the crude fat (% TS), and CA is the crude ash (%TS).

All abbreviations used in this publication are listed in Table 3.

Table 3. Abbreviations Used in the Text

Term	Abbreviation
Acid detergent fiber	ADF
Acid detergent lignin	ADL
Cellulose	CL
Crude ash	CA
Crude fat	CF
Crude protein	CP
Hemicellulose	HCL
First swath common for two and three-cut system	I/II/III
Neutral detergent fiber	NDF
Non-fiber carbohydrates	NFC
Organic carbon	C_{org}
Reed Canary Grass	RCG
Second swath in two-cut system	II/II
Second swath in three-cut system	II/III
Three swath in three-cut system	III/III
Total nitrogen	N_{tot}
Total solids	TS
Virginia Mallow	VM
Volatile solids	VS

Statistical analysis

The statistical analyses were performed in Statistica 12 software (Stat Soft Inc., Tulsa, OK, USA). The results of chemical analyses were expressed as the mean of the three replications for each silage, which was previously prepared from biomass combined from four plots.

Normality of the data was confirmed by the Shapiro-Wilk and Lillefors tests. To assess the equality of variances, the Levene's test was used. Significance of the influence of the nitrogen fertilization level, species, as well as nitrogen fertilization level and swaths of RCG or VM were evaluated by a two-way analysis of variance. Tukey's post-hoc test at $p < 0.05$ was used to determine the significance of the differences between the tested species, swaths, and nitrogen fertilization levels.

RESULTS AND DISCUSSION

Comparison of Chemical Composition of Energy Crops

The tested energy crops differed significantly in the TS content ($p < 0.05$) and in each of the determined chemical properties (Table 4). The lowest VS was obtained by reed canary grass, while the highest was obtained by sorghum. The CA was inversely proportional to the VS.

The tested energy crops differed slightly in C_{org}. The average C_{org} in the tested silages was 49.2% ± 3.0% TS. The lowest content of this element occurred in the reed canary grass harvested in a three-cut system, and the highest content occurred in reed canary grass harvested in a two-cut system.

The concentration of N_{tot} ranged between 1.1% and 2.2% TS, with an average distribution of 1.6% ± 0.3% TS. A significantly higher N_{tot} was observed in the perennial plants, *i.e.* reed canary grass and Virginia mallow. There were not any significant differences between sunflower and triticale, or between maize and sorghum, for which the lowest concentrations of N_{tot} were observed. An analogous dependence relationship occurred in the case of crude protein (CP), which was calculated based on the N_{tot} content.

In view of the differences in $C_{org.}$ and $N_{tot.}$, the C/N ratio was also subjected to changes depending on the species, and it was the highest for maize and sorghum. Despite the high $C_{org.}$, reed canary grass was characterized by a significantly lower C/N ratio than the other energy crops, due to a high N_{tot}. The lowest C/N ratio was obtained for reed canary grass harvested in a three-cut system and for Virginia mallow (28.2 ± 0.5 and 29.1 ± 0.4, respectively).

All of the tested plant species, except for sunflower, had a relatively low CF content that ranged from 1.7% ± 0.0% TS for maize cultivated in a medium nitrogen fertilization level to 5.6% ± 0.2% TS for triticale fertilized in the highest dose of nitrogen. Sunflower, as an oil plant, was characterized by a much higher CF content (from 11.4% ± 0.3% to 14.2% ± 0.1% TS dependent upon the nitrogen fertilization level).

The highest diversity was observed for the components that belonged to the group of carbohydrates, *i.e.* non-fiber carbohydrates, and structural carbohydrates such as cellulose, hemicellulose, and lignin (ADL). Lignin, CL, and HCL constitute lignocellulose.

Table 4. Chemical Composition of Tested Energy Crops Depending on Species and Nitrogen Fertilization Level

	TS (%)	VS (%TS)	CA (%TS)	$C_{org.}$ (%TS)	N_{tot} (%TS)	C/N	CP (%TS)	CF (%TS)	NFC (%TS)	CL (%TS)	HCL (%TS)	ADL (%TS)	(HCL+CL)/ ADL
Species													
Maize	31.7^{e}	96.1^{d}	3.8^{b}	50.4^{bc}	1.3^{a}	39.6^{d}	8.0^{a}	2.5^{a}	33.6^{d}	24.6^{b}	23.0^{c}	4.5^{a}	10.8^{f}
Sunflower	20.3^{ab}	89.2^{b}	10.8^{d}	51.0^{cd}	1.5^{b}	34.4^{c}	9.4^{b}	12.6^{e}	19.4^{c}	29.0^{c}	9.3^{a}	9.5^{f}	4.1^{a}
Sorghum	21.1^{b}	97.2^{e}	2.8^{a}	49.2^{b}	1.2^{a}	39.9_{d}	7.7^{a}	3.8^{d}	43.1^{e}	17.8^{a}	20.6^{c}	4.3^{a}	9.0^{d}
Triticale	20.3^{ab}	89.5^{b}	10.5^{d}	48.9^{b}	1.5^{b}	32.7^{bc}	9.4^{b}	3.4^{c}	11.8^{b}	31.2^{d}	25.5^{d}	8.3^{d}	6.9^{b}
RCG II C*	30.1^{d}	89.8^{b}	10.2^{d}	52.7_{d}	1.9^{d}	31.1^{b}	11.7^{d}	2.6^{ab}	7.7^{a}	32.7^{de}	26.6^{d}	8.6^{d}	9.0^{d}
RCG III C	22.7^{c}	78.0^{a}	22.0^{e}	43.6^{a}	1.9^{d}	28.2^{a}	11.7^{d}	2.9^{b}	6.9^{a}	28.2^{c}	22.9^{c}	5.6^{b}	9.7^{e}
VM II C	20.0^{a}	91.3^{c}	8.7^{c}	48.8^{b}	1.8^{c}	29.1^{a}	11.1^{c}	2.8^{b}	19.9^{c}	34.3^{e}	15.8^{b}	7.4^{c}	7.4^{c}
p value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	742	1451	1452	48	173	124	173	4611	1188	129	122	480	482
N level													
N I	23.9^{a}	90.1^{a}	9.9^{b}	49.1^{a}	1.5^{a}	34.2^{b}	9.5^{a}	4.0^{a}	20.5^{a}	28.1^{ab}	21.0^{a}	7.0^{b}	7.9^{a}
N II	23.8^{a}	90.6^{b}	9.4^{a}	49.0^{a}	1.6^{b}	34.1^{b}	9.9^{b}	4.1^{a}	20.0^{a}	29.1^{b}	20.2^{a}	7.3^{b}	7.8^{a}
N III	23.6^{a}	89.8^{a}	10.2^{b}	49.5^{a}	1.6^{b}	32.4^{a}	10.1^{b}	5.0^{b}	20.5^{a}	27.5^{a}	20.4^{a}	6.4^{a}	8.6^{b}
p value	0.451	0.000	0.000	0.368	0.000	0.000	0.000	0.000	0.399	0.003	0.209	0.000	0.000
F	0.8	12.1	12.1	1.0	12.4	12.5	12.4	222.0	0.9	6.8	1.6	55.0	43.9
Species × N level													
Maize													
N I	30.8	96.5	3.5	51.1	1.3	39.3	8.1	2.7	27.8	25.8	27.3	4.8	11.0
N II	30.9	95.6	4.4	50.2	1.2	42.9	7.3	1.7	31.5	28.0	21.7	5.5	9.0
N III	33.5	96.4	3.7	49.9	1.4	26.5	8.5	3.2	41.4	19.9	20.0	3.3	12.3
Sunflower													
N I	22.0	87.9	12.2	52.0	1.3	39.3	8.3	11.4	20.5	29.2	9.2	9.4	4.1
N II	20.0	90.9	9.1	51.2	1.5	33.2	9.6	12.3	19.0	30.4	9.4	10.2	3.9
N III	19.0	88.7	11.3	49.9	1.6	30.6	10.2	14.2	18.6	27.5	9.4	8.9	4.2
Sorghum													
N I	20.7	97.1	2.9	48.6	1.2	39.4	7.7	3.4	46.4	15.8	19.5	4.3	8.3
N II	23.1	97.8	2.2	49.5	1.2	41.9	7.4	3.6	39.8	21.0	21.5	4.5	9.4
N III	19.2	96.8	3.2	49.5	1.3	38.4	8.0	4.4	43.0	16.6	20.9	4.0	9.4
Triticale													
N I	22.6	91.9	8.1	49.9	1.5	34.1	9.2	2.2	12.9	33.0	26.5	8.1	7.4
N II	19.6	89.4	10.7	47.8	1.4	33.2	9.0	2.3	14.0	30.0	24.6	9.4	5.8
N III	18.8	87.4	12.6	49.1	1.6	30.9	9.9	5.6	8.5	30.5	25.3	7.5	7.5
RCG II C													
N I	29.3	90.0	9.9	51.7	1.9	29.1	11.6	2.8	7.8	31.9	27.3	8.6	9.2
N II	31.6	90.4	9.6	52.3	1.9	30.4	12.1	2.7	8.6	32.4	26.0	8.6	8.7
N III	29.5	89.0	11.0	54.1	1.8	33.9	11.4	2.4	6.7	33.6	26.4	8.5	9.0
RCG III C													
N I	23.0	76.7	23.3	41.9	1.8	28.3	11.4	3.0	6.6	27.5	23.1	5.2	10.0
N II	21.3	79.0	21.1	44.0	1.9	29.7	11.9	2.9	7.0	28.3	23.1	5.8	9.5
N III	23.8	78.4	21.7	45.0	1.9	26.9	11.7	2.6	7.1	28.7	22.6	5.7	9.5
VM II C													
N I	18.4	90.8	9.2	48.9	1.7	29.6	10.4	3.0	21.2	33.3	14.4	8.7	5.5
N II	19.9	90.9	9.1	48.2	1.9	27.9	12.0	3.1	20.4	34.0	14.8	6.8	8.2
N III	21.7	92.1	7.9	49.4	1.8	29.6	10.9	2.5	18.2	35.6	18.2	6.7	8.4
p value	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000
F	31.27	19.32	19.32	2.22	6.71	11.48	6.71	92.20	26.03	6.29	3.69	16.47	29.39

*II C – two-cut system, III C – three-cut system; the means with the same superscript letters do not differ significantly in HSD Tukey test at $p < 0.05$

The average NFC content was 20.3% TS with minimum and maximum values of 5.8% and 47.0% TS, respectively. The highest NFC was noted for sorghum (43.1% ± 1.0% TS), while the lowest was noted for reed canary grass harvested in both two-cut and three-cut systems (7.7% ± 0.4% and 6.9% ± 0.2% TS, respectively). A high content of NFC was also determined for maize (33.6% ± 2.0% TS). There were no significant differences between sunflower and Virginia mallow as well as between both cut-systems of the reed canary grass.

Sorghum and maize were characterized by the lowest ADL content, 4.3% ± 0.1% and 4.5% ± 0.3% TS, respectively. Though slight, there was a statistically significantly higher content of ADL noted for the reed canary grass harvested in a three-cut system (56% ± 0.1% TS). A much higher ADL content was determined in the other energy crops, the highest content was recorded for sunflower (9.5% ± 0.2% TS).

The lower contents were observed in the case of CL and HCL. The highest cellulose content was noted for Virginia mallow and reed canary grass harvested in a two-cut system (34.3% ± 0.8% and 32.7% ± 0.3% TS, respectively), and the lowest for sorghum (17.8% ± 0.8% TS). The highest contents of HCL were determined for reed canary grass harvested in a two-cut system and triticale, and the lowest content was for sunflower.

In addition to the analysis of the individual components of lignocellulose (CL, HCL, and ADL), the ratio of (HCL + CL)/ADL was also calculated. This parameter may indirectly indicate the digestibility of the tested biomass. The highest ratio of (HCL + CL)/ADL was observed for maize, and then for reed canary grass harvested in the three-cut system. In contrast, sunflower was characterized by the lowest ratio of (HCL + CL)/ADL.

The Influence of Nitrogen Fertilization Level on Chemical Composition of Energy Crops

The obtained results indicated that the nitrogen fertilization level significantly influenced on some chemical properties of tested energy crops. An increase in the nitrogen dose caused increases in total N_{tot}, CP, and CF, as well as decreases in ADL and C/N ratio. For ADL, statistically significant decrease occurred only after application the highest N level. The impact of the nitrogen fertilization level on VS, CA and CL was unclear. The concentrations of these components were the lowest or the highest for the medium nitrogen fertilization level, while there were no significant differences between the lowest and the highest nitrogen fertilization levels. The results of a two-way analysis of variance indicated that nitrogen fertilization level did not significantly affect the TS, C_{org}, NFC, and HCL (Table 4).

It should be emphasized that the impact of nitrogen fertilization level was not the same for all of the tested species. This was evidenced by the statistically significant interaction between nitrogen fertilization level and species in the case of each analysis chemical property ($p>0.05$), as well as by the results of the Tukey's test done separately for each individual species.

The variation of nitrogen fertilizer doses significantly impacted the proportion of the lignocellulose components, HCL, CL, and ADL, *i.e.* the ratio of (HCL + CL) / ADL that provided evidence about the susceptibility to digestibility in the methane fermentation process. Generally, in the case of the tested energy crops, the ratio of (HCL + CL) / ADL was highest for the highest nitrogen fertilization level. Only in the case of reed canary grass harvested in the three-cut system, the increase in nitrogen fertilization level had a negative effect on the (HCL + CL) / ADL ratio. In contrast, there were no significant differences between the highest and the lowest nitrogen fertilization level in the case of sunflower, triticale, and reed canary grass harvested in a two-cut system. A general increase in the ratio of (HCL + CL) / ADL indicated a positive influence of nitrogen fertilization on lignocellulose susceptibility to anaerobic digestion.

Influence of Swath on Chemical Composition of Energy Crops

The chemical composition of perennial species, such as reed canary grass and Virginia mallow, was also dependent on the swath from which the biomass originated. Greater differences between swaths were observed in the case of reed canary grass than Virginia mallow. In terms of reed canary grass, the swath differed in all investigated chemical properties. Swaths of Virginia mallow differed significantly in VS, CA, C_{org}, N_{tot}, C/N ratio, CP, CF, CL, and HCL. No significant differences were observed in the case of TS (Table 5 and 6).

Among the reed canary grass swaths, the highest TS was noted for the second swath of the two-cut system (II/II) (35.3% ± 0.7%), and the lowest for the third swath of the three-cut system (III/III) (17.5% ± 0.8%). There were no significant differences between the first and second swath of the three-cut system (I/II/III and II/III). The highest VS as well as the lowest CA was noted for the first swath (common to both two and three-cut systems); (I/II/III) (91.6% ± 0.3% and 8.5% ± 0.3% TS, respectively). The lowest VS and, simultaneously, the highest CA were determined for III/III of the reed canary grass. Thus, the high CA was probably caused by soil contamination during the harvest, which was impeded due to low plant regrowth. As for Virginia mallow, a higher content of VS and, simultaneously, a lower content of CA was noted in the case of the swath of II/II.

The C_{org} was characterized by high diversity. In the case of reed canary grass, the greatest content of this element was noted for II/II (55.0% ± 0.6 % TS) and the lowest for III/III (28.6 ± 1.0% TS). The swath of III/III was characterized also by high N_{tot} content. For this reason, the C/N ratio was much lower when compared to other swaths. The highest ratio of C/N was noted for II/III (49.7 ± 1.7). The difference in C_{org} between swaths of Virginia mallow was small, though statistically significant. A higher C_{org} was noted in the case of II/II. This swath also had a higher content of N_{tot}, whereby the C/N ratio proved to be much lower than I/II.

Among the swaths of reed canary grass, the highest CF was observed for II/III (3.8% ± 0.1% TS), and the lowest for III/III (2.1% ± 0.1% TS). There were no significant differences between the first and second swath of reed canary grass harvested in the two-cut system. Among two swaths of Virginia mallow, significantly higher CF content was noted for II/II.

The swaths also differed significantly in terms of individual components of lignocelluloses (CL, HCL, and ADL) and NFC. In the case of reed canary grass, the highest NFC was reported for both II/II and II/III and the lowest for I/II/III. All swaths of reed canary grass differed significantly between each other in term of CL and ADL.

The CL content decreased and ADL increased along with succeeding swaths. The highest content of HCL was determined in the biomass of swath II/III and the lowest in III/III. There were no significant differences in the HCL content between I/II/III and II/II.

As for the swaths of Virginia mallow, a higher content of lignocellulose components (HCL, CL, and ADL) was determined in first swath (I/II), while a higher content of NFC was determined in the second swath (II/II). Significant differences in the proportions of these components were also observed, both for reed canary grass and Virginia mallow. In terms of reed canary grass, the (HCL + CL)/ADL ratio decreased and in terms of Virginia mallow the ratio increased along with the successive swaths.

Table 5. Chemical Composition of RCG Depending on Swath and Nitrogen Fertilization Level

	TS (%)	VS (%TS)	CA (%TS)	$C_{org.}$ (%TS)	$N_{tot.}$ (%TS)	C/N	CP (%TS)	CF (%TS)	NFC (%TS)	CL (%TS)	HCL (%TS)	ADL (%TS)	(HCL + CL)/ ADL
Swath													
I/II/III*	25.0^{b}	91.6^{d}	8.5^{a}	50.4^{b}	2.38^{d}	21.3^{b}	14.9^{d}	2.6^{b}	3.4^{a}	39.4^{d}	26.5^{b}	4.8^{a}	13.7^{d}
II/III	25.7^{b}	89.5^{c}	10.6^{b}	51.9^{b}	1.05^{a}	49.7^{d}	6.9^{a}	3.8^{c}	11.1^{c}	33.4^{c}	29.1^{c}	5.5^{b}	11.4^{c}
III/III	17.5^{a}	53.0^{a}	47.0^{d}	28.6^{a}	2.16^{c}	13.4^{a}	13.5^{c}	2.1^{a}	6.2^{b}	11.7^{a}	13.2^{a}	9.4^{c}	3.9^{a}
II/II	35.3^{c}	88.1^{b}	11.9^{c}	55.0^{c}	1.36^{b}	40.9^{c}	8.5^{b}	2.7^{b}	12.0^{c}	25.9^{b}	26.6^{b}	12.4^{d}	4.3^{b}
p value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	1012	2888	2888	826	288	1085	288	725	109	8991	2012	4063	19044
N level													
N I	25.7^{a}	79.7^{a}	20.3^{b}	44.8^{a}	1.75^{a}	30.1^{a}	10.9^{a}	2.9^{b}	8.3^{a}	26.6^{a}	24.0^{a}	7.0^{a}	8.5^{b}
N II	25.4^{a}	81.0^{b}	18.5^{a}	46.7^{b}	1.76^{a}	32.1^{b}	11.0^{a}	2.9^{b}	8.5^{a}	27.7^{b}	23.9^{a}	7.4^{b}	8.2^{a}
N III	26.4^{b}	80.4^{a}	19.6^{b}	47.9^{c}	1.70^{a}	31.8^{b}	10.6^{a}	2.6^{a}	7.8^{a}	28.5^{c}	23.6^{a}	7.3^{b}	8.3^{a}
p value	0.004	0.001	0.001	0.000	0.410	0.007	0.410	0.000	0.281	0.000	0.119	0.000	0.000
F	6.82	9.06	9.06	18.98	0.92	6.03	0.92	73.04	1.34	71.78	2.32	22.92	28.51
Swath x N level													
I/II/III													
N I	25.0	91.3	8.7	49.9	2.2	22.8	13.7	3.0	2.6	39.7	27.7	4.7	14.3
N II	25.3	91.8	8.2	50.0	2.5	20.2	15.7	2.5	4.1	39.0	25.5	4.9	13.2
N III	24.6	91.6	8.4	51.3	2.4	21.0	15.2	2.4	3.4	39.3	26.3	4.8	13.6
II/III													
N I	24.3	88.2	11.8	51.2	1.0	51.5	6.2	4.0	9.8	32.9	30.0	5.4	11.6
N II	24.1	90.1	10.0	51.5	1.0	53.9	6.0	3.7	10.0	34.7	29.8	5.5	11.7
N III	28.6	90.1	9.9	52.9	1.2	43.7	7.6	3.6	13.1	32.7	27.6	5.5	10.9
III/III													
N I	19.7	50.6	49.4	24.6	2.3	10.7	14.4	2.0	7.6	9.8	11.5	5.4	4.0
N II	14.5	55.0	45.0	30.6	2.2	13.7	13.9	2.6	6.4	11.3	13.9	6.9	3.6
N III	18.2	53.4	46.6	30.8	1.9	15.9	12.1	1.8	4.7	13.9	14.1	6.8	4.1
II/II													
N I	33.7	88.7	11.3	53.5	1.5	35.4	9.5	2.6	13.1	24.1	26.9	12.6	4.1
N II	37.8	89.1	10.9	54.6	1.8	40.7	8.4	2.9	13.2	25.8	26.4	12.4	4.2
N III	34.3	86.5	13.5	56.8	1.2	46.7	7.6	2.4	9.9	27.9	26.5	12.1	4.5
p value	0.000	0.002	0.002	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	36.8	4.7	47	4.2	8.3	27.4	8.3	34.4	6.3	41.0	21.5	23.7	44.3

*I/II/III – first swath, common for two-cut system and three-cut system, II/III – second swath of three-cut system, III/III – third swath of tree-cut system, II/II – second swath of two-cut system; the means with the same superscript letters do not differ significantly at $p < 0.05$

The influence of nitrogen fertilization level on plant material quality was the subject of many scientific works. It was shown that nitrogen doses influence the morphological characteristics of plants, which substantially determines the chemical composition (Gołębiewska and Wróbel 2010; Ciepiela 2014). Moreover, nitrogen fertilization may cause a lengthening of the growing season, which in turn results in a lower share of generative parts at harvest and, consequently, changes in the chemical composition of biomass (Ali *et al.* 2013).

Table 6. Chemical Composition of VM Depending on Swath and Nitrogen Fertilization Level

	TS (%)	VS (%TS)	CA (%TS)	$C_{org.}$ (%TS)	$N_{tot.}$ (%TS)	C/N	CP (%TS)	CF (%TS)	NFC (%TS)	CL (%TS)	HCL (%TS)	ADL (%TS)	(HCL +CL)/ ADL
Swath													
I/II*	20.4^{a}	91.9^{b}	8.1^{a}	48.6^{a}	1.4^{a}	34.7^{b}	8.8^{a}	2.2^{a}	13.3^{a}	38.6^{b}	19.6^{b}	9.4^{b}	6.3^{a}
II/II	19.6^{a}	90.6^{a}	9.4^{b}	49.0^{b}	2.1^{b}	23.4^{a}	13.4^{b}	3.5^{b}	26.5^{b}	29.9^{a}	11.9^{a}	5.4^{a}	8.4^{b}
p value	0.227	0.008	0.008	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	1.6	9.7	9.7	5.2	563.8	333.5	563.8	306.3	101.6	24.7	23.4	171.9	55.4
N level													
N I	18.4^{a}	90.8^{a}	9.2^{a}	48.9^{b}	1.7^{a}	29.6^{a}	10.4^{a}	3.0^{a}	21.2^{a}	33.3^{a}	14.4^{a}	8.7^{b}	5.5^{a}
N II	19.9^{a}	90.9^{a}	9.1^{a}	48.2^{a}	1.9^{b}	27.9^{a}	12.0^{b}	3.1^{b}	20.4^{a}	34.0^{a}	14.8^{a}	6.8^{a}	8.2^{b}
N III	21.7^{b}	92.1^{a}	7.9^{a}	49.4^{b}	1.8^{a}	29.6^{a}	10.9^{a}	2.5^{a}	18.2^{a}	35.6^{a}	18.2^{a}	6.7^{a}	8.4^{b}
p value	0.002	0.043	0.043	0.000	0.000	0.000	0.000	0.000	0.208	0.558	0.143	0.000	0.000
F	10.38	4.1	4.1	14.0	23.98	3.45	23.98	23.43	1.79	0.61	2.29	17.76	39.93
Swath × N level													
I/II													
N I	19.6	92.0	8.0	49.0	1.5	31.8	9.7	2.2	9.7	37.4	16.0	9.5	5.6
N II	19.1	91.5	8.5	48.0	1.3	36.6	8.2	2.2	17.2	40.8	20.7	10.0	6.2
N III	22.4	92.3	7.7	48.9	1.4	35.8	8.6	2.1	13.1	37.8	22.2	8.6	7.0
II/II													
N I	17.3	89.7	10.3	48.8	1.8	27.5	11.1	3.8	31.0	29.1	12.7	7.8	5.4
N II	20.6	90.3	9.7	48.5	2.5	19.2	15.8	3.9	25.2	27.2	8.9	3.6	10.2
N III	21.0	91.9	8.1	49.8	2.1	23.5	13.3	2.8	23.4	33.4	14.1	4.9	9.8
p value	0.052	0.225	0.225	0.081	0.000	0.000	0.000	0.000	0.003	0.143	0.138	0.000	0.000
F	3.8	1.7	1.7	3.1	84.3	37.6	84.3	19.2	9.7	2.3	2.3	19.9	18.1

* I/II – first swath for two-cut system; II/II – second swath of two-cut system; the means with the same superscript letters do not differ significantly at $p < 0.05$

Table 7 presents the review of the results of previous researches on the influence of nitrogen fertilization level on the different chemical properties of selected energy crops. As shown in the table, previous studies indicate that the differentiation of nitrogen doses may cause differences in the dry matter of plants at harvest. Lewandowski and Kauter (2003) reported that the moisture of wheat and rice straw increased (from 20.9% to 50.1% TS and from 25.4% to 50.4% TS, respectively) under the influence of increasing nitrogen fertilization levels from 0 kg N ha^{-1} to 140 kg N ha^{-1}. Likewise, Peyraud and Astigarraga (1998) recorded the highest dry matter content to be in poorly fertilized grasses. Kacprzak *et al.* (2012) investigated biogas production from reed canary grass cultivated at varying levels of nitrogen fertilization and harvested in a two-cut system, and found no significant differences in TS. Observations of Kacprzak *et al.* (2012) are in accordance with the results of this study.

The next important issue is the influence of the nitrogen fertilization level on CA content in biomass. In this study, no clear effect of nitrogen dose was apparent. The concentrations of CA were the lowest for the medium N level, while there were no significant differences between the lowest and the highest doses. Gołębiewska and Wróbel (2010) confirmed no effect of nitrogen dose on crude ash content in maize that was intended for silage. In contrast, Blümmel *et al.* (2003) and Reddy *et al.* (2003) stated the increase of CA in sorghum biomass under the influence of increasing doses of fertilizer. Nevertheless, there are also reports that point to increasing doses of nitrogen fertilizer as a cause for decreases in CA (Lewandowski and Kauter 2003; Lemus *et al.* 2008; Allison *et* *al.* 2009). Lewandowski and Kauter (2003) explained it by the so-called ‘dilution effect’, which is caused by the growth of biomass yield. Furthermore, a lower content of CA may be also associated with a higher content of VS. Nitrogen, being a forming element of chlorophyll, directly impacts the efficiency of photosynthesis and assimilation of CO_2, which then contributes to the accumulation of organic carbon and organic dry matter (Ali *et al.* 2013). Unclear effect stated in present study may be the resultant of two factors: ‘dilution effect’ (which decrease the concentration of CA) and intensified uptake of microelements (components of CA) occurred in lowered pH of soil after fertilization.

Table 7. The Review of the Results of the Studies Concerning the Influence of Nitrogen Fertilization Level on Chemical Properties of Energy Crops.

TS	VS	CA	C_{org}	N_{tot}	C/N	CP	CF	NFC	CL	HCL	ADL	References
0	0	0	0	+	-	+	+	0	0	0	-	Present study
-	+	-		+		+						Lewandowski and Kauter (2003)
-				+		+		-				Peyraud and Astigarraga (1998)
0												Kacprzak *et al.* (2012)
	0	0					-	0				Gołębiewska and Wróbel (2010)
	-	+									-	Blümmel *et al.* (2003)
	-	+					-					Reddy *et al.* (2003)
	+	-		+		+						Lemus *et al.* (2008)
	+	-	+	+		+						Allison *et al.* (2009)
			+	+	-	+						Jung and Lal (2011)
				+		+	0					Księżak *et al.* (2012)
							-					Ali *et al.* (2013)
			+									Pocienė *et al.* (2013)
								-				Almodares *et al.* (2009)
								-	-	-	-	Ciepiela (2014)
											-	Hodgson *et al.* (2010)

Minus sign (–) means decrease in the content of tested components in biomass, caused by increase in nitrogen fertilization level, plus sign (+) means increase, while (0) means lack of the influence or unclear effect

Many authors have noted an increase in N_{tot} and CP after applying higher doses of nitrogen fertilizer (Lewandowski and Kauter 2003; Lemus *et al.* 2008; Allison *et al.* 2009; Gołębiewska and Wróbel 2010; Jung and Lal 2011; Księżak *et al.* 2012). However, in the present study, increases in N_{tot} and CP were minimal. Peyraud and Asstigarraga (1998) explained that the effect of nitrogen fertilization level on CP is greatest two weeks after fertilization. Then, the dilution effect occurs associated with an increase in the biomass yield. Interestingly, with an increase of the nitrogen fertilization level, a decrease in the cell wall was observed. This indicates improvement in protein digestibility.

Available literature data from previous studies are not compatible with each other as to the impact of nitrogen fertilization on lipid content in plant material. Ali *et al.* (2013), having studied the effect of nitrogen fertilization on the chemical composition of sunflower, reported a decrease in crude fat content and a simultaneous increase in its biomass yield. Reddy *et al.* (2003) noted a decrease in CF content with increasing levels of nitrogen fertilization in the case of sorghum. Gołębiewska and Wróbel (2010) confirmed the same effect also occurring in maize. However, in the study of Księżak *et al.* (2012), the CF content declined in maize biomass and in sorghum grown under the influence of nitrogen fertilizer application. The results of present study indicated that the influence of N level on CF content depended on the species. In the case of annuals, an increase in CF was noted, while for perennials (RCG and VM) a decrease in CF was observed with an increase in N level.

The results of previous research indicated that nitrogen fertilization level has little effect on C_{org}. Allison *et al.* (2009) and Pocienė *et al.* (2013) observed a slight but statistically significant increase of C_{org} content in reed canary grass caused by increasing doses of nitrogen fertilization. Jung and Lal (2011) noted a significant increase in the C_{org} of switchgrass with increasing nitrogen fertilizer doses of 0 kg N ha^{-1}, 50 kg N ha^{-1}, 100 kg N ha^{-1}, and 200 kg N ha^{-1}. However, this increase was only seen in one of three tested localizations. Furthermore, a significant difference was observed only after the application of the highest dose. The authors also added that a slight increase in C_{org} was accompanied by a simultaneous increase in N_{tot}, which resulted in a significant decrease in the C/N ratio. A similar dependence was observed in the present study. It would be mentioned that, in the case of biogas production, the optimal value of C/N is 25 to 30 (Ward *et al.* 2008). This means that the C/N obtained for the highest nitrogen fertilization level (32.4 ± 0.9) was the closest to the optimal value.

From the biogas production point of view, the influence of nitrogen fertilization level is particularly important on NFC, structural carbohydrates (CL and HCL), and ADL. It is commonly known that ADL content directly affects the decomposition of the cell wall in various biotechnological processes (Smuga-Kogut *et al.* 2016).

In the case of NFC, Gołębiewska and Wróbel (2010) found no significant impact of increasing doses of nitrogen fertilizer on nitrogen-free extract or a tendency to decrease in crude fiber in maize cultivated for silage. Almodares *et al.* (2009) confirmed a decrease in the water-soluble carbohydrates in maize and sorghum caused by increases in nitrogen fertilization level. Ciepiela (2014) reported a decrease in NFC in meadow grasses from 22.2% TS for a control to 18.9% TS for plants fertilized with a dose of 150 kg N ha^{-1}. Ciepiela (2014) and Peyraud and Astigarraga (1998) paid attention to the direct correlation between the reduction of NFC and the rise of CP due to increases in nitrogen fertilization level. The decrease in sugar content was associated with the sugar consumption in protein synthesis and energy production for nitrate reduction. A lack of significant differences in NFC obtained in the present study may have resulted from two simultaneous processes: the decrease in sugars due to intensive synthesis of proteins and the increase in sugar percentage caused by a decrease in structural carbohydrates and lignin.

In addition to differences in NFC, Ciepiela (2014) noted a decrease in cellulose (CL) content in meadow grasses, from 30.4% to 28.8% TS by an increase in nitrogen fertilization level from 50 kg N ha^{-1} to 150 kg N ha^{-1}. These results are consistent with data of this study concerning the decrease in CL content between the medium nitrogen fertilization level and the highest nitrogen fertilization level, which was recorded for all tested crops except reed canary grass. According to Ciepiela (2014), the reason for this is a higher leaf to stem ratio in the case of crops fertilized with higher doses of nitrogen. Moreover, a significant decrease in HCL and ADL was observed. Bümmel *et al.* (2003) noted that with a rise in the nitrogen fertilizer level from 9 kg ha^{-1} to 90 kg ha^{-1} in sorghum that had been cultivated for fodder, there was a decrease in ADL from 7.8% to 7.5% TS, as well as a decrease in ADF, the sum of ADL and CL, from 61.8% to 60.6% TS. Hodgson *et al.* (2010) also a confirmed a positive correlation of high nitrogen fertilizer doses with the reduction of ADL for *Miscanthus giganteus*. Peyraud and Astigarraga (1998) and Masoero *et al.* (2011) reported improvements in the digestibility of silage as a fodder with the increasing doses of nitrogen fertilization. The above results are consistent with this study, where a significant decrease in ADL was also noted. Moreover, the increase in (HCL

+ CL)/ADL ratio also proved to create a greater susceptibility of lignocellulose to anaerobic decomposition during the methane fermentation process.

The influence of harvest time and particular swath on the biogas yield of perennial crops has also been the subject of many studies. Kacprzak *et al.* (2012) and Seppälä *et al.* (2009) reported significantly lower biogas yields from the second swath of reed canary grass than from the first one, but they did not refer to the chemical composition of biomass. In contrast, Kandel *et al.* (2013) observed an increase in both lignin and cellulose content with maturity, resulting in a decrease in methane yield. These results are in accordance with the presented study and may explain the differences between the swath of II/III (harvested in August) and the swath of II/II (harvested in October). A positive effect of agrotechnical procedures, such as regular cutting and fertilization, on the usefulness of reed canary grass for biogas production was stated by Oleszek *et al.* (2014). The authors proved that cultivated varieties contained much less lignin and crystalline cellulose than wild varieties. Mähnert *et al.* (2005) emphasized that cutting frequency significantly affects biomass digestibility and crude fiber composition, which in turn may influence biogas yield.

CONCLUSIONS

1. The enhancement of the nitrogen fertilization level had a significant influence on increases in total nitrogen (N_{tot}), crude protein (CP), and crude fat (CF), as well as decreases in lignin (ADL), cellulose (CL), and the C/N ratio in the biomass of tested energy crops. In addition, the ratio of (HCL + CL)/ADL also increased, which indicated an improvement in the susceptibility of lignocellulose to anaerobic decomposition through the methane fermentation process.
2. The tested energy crops differed significantly in respect to each analyzed chemical property. In the case of reed canary grass and Virginia mallow, significant differences between the individual swaths were also found. The highest ratio of (HCL + CL)/ADL was noted for maize and the lowest was for sunflower. This parameter decreased in the case of reed canary grass, but in the case of Virginia mallow, it increased with each successive swath.
3. The results suggest that the application of high levels of nitrogen fertilizer is able to improve the suitability of biomass for biogas production. Nevertheless, the next recommended step is to study the biochemical methane potential to verify this assumption.

ACKNOWLEDGMENTS

This work was supported by the Polish National Science Centre, Grant No. 2014/15/N/NZ9/01127.

REFERENCES CITED

Ali, A., Ahmad, A., Khaliq, T., Ali, A., and Ahmad, M. (2013). "Nitrogen nutrition and planting density effects on sunflower growth and yield: A review," *Pakistan Journal of Nutrition* 12(12), 1024-1035. DOI: 10.3923/pjn.2013.1024.1035

Allison, G. G., Morris, C., Hodgson, E., Jones, J., Kubacki, M., Barraclough, T., Yates, N., Shield, I., Bridgwater, A. V., and Donnison, I. S. (2009). "Measurement of key compositional parameters in two species of energy grass by Fourier transform infrared spectroscopy," *Bioresource Technology* 100(24), 6428-6433. DOI: 10.1016/j.biortech.2009.07.015

Allison, G. G., Morris, C., Lister, S. J., Barraclough, T., Yates, N., Shield, I., and Donnison, I. S. (2012). "Effect of nitrogen fertilizer application on cell wall composition in switchgrass and reed canary grass," *Biomass and Bioenergy* 40, 19-26. DOI: 10.1016/j.biombioe.2012.01.034

Almodares, A., Jafarinia, M., and Hadi, M. R. (2009). "The effects of nitrogen fertilizer on chemical compositions in corn and sweet sorghum," *American-Eurasian Journal of Agricultural & Environmental Sciences* 6, 441-446.

Bayané, A., and Guiot, S. R. (2011). "Animal digestive strategies *versus* anaerobic digestion bioprocesses for biogas production from lignocellulosic biomass," *Reviews in Environmental Science and Biotechnology* 10(1), 43-62. DOI: 10.1007/s11157-010-9209-4

Blümmel, M., Zerbini, E., Reddy, B. V. S., Hash, C. T., Bidinger, F., and Ravi, D. (2003). "Improving the production and utilization of sorghum and pearl millet as livestock feed: Methodological problems and possible solutions," *Field Crops Research* 84(1), 123-142. DOI: 10.1016/S0378-4290(03)00145-X

Ciepiela, G. A. (2014). "Content of structural and nonstructural carbohydrates and lignin in *Dactylis glomerata* L. and *Festulolium Braunii* (K. Richt.) A. *Camus* supplied by biostimulator Kelpak sl and nitrogen (in Polish)," *Science Nature Technologies* 1(8), 1-11.

Dandikas, V., Heuwinkel, H., Lichti, F., Drewes, J. E., and Koch, K. (2014). "Correlation between biogas yield and chemical composition of energy crops," *Bioresource Technology* 174, 316-320. DOI: 10.1016/j.biortech.2014.10.019

Gansberger, M., Montgomery, L. F., and Liebhard, P. (2015). "Botanical characteristics, crop management and potential of *Silphium perfoliatum* L. as a renewable resource for biogas production: A review," *Industrial Crops and Products* 63, 362-372. DOI: 10.1016/j.indcrop.2014.09.047

Goliński, P., and Jokś, W. (2007). "Chemical and biological properties of grasses and biogas production (in Polish)," *Grassland Science in Poland* 10, 37-47.

Gołębiewska, M., and Wróbel, E. (2010). "Effect of nitrogen fertilization on yield and quality of whole maize plants for silage (in Polish)," *Bulletin of The Institute of Plant Breeding and Acclimatization* 256, 117-132.

Hodgson, E. M., Fahmi, R., Yates, N., Barraclough, T., Shield, I., Allison, G., Bridgwater, A. V., and Donnison, I. S. (2010). "*Miscanthus* as a feedstock for fast-pyrolysis: Does agronomic treatment affect quality?," *Bioresource Technology* 101(15), 6185-6191. DOI: 10.1016/j.biortech.2010.03.024

Jacobi, H. F., Ohl, S., Thiessen, E., and Hartung, E. (2012). "NIRS-aided monitoring and prediction of biogas yields from maize silage at a full-scale biogas plant applying lumped kinetics," *Bioresource Technology* 103(1), 162-172. DOI: 10.1016/j.biortech.2011.10.012

Jung, J. Y., and Lal, R. (2011). "Impacts of nitrogen fertilization on biomass production of switchgrass (*Panicum virgatum* L.) and changes in soil organic carbon in Ohio," *Geoderma* 166(1), 145-152. DOI: 10.1016/j.geoderma.2011.07.023

Kacprzak, A., Matyka, M., Krzystek, L., and Ledakowicz, S. (2012). "Evaluation of biogas collection from reed canary grass, depending on nitrogen fertilization levels," *Chemical & Process Engineering* 33(4), 697-701. DOI: 10.2478/v10176-012-0059-1

Kalač, P. (2011). "The required characteristics of ensiled crops used as a feedstock for biogas production: A review," *Journal of Agrobiology* 28(2), 85-96. DOI: 10.2478/v10146-011-0010-y

Kandel, T. P., Sutaryo, S., Møller, H. B., Jørgensen, U., and Lærke, P. E. (2013). "Chemical composition and methane yield of reed canary grass as influenced by harvesting time and harvest frequency," *Bioresource Technology* 130, 659-666. DOI: 10.1016/j.biortech.2012.11.138

Kjeldahl, J. G. C. (1883). "A new method for the determination of nitrogen in organic matter," *Fresenius Journal of Analytical Chemistry* 22, 366-372.

Krzemińska, I., Nawrocka, A., Piasecka, A., Jagielski, P., and Tys J. (2015). "Cultivation of *Chlorella protothecoides* in photobioreactors: The combined impact of photoperiod and CO_2 concentration," *Engineering in Life Sciences* 15(5), 533-541. DOI: 10.1002/elsc.201400174

Księżak, J., Bojarszczuk, J., and Staniak, M. (2012). "The productivity of maize and sorghum yields of according level of nitrogen fertilization (in Polish)," *Polish Journal of Agronomy* 8, 20-28.

Lemus, R., Brummer, E. C., Burras, C. L., Moore, K. J., Barker, M. F., and Molstad, N. E. (2008). "Effects of nitrogen fertilization on biomass yield and quality in large fields of established switchgrass in southern Iowa, USA," *Biomass and Bioenergy* 32(12), 1187-1194. DOI: 10.1016/j.biombioe.2008.02.016

Lewandowski, I., and Kauter, D. (2003). "The influence of nitrogen fertilizer on the yield and combustion quality of whole grain crops for solid fuel use," *Industrial Crops and Products* 17(2), 103-117. DOI: 10.1016/S0926-6690(02)00090-0

Masoero, F., Gallo, A., Zanfi, C., Giuberti, G., and Spanghero, M. (2011). "Effect of nitrogen fertilization on chemical composition and rumen fermentation of different parts of plants of three corn hybrids," *Animal Feed Science and Technology* 164, 207-216. DOI: 10.1016/j.anifeedsci.2011.02.001

Mähnert, P., Heiermann, M., and Linke, B. (2005). "Batch- and semi-continuous biogas production from different grass species," *Agricultural Engineering International: The CIGR Ejournal.* Manuscript EE 05 010, 7, 1-11.

Oleszek, M., and Tys, J. (2013). "Lab scale measurement of biogas yield (in Polish)," *Przemysł Chemiczny* 92(1), 126-130.

Oleszek, M., Król, A., Tys, J., Matyka, M., and Kulik, M. (2014). "Comparison of biogas production from wild and cultivated varieties of reed canary grass," *Bioresource Technology* 156, 303-306. DOI: 10.1016/j.biortech.2014.01.055

Oleszek, M., Tys, J., Wiącek, D., Król, A., and Kuna, J. (2016). "The possibility of meeting greenhouse energy and CO_2 demands through utilization of cucumber and tomato residues," *BioEnergy Research* 9(2), 624-632. DOI: 10.1007/s12155-015-9705-z

Oleszek, M., and Krzemińska, I. (2017). "Enhancement of biogas production by co-digestion of maize silage with common goldenrod rich in biologically active compounds," *BioResources* 12(1), 704-714. DOI: 10.15376/biores.12.1.704-714

Oszust, K., Pawlik, A., Janusz, G., Ziemiński, K., Cyran, M., Siczek, A., Gryta, A., Bilińska-Wiergus, N., and Frąc, M. (2017). "Characterization and influence of a multi-enzymatic biopreparation for biogas yield enhancement," *BioResources* 12(3), 6187-620. DOI: 10.15376/biores.12.3.6187-6206

Peyraud, J. L., and Astigarraga, L. (1998). "Review of the effect of nitrogen fertilization on the chemical composition, intake, digestion and nutritive value of fresh herbage: Consequences on animal nutrition and N balance," *Animal Feed Science and Technology* 72(3), 235-259. DOI: 10.1016/S0377-8401(97)00191-0

Pocienė, L., Šarūnaitė, L., Tilvikienė, V., Šlepetys, J., and Kadžiulienė, Ž. (2013). "The yield and composition of reed canary grass biomass as raw material for combustion," *Biologija* 59(2), 195-200. DOI: 10.6001/biologija.v59i2.2752

Reddy, B. V. S., Reddy, P. S., Bidinger, F., and Blümmel, M. (2003). "Crop management factors influencing yield and quality of crop residues," *Field Crops Research* 84(1), 57-77. DOI: 10.1016/S0378-4290(03)00141-2

Seppälä, M., Paavola, T., Lehtomäki, A., and Rintala, J. (2009). "Biogas production from boreal herbaceous grasses–specific methane yield and methane yield per hectare," *Bioresource Technology* 100(12), 2952-2958. DOI: 10.1016/j.biortech.2009.01.044

Smuga-Kogut, M., Zgórska, K., and Szymanowska-Powałowska, D. (2016). "Influence of the crystalline structure of cellulose on the production of ethanol from lignocellulose biomass," *International Agrophysics* 30(1), 83-88. DOI: 10.1515/intag-2015-0072

Triolo, J. M., Sommer, S. G., Møller, H. B., Weisbjerg, M. R., and Jiang, X. Y. (2011). "A new algorithm to characterize biodegradability of biomass during anaerobic digestion: Influence of lignin concentration on methane production potential," *Bioresource Technology* 102(20), 9395-9402. DOI: 10.1016/j.biortech.2011.07.026

Tsavkelova, E. A., and Netrusov, A. I. (2012). "Biogas production from cellulose-containing substrates: A review," *Applied Biochemistry and Microbiology* 48(5), 421-433. DOI: 10.1134/S0003683812050134

Van Soest, P. V., Robertson, J. B., and Lewis, B. A. (1991). "Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition," *Journal of Dairy Science* 74(10), 3583-3597. DOI: 10.3168/jds.S0022-0302(91)78551-2

Ward, A. J., Hobbs, P. J., Holliman, P. J., and Jones, D. L. (2008). "Optimization of the anaerobic digestion of agricultural resources," *Bioresource Technology* 99(17), 7928-7940. DOI: 10.1016/j.biortech.2008.02.044

Article submitted: May 29, 2017; Peer review completed: September 17, 2017; Revised version received and accepted: September 22, 107; Published: September 28, 2017.
DOI: 10.15376/biores.12.4.8565-8580