Use of Wild Plant Species: A Potential for Methane Production in Biogas Plants

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Abstract- Sources of the input biomass for biogas plant (BGP) are very often communal biological wastes, farm materials such as slurry, dung or biomass of purposefully grown plants. Efforts aiming to support biogas yield from grass biomass with using additionally sown wild species can affect efficiency of the whole production process and improve its environmental impact. This was why 14 wild plant species were selected as a potential source of biomass for BGP: *Trifolium alpestre* L.; *Trifolium rubens* L.; *Galega orientalis* Lamb.; *Medicago sativa* L.; *Onobrychis viciifolia* Scop.; *Vicia sylvatica* L.; *Astragalus cicer* L.; *Trifolium pannonicum* Jacq.; *Lathyrus pratensis* L.; *Melilotus alba* Medic.; *Trigonella foenum-graecum* L.; *Lathyrus sylverstris* L.; *Securigera varia* L. and *Dorycnium germanicum* (Gremli) Rikli. The potential of individual crops for being used in BGP was evaluated based on calculating a theoretical methane yield (TMY). The calculated TMY values ranged from 0.130 m^3/kg_{VS} to 0.182 m^3/kg_{VS}. The demonstrably lowest ($p<0.05$) value of TMY was calculated for *Medicago sativa* L., which showed the lowest content of lipids and the highest content of ADF. By contrast, the highest TMY was recorded in *Securigera varia* L., which exhibited the highest content of carbohydrates and starch and the lowest content of ADF and NDF. An analysis of the biomass of grown species as well as the TMY calculation demonstrated significant differences between the respective plant species and a need to study in details the characteristics of wild plant species prior to their use for the production of biomass for BGP.

Keywords legumes; wild plant species; permanent grass stands; methane yield; biogas plant

1. Introduction

Changing climatic conditions, newly emerging diseases and pests as well as higher demands for yields and genotype uniformity of cultivated crops force breeders to seek new genetic sources. In order to meet future requirements of society, not only food production but for example environment friendly energies, farmers will need a wide species diversity [1-4]. It is desirable that sustainable agriculture can use new species and varieties that are resistant to environmental stresses. A solution can be seen in using wild plant species which could enhance adaptive features of crops worldwide and thus play a key role in this field [5, 6].

Wild plant species used to represent primarily a source of livelihood for human population. Due to agricultural activities, they were gradually converted into cultural crops with values added in line with the requirements of humans. An example can be altered morphological and physiological characteristics leading to higher yields or changed contents of substances [7]. Wild plant species are currently defined as plants which spontaneously reproduce in the open without humans interfering with their growth and development [8]. Their habitats are sites in diverse regions in the world where they have to face unfavourable effects of the environment [9]. Thus, they become rich sources of genetic information containing particularly the capacity to resist biotic and abiotic stresses [10,11].

Both biotic and abiotic stresses have a negative influence on the growth and development of plants [12, 13] the vitality of which then reflects in the yield and quality of harvested crops [14,15]. Wild species are currently used to create varieties resilient to abiotic stress such as drought [16-18], high temperatures [19] and saline soils [20-22]. As to biotic stresses, wild plant species are investigated for their resistance to fungal diseases, viruses and other pathogens [11] – e.g. against corn (*Zea mays* L.) blight; [23], tomato (*Solanum lycopersicum* L.) yellow leaf curl virus; [24], wheat (*Triticum aestivum* L.) curl mite; [25-27] or potato (*Solanum tuberosum* L.) blight; [28]. Wild plant species play a role for example in increasing rice (*Oryza sativa* L.) yields; [29]. They also deserve attention for enriching plants with desirable nutritional substances. This was how durum wheat (*Triticum durum* Desf.) richer in gluten was obtained [30], potatoes (*Solanum tuberosum* L.) with a higher content of calcium [31] and tomatoes (*Solanum lycopersicum* L.) enriched significantly with pro-vitamin A [32]. Wild plant species can also play an important role in the renewal of natural functions of ecosystems that have been disturbed by anthropogenic [33,34].

A recent study showed that anaerobic digestion would be probably one of the most promising technologies to gain energy from biomass, especially in farms [35,36]. The dynamic development of biotechnologies is expected to bring higher use of utilizing wild plant species in breeding programmes with multiple objectives, e.g. for their cultivation in mixed cultures with conventional crops to produce biomass for biogas plants [11,37,38]. Biomass produced by grass stands is a reservoir of chemically deposited energy obtained from the sun [39,40].

The main goal of this study was to verify a potential of selected perspective wild plant species from the *Fabaceae* family for the production of biogas in biogas plants and based on the results to put together a list of species that can be recommended for sowing into permanent grass stands for the purpose of further plant biomass use in BGP.

2. Materials and methods

2.1. Plant sampling - cultivation of selected plant species

The plant sampling was performed on the study site Troubsko (Czech Republic). The Troubsko locality belongs to warm and dry growing area with an altitude of 270 m a. s. l. The average annual rainfall is 512 mm and the average annual temperature is 9.4 °C. The green mass of selected plants (see Table 1) was monitored. Dry matter samples were taken for chemical analyses. We evaluated the growing season of year 2019.

Table 1. Tested plants

Treatment no.	Monitored crop	
	Vernacular names	**Latin name**
1	Purple-globe clover	*Trifolium alpestre* L.
2	Red feather clover	*Trifolium rubens* L.
3	Fodder galega	*Galega orientalis* Lamb.
4	Alfalfa	*Medicago sativa* L.
5	Common sainfoin	*Onobrychis viciifolia* Scop.
6	Wood vetch	*Vicia sylvatica* L.
7	Chickpea milk-vetch	*Astragalus cicer* L.
8	Hungarian clover	*Trifolium pannonicum* Jacq.
9	Meadow vetchling	*Lathyrus pratensis* L.
10	White sweet clover	*Melilotus alba* Medic.
11	Fenugreek	*Trigonella foenum-graecum* L.
12	Flat pea	*Lathyrus sylverstris* L.
13	Crown vetch	*Securigera varia* L.
14	Dorycnium germanicum	*Dorycnium germanicum* (Gremli) Rikli

Biomass samples were collected in the growth stage of butonization. Three plant biomass samples were taken from each species and selected characteristics were measured. These characteristics were following: content of carbohydrates (CAR), proteins and lipids. The content of carbohydrates was determined by colorimetry. KjeltecTM 2300 Analyzer (FOSS Analytical, DK) was used for the determination of nitrogen content according to Kjeldahl method. The data were used to determine the content of crude proteins. Contents of lipids were measured using a water-cooled Soxhlet extractor.

2.2. Theoretic methane yield calculation

Results of nutrient analysis were used to calculate a theoretical methane yield (TMY) according to Lesteur et al. [41]; and Pham et al. [42]. In this calculation, biodegradable compounds, lipids, proteins and carbohydrates are used for the Buswell formula (1). The result of this calculation is TMY in m^3 per kilogram of volatile solids (VS).

$$\text{(1)} \qquad TMY = \frac{(0.415\times\%\ \text{carbohydrates}) + (0.496\times\%\ \text{proteins}) + (1.014\times\%\ \text{lipids})}{100}\ [m^3/kg_{VS}]$$

2.3. Statistical processing of resulting data

The measured values of green matter and hay yield together with TMY were graphically processed and statistically assessed in Statistica 12 (Dell Inc., Round Rock, Texas, USA) programme using one-way ANOVA in combination with LSD Fischer´s test at a level of significance $\alpha = 0.05$.

3. Results

To calculate TMY of gained plant material and to assess individual crops, selected indicators for VS, CAR (carbohydrates), proteins, starch, lipids, CF (crude proteins), NDF (neutral detergent fibre) and ADF (acid detergent fibre) were determined whose values are presented in Tables 2 and 3.

VS values were over 90 % in a majority of tested plants, only in *Melilotus alba* the VS value was below this limit. This is why the species was the only one exhibiting a statistically significant difference in VS as compared with the other plant species.

More distinctive differences were recorded in the contents of CAR, proteins, starch and lipids (Table 2). CAR in the plant biomass were determined to range from 5.05 $\%_{VS}$ to 17.87 $\%_{VS}$. The highest CAR content was found in *Securigera varia* while the lowest values were demonstrably recorded in the biomass of plant species *Trifolium alpestre*, *Galega orientalis* and *Lathyrus pratensis*. Proteins occurred in the plant biomass from 12.47 $\%_{VS}$ to 21.34 $\%_{VS}$. The values fluctuated less than those of CAR. The highest value was recorded in the biomass of plant species *Galega orientalis* and *Lathyrus pratensis*, the difference being statistically significant as compared with the other plant species. On the other hand, the demonstrably lowest yield was found in analysing the species of *Onobrychis viciifolia* and *Dorycnium germanicum*. Although the other species showed partial statistical differences, their biomass contained high amounts of proteins in a relatively narrow range from 14.24 $\%_{VS}$ to 17.84 $\%_{VS}$. Similarly, as in the case of protein contents, the starch parameter exhibited many significant differences. The substance was contained in all plant species at different amounts which were in a majority of cases statistically significant across the species spectrum. Compared with all other variants, the highest value was demonstrably recorded in *Securigera varia* (67.57 $\%_{VS}$) while the lowest values were measured in the biomass of *Trifolium pannonicum* (45.13 $\%_{VS}$) with the difference being statistically significant as compared with the remaining variants. Starch content values in the other species then ranged from 46.86 $\%_{VS}$ (*Trifolium rubens*) to 53.96 $\%_{VS}$ (*Vicia sylvatica*). Contents of lipids in the plant biomass were less fluctuating as compared with the other parameters. The highest contents of lipids were detected in *Melilotus alba* (4.43 $\%_{VS}$) with differences being statistically significant compared with the other variants. The lowest values ($\leq 2.80\ \%_{VS}$) were found in *Trifolium rubens*, *Medicago sativa* and *Onobrychis viciifolia*, with statistically significant differences as compared with all other variants except for *Securigera varia* and *Dorycnium germanicum*.

The remaining parameters CF, NDF, ADF are presented in Table 3. All three parameters exhibit significant differences across the species spectrum. The biomass of *Galega orientalis* exhibited always the highest values of these parameters while the biomass of *Securigera varia* showed the lowest values of these substances.

Table 2. Qualitative parameters of harvested model crop shreddings – Part A

Sample	VS		CAR		Proteins		Starch		Lipids	
	% ± SD	LSD	$\%_{VS}$	LSD	$\%_{VS}$	LSD	$\%_{VS}$	LSD	$\%_{VS}$	LSD
Trifolium alpestre	92.57 ± 1.05	A	5.19 ± 0.24	A	15.08 ± 0.01	D	48.05 ± 0.18	C	4.04 ± 0.32	EF
Trifolium rubens	95.06 ± 3.50	A	7.62 ± 0.11	CD	15.21 ± 0.06	D	46.86 ± 0.40	B	2.78 ± 0.06	A
Galega orientalis	93.66 ± 2.01	A	5.05 ± 1.04	A	21.31 ± 0.08	H	49.37 ± 0.46	D	3.34 ± 0.49	BC
Medicago sativa	94.98 ± 3.24	A	6.39 ± 0.34	B	15.33 ± 0.02	D	49.81 ± 0.71	D	2.75 ± 0.22	A
Onobrychis viciifolia	92.18 ± 0.73	A	12.52 ± 0.22	H	12.47 ± 0.43	A	52.85 ± 0.55	EF	2.77 ± 0.21	A
Vicia sylvatica	95.09 ± 4.50	A	7.77 ± 0.69	DE	17.84 ± 0.33	F	53.96 ± 0.48	F	3.63 ± 0.19	CE
Astragalus cicer	94.80 ± 3.59	A	8.26 ± 1.00	E	17.11 ± 0.39	F	52.12 ± 0.23	E	3.37 ± 0.21	B
Trifolium pannonicum	94.86 ± 4.51	A	7.20 ± 0.86	BCD	13.57 ± 0.22	C	45.13 ± 1.78	A	3.81 ± 0.35	DE
Lathyrus pratensis	91.79 ± 0.78	AB	5.38 ± 0.31	A	21.34 ± 0.10	H	53.10 ± 1.14	EF	3.69 ± 0.12	CE
Melilotus alba	87.23 ± 4.91	B	10.85 ± 0.24	G	16.68 ± 0.02	E	49.55 ± 0.18	D	4.43 ± 0.32	F
Trigonella foenum-graecum	94.90 ± 0.63	A	9.35 ± 0.11	F	18.75 ± 0.06	G	52.15 ± 0.40	E	3.54 ± 0.06	B
Lathyrus sylverstris	92.99 ± 2.80	A	6.73 ± 1.04	BC	14.24 ± 0.08	B	48.54 ± 0.46	C	3.68 ± 0.49	CE
Securigera varia	94.73 ± 0.37	A	17.87 ± 0.34	K	15.46 ± 0.01	D	67.57 ± 0.71	G	3.05 ± 0.22	AB
Dorycnium germanicum	95.72 ± 1.13	A	14.42 ± 0.12	J	12.53 ± 0.43	A	51.99 ± 0.55	E	3.07 ± 0.21	AB

Comment for Table 2: Percentage by weight; VS – volatile solids; CAR – carbohydrates. The values are represented as mean ± SD; for $n = 3$. Different letter indexes show significant differences in individual parameters between individual plant species VS at a level of significance $\alpha = 0.05$, post-hoc Fischer LSD test.

Table 3. Qualitative parameters of harvested model crop shreddings – Part B

Sample	CF		NDF		ADF	
	%vs ± SD	**LSD**	**%vs**	**LSD**	**%vs**	**LSD**
Trifolium alpestre	24.74 ± 0.46	D	41.69 ± 0.52	F	32.09 ± 0.86	D
Trifolium rubens	26.62 ± 0.45	D	42.98 ± 0.07	G	33.82 ± 0.11	E
Galega orientalis	26.09 ± 0.32	D	55.14 ± 0.25	J	39.78 ± 1.5	F
Medicago sativa	25.30 ± 0.87	C	47.83 ± 1.15	H	38.27 ± 0.07	F
Onobrychis viciifolia	26.08 ± 0.60	C	46.97 ± 0.58	H	38.48 ± 0.40	F
Vicia sylvatica	17.43 ± 0.05	AB	32.27 ± 0.63	B	27.70 ± 0.37	B
Astragalus cicer	21.77 ± 0.03	CD	38.58 ± 1.43	E	30.68 ± 0.41	C
Trifolium pannonicum	26.87 ± 0.16	D	48.18 ± 0.51	HI	37.98 ± 0.38	F
Lathyrus pratensis	22.52 ± 3.84	CD	42.13 ± 1.54	F	30.04 ± 0.86	C
Melilotus alba	19.89 ± 0.43	ABC	35.16 ± 0.07	D	27.82 ± 0.11	B
Trigonella foenum-graecum	18.53 ± 1.02	A	33.49 ± 0.55	C	27.10 ± 2.27	B
Lathyrus sylvestris	23.48 ± 4.78	C	48.68 ± 1.34	I	41.15 ± 0.15	G
Securigera varia	15.54 ± 9.28	AB	28.14 ± 0.65	A	20.69 ± 1.2	A
Dorycnium germanicum	15.46 ± 4.57	AB	41.02 ± 0.99	F	28.29 ± 0.06	B

Comment for Table 3: CF –crude fibre; NDF – neutral detergent fibre; ADF – acid detergent fibre. The values are represented as mean ± SD; for n = 3). Different letter indexes show significant differences in individual parameters between individual plant species VS at a level of significance α = 0.05, post-hoc Fischer LSD test.

Table 4. Correlation matrix calculated within factor analysis

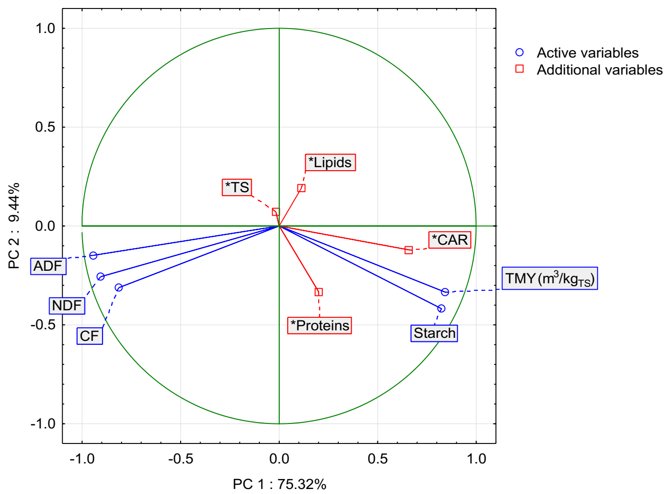
	VS	CAR	Proteins	Starch	Lipids	CF	NDF	ADF	TMY
VS	1.00	0.07	-0.09	0.07	-0.47	-0.01	-0.00	-0.01	-0.17
CAR	0.07	1.00	-0.44	0.70	-0.30	-0.54	-0.56	-0.58	0.48
Proteins	-0.09	-0.44	1.00	0.09	0.26	-0.04	-0.06	-0.16	0.52
Starch	0.07	0.70	0.09	1.00	-0.20	-0.58	-0.65	-0.69	0.68
Lipids	-0.47	-0.30	0.26	-0.20	1.00	-0.11	-0.16	-0.14	0.27
CF	-0.01	-0.54	-0.04	-0.58	-0.11	1.00	0.67	0.70	-0.59
NDF	-0.00	-0.56	-0.06	-0.65	-0.16	0.67	1.00	0.94	-0.64
ADF	-0.01	-0.58	-0.16	-0.69	-0.14	0.70	0.94	1.00	-0.75
TMY	-0.17	0.48	0.52	0.68	0.27	-0.59	-0.64	-0.75	1.00

Comments for Table 4: Correlation coefficients are shown calculated within factor analysis. Scope of input set included 42 values for each parameter (n = 42). Values in red are statistically significant at a level α > 0.05. VS – volatile solid; CAR – carbohydrates; CF – crude fibre; NDF – neutral detergent fibre; ADF – acid detergent fibre; TMY – theoretical methane yield.

As mentioned above, the described parameters were selected for the calculation of TMY according to Eq. 1. In order to determine their influence on the calculation and relations among them, a correlation matrix was constructed within the factor analysis. The matrix (Table 4) shows that the demonstrably greatest influence on the calculated TMY value (Figure 1) was that of starch (R = 0.68), NDF (R = -0.64), ADF (R = -0.75) and the CF parameter which was on the verge of provability (R = -0.59). This influence was of either positive or negative character. It can be summarized that the TMY values were decreasing with the increasing contents of CF, NDF and ADF while a significant TMY increase could be expected with the increasing content of starch.

In addition, relations of selected parameters were analysed using the PCA analysis, the results of which are shown using the biplot graph (Figure 1). Based on these results and the correlation matrix (Table 4, Table 5, Annex 1), a strong positive correlation apparently existed between the starch content and CAR in the plant biomass. In contrast, a moderately strong negative correlation was detected in the relation between starch and CF, NDF, ADF. Therefore, it can be assumed that the content of starch in plant biomass is decreasing with the increasing values of these parameters. Other demonstrable correlations among the individual plant biomass parameters were not recorded. The PCA analysis revealed the presence of four factors - components (Annex 2) with the first two of them explaining over 84 % of variability and being therefore considered as the first two main components (PC 1: 75.32 %; PC 2: 9.44 %). PC 1 positively correlated with the contents of CAR, starch and TMY value, showing by contrast a minimum correlation to the values of proteins, lipids and VS. PC 1 along with PC 2 exhibited a negative correlation to the values of ADF, NDF and CF. In case of PC 1, it was a very strong negative correlation (R > -0.8) while PC 2 showed a lower R value (from -0.3 to -0.4).

Figure 1. Biplot graph – projection of variables related to the biomass composition and TMY



When PC 1 positively correlates namely with the TMY values, it presumably characterises variability caused by plant species and their natural capability of creating reserves of starch and CAR in the biomass, which positively affect the TMY value. In contrast, PC 2 correlated slightly positively with the parameter of lipids, exhibiting either none apparent dependence or a weakly negative correlation (R ≤ -0.4; Table 4) in the other parameters.

Table 5. Factor coordinates of variables by correlations

	PC 1	PC 2
TMY	0.843	-0.334
CF	-0.813	-0.311
NDF	-0.905	-0.256
ADF	-0.945	-0.149
Starch	0.824	-0.418
*VS	-0.010	0.071
*CAR	0.658	-0.120
*Proteins	0.201	-0.332
*Lipids	0.113	0.192

The calculated TMY values (Figure 2) ranged from 0.130 m^3/kg$_{VS}$ to 0.182 m^3/kg$_{VS}$ and significant differences were found across the respective plant species. In terms of the development of calculated TMY values, main zones of the distribution of values were identified, which were delineated based on the prepared histogram (Figure 3) by the following TMY intervals: 1) 0.12 – 0.14 m^3/kg$_{VS}$; 2) 0.141 – 0.160 m^3/kg$_{VS}$; 3) 0.161 – 0.172 m^3/kg$_{VS}$ and 4) > 0.180 m^3/kg$_{VS}$. The values of most tested plant species occurred in these intervals and statistically significant differences existed among them (Figure 2). The highest TMY value was calculated for a single species (*Securigera varia*) which did not occur in any of the intervals and exhibited the highest TMY value (0.182 m^3/kg$_{VS}$) of all other plant species, thus deviating from the distribution of values.

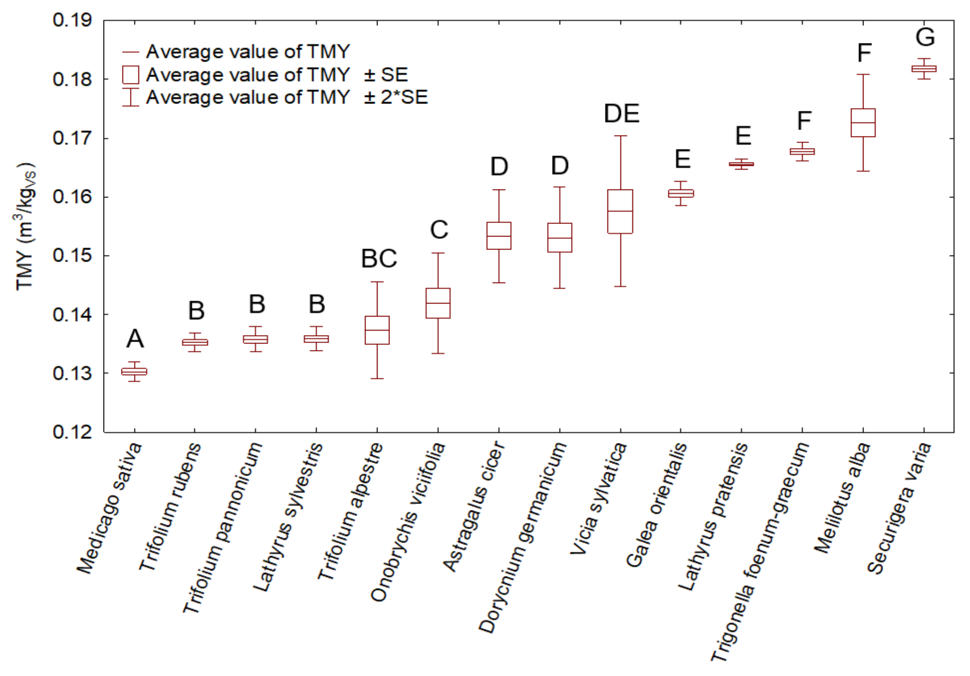


Figure 2. Theoretical methane yield (m^3/kg$_{VS}$) from individual variants in m^3 per kg of individual plants VS. Different letter indexes show significant differences in TMY among individual plants at a level of significance α = 0.05, post-hoc Fischer LSD test.

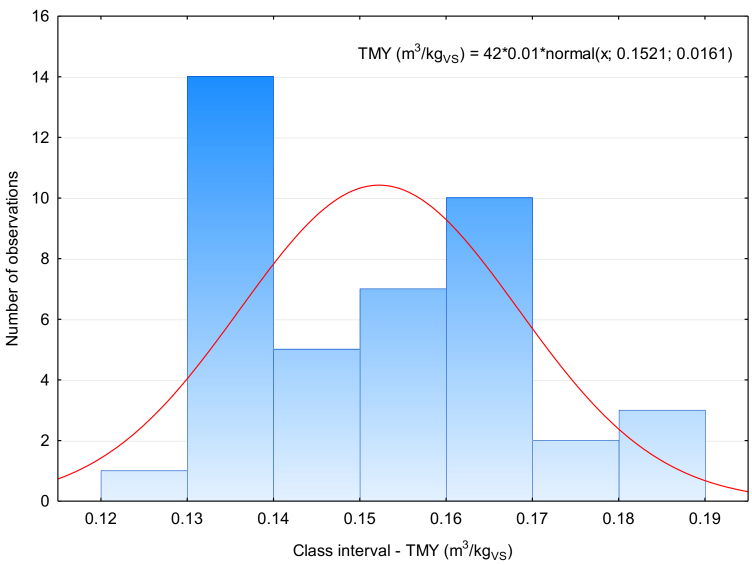


Figure 1. Histogram of the number of observations for TMY (m^3/kg$_{VS}$)

The provably and absolutely lowest TMY yield value in Interval 1 (0.12 – 0.14 m^3/kg$_{VS}$) was calculated for *Medicago sativa*. Very low values of TMY were recorded also in *Trifolium pannonicum, Lathyrus sylvestris, Trifolium rubens* and *Trifolium alpestre*. Although, the species exhibited demonstrably higher TMY values than *Medicago sativa*, they belonged in Interval 1 with the lowest TMY anyhow. The second interval of values with TMY ranging from 0.141 to 0.160 m^3/kg$_{VS}$ can be denoted as moderately high. These values were recorded in the following species ordered according to the TMY value: *Onobrychis viciifolia* < *Astragalus cicer, Dorycnium germanicum* < *Vicia sylvatica*. Compared to the first interval, these plant species exhibited statistically significant differences as well as partial differences were existing among them, too. Interval 3 with the calculated TMY values ranging from 0.161 to 0.172 m^3/kg$_{VS}$ included the following plant species: *Galega orientalis, Lathyrus pratensis, Trigonella foenum-graecum* and *Melilotus alba*. The last two species exhibited statistically significantly higher TMY values as compared with *Galega orientalis* and *Lathyrus pratensis*. The absolutely highest TMY (P <

0.05 compared with all the other plant species) was recorded in *Securigera varia* in which the TMY value exceeded 0.180 m^3/kg_{VS} as mentioned above.

4. Discussions

The mutual link between degradability (biomethane production) and chemical composition of agricultural crops was characterized in several studies [39,43,44]. Calculation of TMY is one of the simplest methods applicable for the primary assessment of biological degradability of feedstock for biomethane production [41]. The obtained TMY values inform of maximum potential yield of biomethane from the given organic substrate upon the condition of its complete degradation according to Buswell equation [45]. According to Boe et al. [46], biomethane production can be considered a qualitative indicator of fermentation process in the biogas plant.

The primary factor affecting degradability of agricultural crops is ripeness at which the crop is harvested. Wahid et al. [47] claim that the contents of fibre and dry matter can be affected by the number of cuts or by harvesting technology, too [48]. The experimental leguminous species showed demonstrable differences in the chemical composition of biomass in terms of their usability as substrates for biogas production. Although the focus of our primary assessment was green biomass, similar characteristics can be expected in biomass conserved by ensiling. The process of ensiling is a traditional method of biomass conservation for use in biogas production [49, 50]. According to Zhao et al. [51], ensiling can be used as a process of very efficient pre-treatment of material for biogas production, which can reduce the content of cellulose and hemicellulose while increasing the content of soluble materials and organic acids with appropriate additives. Franco et al. [52] inform that in specific conditions, ensiling can increase the potential of methane production even taking into account storage losses. One of possible explanations is that the gained biochemical availability can overcome losses of organic matter during storage. Based on the performed calculations, we can state that the theoretical yield of biomethane was adversely affected by the increasing representation of CF, NDF and ADF, which is in line with Suha Uslu et al. [43] and Slepetiene et al. [44]. Biomass with a high content of lignocellulose shows low biogas yield and biodegradability due to low lignin biodegradability [53,54]. To increase the degradability of polymer such as lignin, Loughrin et al. [55] recommend the technology of micro-aeration during which facultatively aerobic bacteria are supported and hence the production of needed enzymes. According to Ozbayram et al. [56], it is also possible to use inoculation or bioaugmentation by means of rumen microbiota to promote lignocellulose biomass break down and production of biomethane. Moreover, NDF digestibility decreases in legumes during ripening. TMY was further favourably affected by higher contents of lipids, polysaccharides (carbohydrates) and proteins, however, the effect was statistically significant only for polysaccharides (Table 4). Lipid, carbohydrate, and protein ratios may also indicate biomethane yield performance. Lipid-rich biomass can significantly increase methane production [57]. On the other hand, a high content of lipids can be responsible for fatty acids accumulation, which are inhibitory for methanogens [58]. Simple sugars and starch are well degradable and thus may positively affect the biomethane yield. During the biological degradation of protein-rich substrates, ammonia in both forms, ammonium (NH_4) and free ammonia (NH_3) are produced. These compounds are reported as strong inhibitors in the process of anaerobic fermentation [59].

Assessing legumes, their capability of biological fixation of nitrogen has to be considered as well as the content of substances in their biomass, which are not taken into account in the TMY calculation but can significantly change the perspective of their biomass use in the production of biomethane. The main energy crop for biogas production is corn silage, grass silage, corn cob mix (CCM) and other crops from the family of *Fabaceae*. Thanks to their capacity of biological fixation of nitrogen, legumes in particular can compensate energy requirements of the production of N-fertilizers needed for the successful cultivation of other crops presuming that digestate obtained from the biomass of legumes is applied to the other crops [60,61,62]. According to Carksson & Huss-Danell [63], biological fixation of nitrogen may amount up to 373 kg $N \cdot ha^{-1} \cdot year^{-1}$ in red clover (*Trifolium pratense* L.), 545 kg $N \cdot ha^{-1} \cdot year^{-1}$ in white clover (*T. repens* L.), and 350 kg $N \cdot ha^{-1} \cdot year^{-1}$ was measured in the stand of alfalfa (*Medicago sativa* L.) in dependence on soil and climatic conditions of the given site. Representatives of *Poaceae* family to which corn (*Zea mays*) belongs usually feature higher contents of starch and sugars than the contents of fibre and proteins, which predetermines them for use in BGP [64] as compared with other plant families. Contents of starch and proteins were compared in Kintl et al. [65] – corn had the starch and protein contents 20.66 %VS and 8.13 $\%_{VS}$, respectively, while white sweet clover (*Melilotus albus*) had 4.51 $\%_{VS}$ of starch and 11.62 $\%_{VS}$ of proteins. Wahid et al. [47] claim the higher representation of N-substances may inhibit the process of fermentation. On the other hand, according to Hutňan et al. [66] the AD process is unstable due to the low content of nitrogen in the corn silage, and this is why they recommend to stabilize it by adding a substrate with the higher N content. Leguminoses meet this requirement. The process is elucidated in Mata-Alvarez et al. [67] based on using AcoD, (anaerobic co-digestion) during which joint digestion of a mixture of two and more substrates with complementary features takes place. The AcoD process allows to increase the production of biogas and to stabilize the process. Kintl et al. [65] confirmed that with the representation of white sweet clover up to 20 % in the silage produced from a mixed culture of white sweet clover and corn, a higher production of both biogas and methane was achieved from AcoD. Tests focused on the applicability of silage from a mixed culture of corn and white lupine similarly showed that the production of methane from corn was 0.327 m^3 CH_4/kg_{VS} and 0.330 m^3

CH_4/kg_{VS} from the silage produced of 90% of corn and 10 % of lupine, however, the difference was not statistically significant [40]. Adamovics et al. [68] claim that one of important fodder crops is also fodder galega (*Galega orientalis* Lam.) which was tested for the production of biogas and methane. Research results support the statement that a feedstock mixture in the AcoD process can help reach higher production. The application of a mixture of fodder galega (*Galega orientalis* Lam.) and meadow fescue (*Festuca pratensis* Huds.) at a ratio of 40/60 % resulted in the highest production of both biogas and methane as compared with the production of single species biomass of tested plant species.

The experimental plant species were also subjected to many analyses in connection with their utilization for cattle nutrition. The analyses could be used also for the evaluation of legumes. The tested species of sainfoin (*Onobrychis viciifolia*), crown vetch (*Securigera varia* L.) and cicer milkvetch (*Astragalus cicer* L.) can eliminate flatulence in ruminants [69]; they can also contain biologically active substances such as tannins [70]. According to McAllister et al. [71] and McSweeney et al. [72], these substances can affect microbial activities in the rumen of ruminant cattle species. The content of these substances can affect utilization of the tested crown vetch (*Securigera varia* L.) in spite of the fact that the statistically highest significant value was found in the calculation of TMY.

A similar problem was explored also in coumarin [72] contained in white sweet clover (*Melilotus albus* MED.). According to Kadaňková et al. [74], there is a direct dependence between the content of coumarin in shreddings and the content of coumarin in the resulting silage. Popp et al. [73] claim that individual plants can contain up to 5 % dry matter of coumarin and that microbiome in the biogas plant fermenter must get used to its presence. This corresponded to the results published by Gatta et al. [75] who found out that the highest yield of biogas and methane was achieved in testing a mixed culture in which the representation of legumes was 30 %.

5. Conclusion

The main goal of this study was to calculate potential on the basis of establishing the TMY parameter a potential of selected perspective wild plant species from the *Fabaceae* family for their use in biogas plants for the production of biogas. Based on the results, a list of wild plant species was developed, which can be recommended for sowing into permanent grass stands for the purpose of further plant biomass use in BGP. Legume species exhibiting the best results in the assessment of biomass by using the Buswell equation were: *Galega orientalis* Lamb.; *Lathyrus pratensis* L.; *Trigonella foenum-graecum* L.; *Melilotus alba* Medic. and *Securigera varia* L. With respect to the favourable results, these five species can be recommended for the complementary sowing into permanent grass stands. Species appearing little perspective with respect to their use for the production of biogas were *Medicago sativa, Trifolium rubens, Trifolium pannonicum* and *Lathyrus sylvestris*.

Although it is possible to obtain a maximum potential biomethane yield (TMY) from the given organic substrate using the Buswell equation, there are other properties of explored biomass, which have to be evaluated as they could be limiting the process. The capacity of biological fixation of nitrogen, which is important for the growth of plant species from the Fabaceae family but also in their use for biogas production and subsequent use of digestate as a fertilizer to other crops such as corn, plays in favour of legumes. It is gentle agro-technological procedures using legumes, intermediate crops and erosion-control technologies that are the cornerstones of phytoenergetics, i. e. renewable biogas production.

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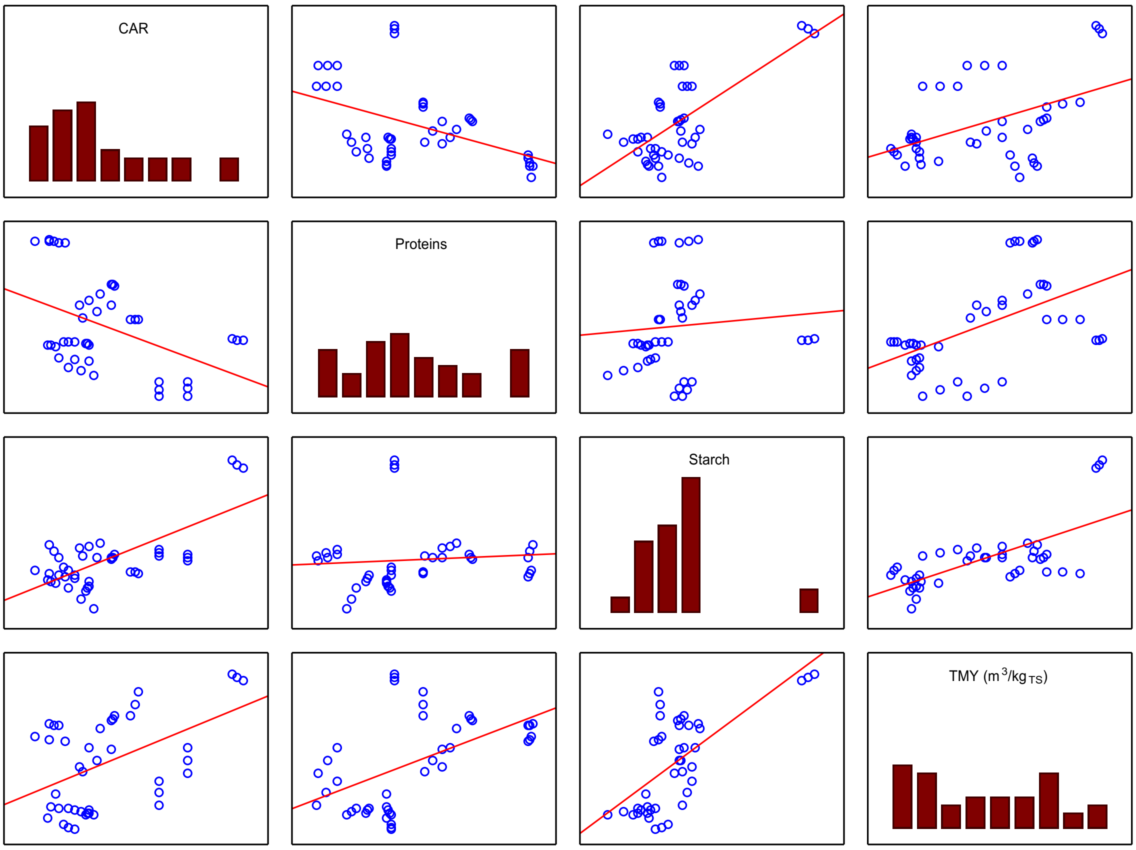
ANNEX

Annex 1. A graphical representation of the correlation matrix



Annex 2. Scree plot of the eigenvalues of principal components