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Possibilities of Using White Sweetclover Grown in Mixture with Maize for Biomethane Production

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Abstract: Methods of growing plant biomass for the production of biogas in anaerobic digestion plants have a decisive influence on arable land and on the evaluation of biogas plant technologies from the environmental point of view. The main benefit of anaerobic digestion is the possibility to use various agricultural crops for energy production. Some of these plant species, e.g., legumes, are generally considered to be beneficial for arable soil quality, as compared with maize monocultures with frequently manifested soil degradation and adverse environmental impact on arable land. A possible change is offered by cultivation systems composed of two and more crops and defined as mixed cropping (MC) systems. The systems are characterized by a more efficient utilization of natural resources of the site as well as by a greater potential for arable soil protection. A question remains as to whether the MC system of growing maize and white sweetclover can be used for biogas yield. In the presented research study, a mixed cropping system was tested with maize (*Zea mays* L.) and white sweetclover (*Melilotus albus* MED.). The goal of our research was to determine an optimum ratio of maize and white sweetclover (s.c.) shreddings in silage for a biogas plant. For this purpose, model micro-silages of monocultures were prepared: maize (100%), white s.c. (100%), as well as variants with different weight shares of these two crops (maize:white s.c.; 3:7, 1:1, 7:3, 8:2, 8.5:1.5, 9:1). The silages were subjected to biomethanation tests, in order to determine the influence of the increased addition of white s.c. biomass on methane yield and methane concentration in biogas. The highest values of biogas yield were recorded in the maize monoculture and in the MC variant of maize and white s.c. at 9:1 (>0.26 m^3/kg_{VS}). The lowest methane yield values were recorded in the white s.c. monoculture (0.16 m^3/kg_{VS}). It was found out that the yield of methane decreased with an increasing share of white sweetclover in the maize silage, due to the increased content of poorly degradable organic substances and the presence of fermentation inhibitors (e.g., coumarin).

Keywords: legumes; white sweetclover; mixed cropping; silage; methane; biogas plant

1. Introduction

The production of biofuels from agricultural raw materials was promoted as a solution of environmental problems in the late 20th century. Biogas represents alternative fuel gained from renewable resources (plant biomass), for the generation of electric and thermal energies, as compared with the use of fossil fuels [1,2]. An advantage of biogas production technology is the ability to provide for a storable and continually generated source of energy [2]. The sustainable production of plant biomass utilizable in biogas plants (BGP) should be critically discussed with regard to the growing share of farmland used for growing energy crops [3]. According to Möller [4], the currently used agricultural systems producing energy crops must not put into danger the capacity of farmland to satisfy the future production of energy or food crops. According to Britz and Delzeit [5], the main reason for extending areas sown with maize (*Zea mays* L.) is the fact that it is the most important agricultural raw material for anaerobic digestion or a so-called energy crop. Intensive cultivation of *Zea mays* for the production of biogas is currently perceived disapprovingly by the social and scientific communities [6].

Adhering to the principles of sustainability, anaerobic digestion represents one of the most promising technologies for gaining renewable bioenergy from plant biomass, namely on agricultural farms [7,8]. However, it is at the same time important that agricultural practices sustainable over a long time are developed. These practices will facilitate growing of crops usable in BGP, but with lower negative environmental impacts on arable land, as compared with the conventional practice [9,10]. In the future, it is also necessary to count on the carbon-neutral production of biohydrogen from biomass [11]. To achieve the optimum and sustainable production of plant biomass, a system of growing two or more crops (mixed cropping) on one site at the same time can be applied [10]. The sustainability of mixed cropping derives from the plant species grown together with maize. It is considered to be very beneficial for crops from the families of *Poaceae* and *Fabaceae*. These plant species have a generally positive effect on the soil environment, and in mixed cropping, they can contribute to the reduction of negative environmental impacts of conventional technologies [12,13]. The main goal of mixed cropping is to reach higher productivity per unit area [14], based on the higher efficiency of using resources, including water, nutrients and solar energy [15–17]. The benefits of mixed cropping were also demonstrated in the legume/maize combination [6].

The implementation of biogas plants in agricultural systems has the potential to reduce overall emissions of greenhouse gases from agricultural systems, on the condition that plant biomass for biogas production is grown in sustainable ways [18]. Biogas plants reduce methane emissions, particularly in the processing of biologically degradable materials from animal production, and agricultural wastes which would otherwise be processed conventionally. Benefits from using legumes as feedstock for biogas production consist of reduced amounts of applied mineral fertilizers. The reason is that the biological fixation of N in the soil by means of legumes and the use of digestate from the biogas plant in which the resulting biomass from the mixed cropping system is utilized. The lower consumption of these fertilizers also brings about their lower production, and this has a direct effect on greenhouse gas emissions [19]. Mixed cropping offers benefits not only in the reduced inputs of mineral fertilizers, but also pesticides and herbicides. The reduced application of such substances in the technology of mixed cropping has a positive effect on the environment [20–22].

One of the possibilities for including legumes in the sowing plan when growing energy crops is the mixed cropping [23] (or culture system; MC) of white sweetclover (*Melilotus albus* MED.) and maize (*Zea mays* L.). Nevertheless, *Melilotus* species plants are specific in their high content of coumarin, and its concentrations reach up to 5% DM depending on the cultivar [24]. Kadaňková et al. [25] confirmed that the content of coumarin in the silage of MC of maize and white s.c. depends on the amount of white s.c. in the silage. The content of coumarin in the biomass of white sweetclover and hence in the silage adversely affects biogas production. White sweetclover biomass can be used for the production of biogas, but microbial communities in the biogas plant fermenter have to adapt to the presence of coumarin first [24]. Gatta et al. [26] maintain that growing a well-balanced mixture of crops with legumes not exceeding 30% is suitable both for agricultural systems and for the technology of biogas production. Nonetheless, the mixed culture system of *Poaceae* with *Fabaceae* is considered to be an important factor in the development of sustainable agricultural systems [27]. It should also be taken into account that there are other biomass sources outside the conventional agricultural production that can be used. Grass from non-production areas can be used after mowing to make haylage or ensiled and then used in BGP [26,27].

The use of the mixed culture system might extend the offer of growing systems for gaining biomass utilizable in biogas yield, thus contributing to the sustainability of phytopower engineering as a combination of industrial and agricultural sectors in the European Union. The main goal of the submitted study was to analyze the potential use of silage from a mixture of a conventional crop (*Zea mays* L.) and a legume for methane yield, and to find an optimal content of selected legumes (*Melilotus albus* MED.) in this silage, in terms of the effect on biomethane yield. Partial objectives of the study were as follows: (a) How the addition of the biomass of *Melilotus albus* MED. affected the quality of maize silage? (b) What was the methane yield from the prepared silages? (c) Was the biogas quality (methane content) affected by the addition of the biomass of *Melilotus albus* MED. in the maize silage? Hypothesis H_0 = Addition of the biomass of white sweetclover to the maize silage will affect neither biogas quality nor methane yield.

2. Materials and Methods

2.1. Localization of the Field Experiment

Plant biomass intended for biogas yield was cultivated in the Experimental Station for Fodder Crops in Vatín. The station is located in the Bohemian-Moravian Highland in the central part of the Czech Republic. The Vatín experimental station is 7 km south of Žd'ár nad Sázavou (Figure 1). The mean altitude is 540 m a. s. l. The area of experimental station belongs to the mildly warm climatic zone. Plant biomass was cultivated on the cambisol sandy loam occurring on the deluvium of biotic orthogneiss. Basic information on the characteristics of arable soil from the experimental site is given in Table 1. Meteorological and climatological parameters are shown in Figure 2.

Table 1. Characteristics of arable soil from the experimental site (represented as mean ± SD; for n = 9)—average contents of plant available nutrients.

Sample	Soil Reaction (pH)	Plant Available Nutrient Content (mg/kg)			
		P	K	Ca	Mg
Arable Soil	5.9	95 ± 4.7	246 ± 39.4	1271 ± 64.7	135 ± 17.0

2.2. Production of Plant Biomass for Silage Preparation

In 2019, a stand of the mixed cropping system was established by technology (Figure 3), which was developed from 2015. Examples of different technologies for establishing mixed cropping stands are presented in Appendix A, Figures A1 and A2. The technology of mixed cropping was used to grow biomass for the preparation of various model silages with different shares ($\%_w$) of individual crops. The selected crops (*Zea mays* L., FAO 270 and *Melilotus albus* MED., Meba variety) were grown to obtain biomass for the preparation of shredding's and model micro-silages utilizable in the laboratory biogas plant. The experimental plot was fertilized with DASA fertilizer (300 kg/ha) applied prior to the sowing. The DASA fertilizer (AGRO CS Ltd., Rikov, Czech Republic) is composed of 26% N (1/3 in the form of nitrate N and 2/3 in the form of ammonium N) and 13% S in the form of ammonium sulfate.

This fertilizer dose provided enough nitrogen for maize nutrition and did not threaten the growth of the legume. Other nutrients (P, K, Mg and Ca) did not have to be applied with respect to the average supply of nutrients in the arable soil (Table 1). The system of stand organization and its individual variants is illustrated in Figure 3. Stand variants were divided into the following three groups:

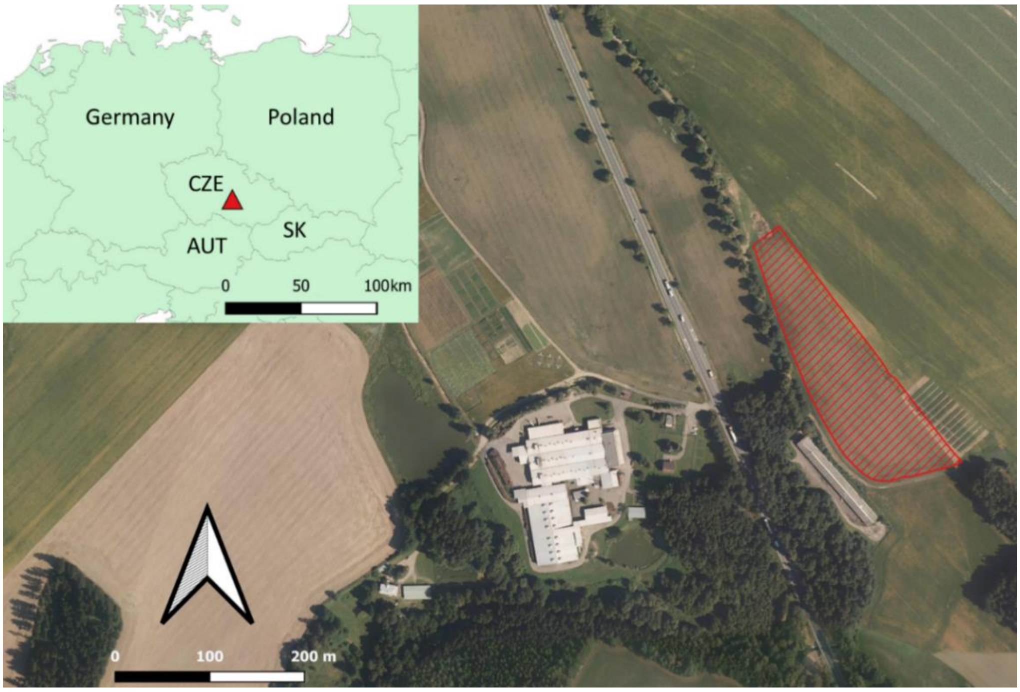

Figure 1. Localization of the Vatín experimental field station in CZE (Czech Republic) and European Union. The experimental plot from 2019 is marked with red hatching.

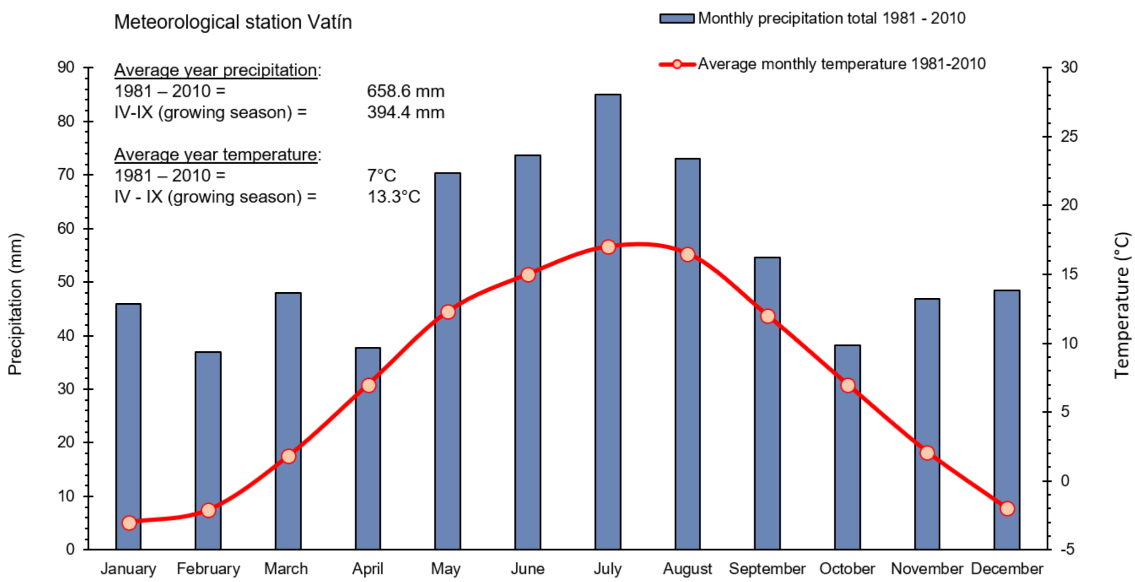

Figure 2. Weather conditions at the Vatín field experimental station—average monthly temperatures and mean annual precipitation amounts for long-term standard (1981–2010).

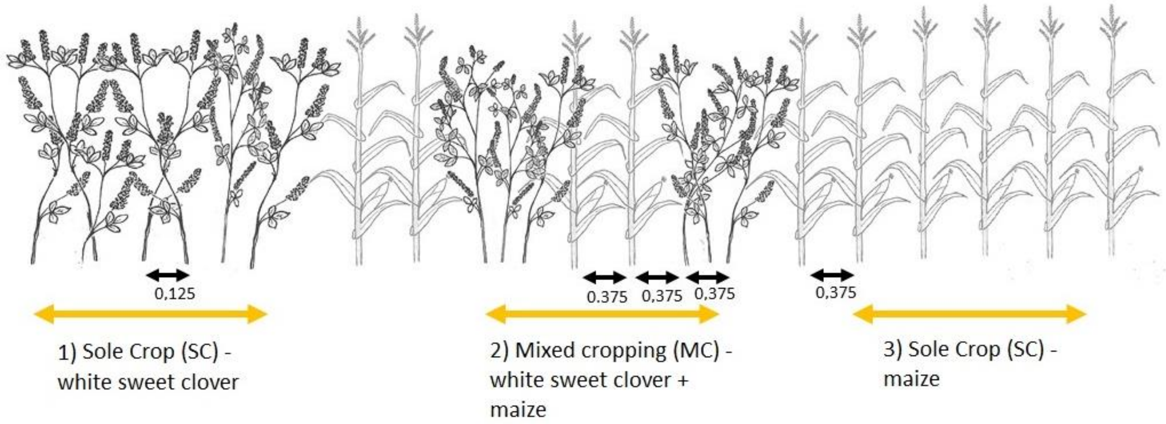

Figure 3. Organization of plant biomass production in 2019.

(1) Sole crop (SC)—White sweetclover

The selected seeds were sown individually on the same date in rows of 0.125 m and depth 0.02 m, by using the parcel no-remainder seeding machine. The crop was sown at 75,000 individuals/ha.

(2) Mixed cropping (MC)—White sweetclover + maize

The combination of maize and legume plants was sown on the same date by using the Kinze 3500 (Kinze Manufacturing, Williamsburg, IA, USA) "interplant system" seeding machine in a single operation. The seeder was modified to be able to sow the same number of maize and melilot at the same time—see Figure 3. The mixed crop was sown so that two rows of maize were replaced by seeds of white sweetclover sown in a strip of 0.375 m in width, which was on each side distant 0.375 m from the nearest rows of maize. The sowing rate of each crop was 75,000 individuals per hectare, with the number of individuals (maize + white sweetclover) totaling 150,000/ha.

(3) Sole crop (SC)—Maize

The selected seeds were sown individually on the same date into rows of 0.375 m in width by using the Kinze 3500 "interplant system" seeding machine. The crop was sown at 75,000 individuals/ha.

2.3. Production of Mixed Culture Silage—Preparation of Model Silage

The plant biomass was collected by hand at a stubble height of 18 cm and then chopped to shreddings (15–20 mm) using the Deutz-Fahr MH 650s (Deutz-Fahr, Lauingen, Germany) cutter. It is an attached single-row chopper with the feeding and cutting mechanism ended with the sweeper. The cutting mechanism is equipped with 12 knives. The biomass shreddings were used to prepare model silages–8 variants of experimental silage, each in triplicate. Two silage variants were prepared only with 100% of maize or melilot. The remaining silage variants were prepared by mixing the shreddings of maize and melilot at different weight ratios (Table 2).

The process of micro-silage preparation was identical for all variants: 8 kg of shreddings were placed in a mini-silo (container of 150 mm in diameter and 1000 mm in height), together with the inoculating agent for each silo (Silo Solve EF, Chr. Hansen Holding Ltd., Starovice, the Czech Republic), dosed at 5 g + 3.5 L H_2O/t. The prepared plant material was compacted by the pneumatic press with a force of 6,000 N/m^2. Maize forage compaction ranged from 155 to 198 kg $_{TS}$/m^3 (Table 2). Then, the mini-silo was hermetically sealed and placed in the dark to prevent exposure to light, at a temperature of 28 °C ± 1 °C. Each container was equipped with a safety valve for the removal of excessive gaseous products [28,29]. After the lapse of the incubation time of 90 days [29], the micro-silages were opened and samples of them were homogenized. Subsequently, the samples were frozen and transported to the laboratory for chemical analyses and fermentation tests.

Table 2. Overview of prepared model silages.

Treatment	Percentage of Maize (*Zea mays* L.) in Silage *	Percentage of White Sweetclover (*Melilotus Albus* MED.) in Silage *	Kilograms of Maize (*Zea Mays* L.) in Silage	Kilograms of White Sweetclover (*Melilotus Albus* MED.) in Silage	Average Density of Model Silage in Dry Matter (kg/m^3)
Maize	100	0	8	0	173.9
White sweetclover	0	100	0	8	198.7
Maize + White s.c. 3:7	30	70	2.4	5.6	171.0
Maize + White s.c. 1:1	50	50	4	4	155.9
Maize + White s.c. 7:3	70	30	5.6	2.4	155.7
Maize + White s.c. 8:2	80	20	6.4	1.6	167.2
Maize + White s.c. 8.5:1.5	85	15	6.8	1.2	161.2
Maize + White s.c. 9:1	90	10	7.2	0.8	173.4

* Percentage *w/w*.

2.4. Silage Characteristics

Individual samples of silages were frozen immediately after delivery. The samples were defrosted prior to the fermentation tests at a laboratory temperature. Contents of dry matter (=total solid, TS) and volatile solids (VS) in the samples were determined gravimetrically—by desiccation in the electric furnace LMH 07/12 (LAC, Czech Republic) at a temperature of 105 °C to constant weight, and by burning and annealing of the dried out samples at 550 °C to constant weight, according to standards CSN EN 15934 [30], and ČSN EN 15169 [31]. The Kjeldahl method was used for the determination of crude protein. Protein content was determined by using the KjeltecTM 2300 Analyzer (FOSS Analytical, Denmark), and subsequently multiplied by the empirical factor of 6.25. Fat content was determined gravimetrically by using the water-cooled Soxhlet extractor by direct sample extraction with diethyl ether. Crude fiber (CF) content was determined by the two-step hydrolysis with sulfuric acid and potassium hydroxide, and then ash content was established. Acid detergent fiber (ADF) was obtained using the solution containing concentrated sulphuric acid and acetyltrimethylammonium bromide. Neutral detergent fiber (NDF) was obtained using the solution of sodium lauryl sulfuric and ethylenediamine tetraecetic acid. The analyses were performed by using the ANKOM 200 Fiber Analyzer (ANKOM Technology, NY, USA). Acid detergent lignin (ADL) was determined according to ISO 13906 [32].

Coumarin content in the silages of all variants was determined after the end of the incubation time, i.e., 90 days. The coumarin concentration analysis was carried out using the gas chromatograph TraceTM 1310 with split injector (Thermo Fisher Scientific Inc., Waltham, MA, USA). Mass detector ISQTM LT Single Quadrupole (Thermo Fisher Scientific Inc., Waltham, MA, USA) SPME-fiber DVB/CAR//PDMS 50/30 µm (Supelco, Bellefonte, PA, USA) with standard Tr = 38.14 min. The process of determination was implemented according to Divišová et al. [33].

2.5. Fermentation Tests

Biomethanation batch tests were performed in an automatic custom-made system (Figure 4). In each system, a set of eight 5 L glass fermenters was placed in the heated water bath that could be set and maintain a constant temperature. Each fermenter had its own gas holder for reading the biogas yield. Each gas holder was also equipped with a port for biogas composition measurement. Each sample was fermented in three repetitions. On the first day of the experiment, all fermenters were filled up with 3 L of filtered inoculum. Two fermenters in each system were used as blank for the determination of endogenous inoculum biogas yield. In each system, the samples mentioned in Table 3 were added to the remaining six fermenters. Initial organic loading rate was 5.5 g vs of introduced substrate/L. Retention time of digester was 21 days. Temperature during the tests was 42 °C ± 0.1 °C. Biogas produced was measured daily, applying the liquid displacement method (according to VDI 4630), with the acidified saturated NaCl solution used as a barrier solution. Biogas volume generated was converted into standard temperature and pressure (273.15 K and 1 bar). Dräger X-am 5600 (Dräger, Germany) was used for the biogas composition analysis. All tests were carried out until the daily biogas yield in three consecutive days was <1% of total biogas yield (VDI 4630).

2.6. Statistical Analysis—Data Treatment

All parameters of the experiment were measured in at least three repetitions. The data were processed in the Czech version of Statistica 12 (Dell Software, Round Rock, TX, USA). At first, exploratory data analysis (EDA) was made to determine basic statistical parameters (expression of symmetry and sharpness at various distances from the median), in order to reveal possible extremes. A one-way analysis of variance (ANOVA) followed, in combination with the post-hoc Tukey's HSD test, which was intended to reveal significant differences in the individual parameters among the variants. This analysis was complemented with the pair t-test and regression analysis, serving to specify relations among the respective parameters across the variants. Possible factors affecting the values of measured parameters were analyzed using principal component analysis (PCA). All analyses were performed at a significance level $p < 0.05$. Graphical documentation was prepared by employing the Origin 6.1 software (OriginLab Corporation, Northampton, MA, USA) and CorelDrawX7 (Corel Corporation, Ottawa, ON, Canada).

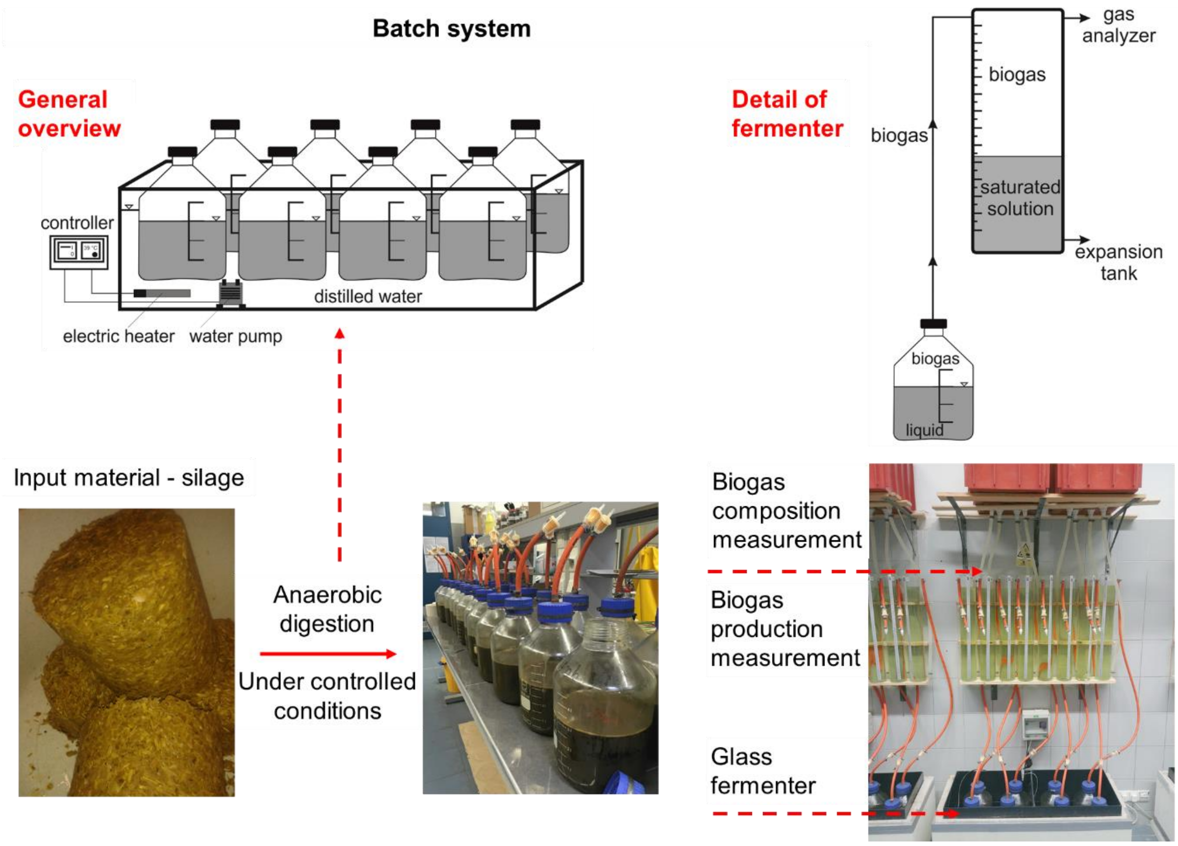

Figure 4. Fermentation test design.

Table 3. Dry matter, organic dry matter, proteins, starch and lipids content in the prepared silages.

	TS		**VS**		**Proteins**		**Starch**		**Lipids**	
	% ± SD	**HSD**	**% ± SD**	**HSD**	**%TS ± SD**	**HSD**	**%TS ± SD**	**HSD**	**%TS ± SD**	**HSD**
Maize	33.28 ± 0.58	A	96.07 ± 0.07	A	8.13 ± 0.14	A,B	20.66 ± 2.83	B,C,D	4.26 ± 0.07	D
White s.c.	36.14 ± 3.30	A	96.75 ± 0.13	A	11.62 ± 0.84	E	4.51 ± 0.15	A	3.83 ± 0.11	D
Maize + White s.c. 3:7	32.17 ± 0.95	A	94.89 ± 0.24	A	11.34 ± 0.24	E	11.13 ± 0.49	A,B	2.59 ± 0.23	A
Maize + White s.c. 1:1	34.10 ± 0.57	A	95.65 ± 0.03	A	10.95 ± 0.21	D,E	16.54 ± 1.61	A,B,C	3.64 ± 0.15	C,D
Maize + White s.c. 7:3	32.06 ± 0.54	A	96.43 ± 0.83	A	9.95 ± 0.23	C,E	25.93 ± 1.57	C	4.03 ± 0.45	C,D
Maize + White s.c. 8:2	32.84 ± 0.55	A	96.21 ± 0.41	A	8.45 ± 0.15	B,C	32.58 ± 0.87	D	3.40 ± 0.15	A,C,D
Maize + White s.c. 8.5:1.5	32.10 ± 0.54	A	96.58 ± 0.19	A	8.32 ± 0.17	B,C	23.81 ± 3.41	B,C,D	3.93 ± 0.09	C,D
Maize + White s.c. 9:1	34.13 ± 0.57	A	96.69 ± 0.21	A	8.10 ± 0.13	B	21.04 ± 5.60	B,C,D	3.01 ± 0.16	B,D

3. Results

3.1. Qualitative Parameters of Silage

The quality of silage prepared in the respective variants was studied with using the following parameters: dry matter content (TS), proteins, starch, lipids, CF (crude fiber), ADF, NDF, ADL and Coumarin (Tables 3 and 4). These parameters were determined at all times upon the end of silage formation process, i.e., after the silage containers were opened. The measured values indicated that no differences could be confirmed in the respective samples for the parameter of dry matter. Marked differences were apparent only in the other parameters. In particular, the contents of proteins, CF, ADF, NDF, ADL and coumarin exhibited considerably different values across all variants.

The contents of proteins and lipids were changing with the changing content of White s.c. in the silage (Table 3). The highest protein contents ($\geq 10\%_{TS}$) were found in the variant of White s.c. ($100\%_{w}$) and in variants with the proportion of White s.c. shreddings reaching at least $30\%_{w}$. These variants did not show any significant mutual differences (Table 3). On the contrary, they contained demonstrably more proteins than the maize silage alone or the silage with only a slight addition of White s.c. (Maize + White s.c. 8:2–9:1). On the other hand, the content of lipids was demonstrably the lowest in the silage Maize + White s.c. 3:7, where it did not exceed $2.6\%_{TS}$. Other variants exhibited only partial differences in the content of lipids with no apparent trend (Table 3). Silage variants MC 1:1, 7:3, 8:2, 8.5:1.5 contained demonstrably higher amounts of lipids than the 9:1 variant, the content of lipids was however lower than in silages consisting either of maize alone ($100\%_{w}$) or White s.c. alone ($100\%_{w}$). Therefore, it is not possible to say that a change in the silage composition, i.e., decreased or increased content of White s.c. resulted in the increased or decreased content of lipids. Compared with the values of proteins and lipids, more pronounced differences were observed in the content of starch, with the highest value ($32.58\%_{TS}$) being found in the variant Maize + White s.c 7:3 and the lowest values found in the silage of White s.c. alone ($4.51\%_{TS}$). The difference between the silages was significant (Table 3). The measured values show that the content of starch was increasing with the increasing maize content in the MC silage, although the increase was demonstrable in the following order only: White s.c. ($100\%_{w}$) < Maize + White s.c. 3:7 < Maize + White s.c. 1:1 ≤ Maize + White s.c. 7:3 < Maize + White s.c. 8:2. The remaining two variants (Maize + White s.c. 8.5:1.5 a 9:1) exhibited lower values of starch content, but due the high dispersion of these values, the differences were not significant if compared with the variant Maize + White s.c. 8:2. Furthermore, the difference between the monoculture silages of Maize and White s.c. was demonstrable with the maize silage exhibiting five-times higher starch values.

Another studied parameter was CF, where the influence of White s.c. shreddings in the maize silage clearly showed. The content of CF ranged from 28 to $16\%_{TS}$. The highest value was observed in the variant of White s.c. ($28\%_{TS}$). This value was demonstrably the highest as compared with all other variants (Table 4), with the exception of variant of Maize + White s.c. 3:7. In the other variants, the content of CF was decreasing along with the decreasing content of White s.c. shreddings in the maize silage with the decrease being significant as compared with the White s.c. variant. The decreasing values of CF content were apparent until the variant with the ratio of maize and White s.c. being 9:1, where a slight increase in the CF content was observed, which was inconclusive though.

Values of ADF and NDF parameters exhibited a similar behavior as the CT parameter, albeit with some differences. The highest values of both ADF ($>38\%_{TS}$) and NDF ($>57\%_{TS}$) were at all times found in the MC silage variant, where the ratio of maize and legume was 3:7. Subsequently, these parameters is silages were demonstrably increasing with the increasing content of legume (Table 4). Further on, the silage consisting of legume shreddings only exhibited demonstrably higher contents of ADF and NDF compared with the pure maize silage.

Table 4. CF, ADF, NDF, ADL and coumarin contents in the prepared silage.

	CF		ADF		NDF		ADL		Coumarin	
	% TS ± SD	**HSD**	**% TS ± SD**	**HSD**	**% TS ± SD**	**HSD**	**% TS ± SD**	**HSD**	**mg/g $_{TS}$ ± SD**	**HSD**
Maize	16.73 ± 0.30	A	20.35 ± 0.43	A,B	42.14 ± 0.90	A	6.16 ± 2.28	A,B	1.84 ± 0.20	A
White s.c.	28.16 ± 1.06	C	31.62 ± 4.15	C,D	48.67 ± 5.60	B,C	14.32 ± 3.89	A,B	17.51 ± 0.84	D
Maize + White s.c. 3:7	25.49 ± 0.56	B,C	38.84 ± 1.70	D	57.10 ± 3.75	C	16.33 ± 0.55	B	14.25 ± 1.18	C
Maize + White s.c. 1:1	23.11 ± 1.18	B	32.07 ± 1.47	C,D	48.27 ± 0.82	B,C	13.08 ± 4.15	A,B	12.33 ± 0.33	C
Maize + White s.c. 7:3	19.74 ± 0.64	A,B	26.53 ± 0.52	B,C	43.01 ± 0.73	A	5.42 ± 0.33	A,B	7.97 ± 0.14	B
Maize + White s.c. 8:2	18.70 ± 0.32	A	25.11 ± 1.10	B,C	41.47 ± 0.89	A	4.20 ± 0.55	A	7.73 ± 0.13	B
Maize + White s.c. 8.5:1.5	17.59 ± 0.46	A	23.44 ± 0.79	B,C	39.17 ± 0.67	A	3.90 ± 0.85	A	7.29 ± 0.63	B
Maize + White s.c. 9:1	18.01 ± 0.81	A	22.49 ± 0.77	B	37.21 ± 1.64	A	11.01 ± 1.89	A,B	4.35 ± 0.35	A

Legend to Tables 3 and 4: Mean of measured values (n = 3) is shown ± Standard Deviation (SD). All parameters were recalculated to sample dry weight (Total Solid—dry matter, TS). Different letters indicate significant differences ($p < 0.05$) among individual variants in the specific parameter. vs (volatile solids), CF (Crude Fibre), ADF (Acid Detergent Fibre), NDF (Neutral Detergent Fibre), ADL (Acid Detergent Lignin).

ADL showed an apparently different behavior, with a lower number of demonstrable differences (Table 4). Although the highest content was again recorded in the variant Maize + White s.c. 3:7 (16.33%$_{TS}$), the value was demonstrable only in relation to variants with the Maize and White s.c. ratios of 8:2, resp. 8.5:1.5, which exhibited the lowest ADL values. The other differences were not statistically significant.

The concentration of coumarin in the prepared silages represented the last studied parameter. Measured results show (Table 4) that the lowest coumarin concentration was demonstrably recorded in the variant of Maize (<2%$_{TS}$), with the lowest supplement of White s.c. (9:1). By contrast, the demonstrably highest concentration of coumarin was measured in the silage consisting of White s.c. shreddings only (>17%$_{TS}$). This value was in statistical terms demonstrable in relation to all other variants. Apart from that, the content of coumarin in the silage was significantly decreasing with the decreasing content of legume crop shreddings (White s.c. > Maize + White s.c. 3:7, 1:1 > Maize + White s.c. 7:3, 8:2, 8.5:1.5 > Maize + White s.c. 9:1; Maize).

3.2. Biomethane Yield

The scatter chart (Figure 5) shows the course of daily methane yield during 21 days of experiment. The course of values indicates that the pure White s.c. silage exhibited the lowest yield of methane already from the first measurement up to the end of the experiment. By contrast, the pure maize silage exhibited the highest biogas yield in the first 11 days of the experiment. In the following days of experiment duration, the difference in biogas yield between the maize silage and silage with the admixture of White s.c. up to 15%$_{w}$ (variants Maize + White s.c. 9:1 and 8.5:1.5) was diminishing. As mentioned above, the maize variant exhibited the demonstrably highest methane yield up to Day 11, which was also corroborated by the statistical analysis. On the other hand, the difference between this variant and variants supplemented with White s.c. silage (variants 9:1 and 8.5:1.5) was decreasing from Day 11 and from Day 13, as well as the difference between these variants plus the variant of Maize + White s.c. at 8:2.

The values of all curves for the experimental period were statistically investigated by using the pair t-test. The variant of pure maize (100%$_{w}$) exhibited demonstrably the highest yield of methane with respect to the overall course of the curve, although in individual days of measurement the values were significant until Day 11. In the following three days, the maize variant exhibited methane yield higher than the variants supplemented with White s.c.; however, the recorded differences were not demonstrable. In addition to the above mentioned maize silage in relation to the other variants, demonstrable differences were also found between the variants supplemented with White s.c. up to 30%$_{w}$ (9:1; 8.5:1.5; 8:2; 7:3), and the remaining variants of MC silage, in which the content of White s.c. exceeded 30%$_{w}$ (3:7; 1:1), including the White s.c. monoculture (100%$_{w}$). Apart from the methane yield, we also monitored the development of biogas during 21 days of the experiment (Appendix B, Figure A3). The pure White s.c. silage exhibited the lowest biogas yield, right from the first measurement to the end of the experiment. By contrast, the pure maize silage exhibited the highest biogas yield in the first 9 days of the experiment. In the following days of experiment, the biogas yield between the maize silage and the silage with admixed White s.c. up to 15%$_{w}$, i.e., variants Maize + White s.c. 9:1 and 8.5:1.5, was further decreasing. Therefore, it is possible to say that the course of biogas yield was similar as in methane.

The course of methane yield values during the experiment shows the development of these parameters across the respective variants and the time of experiment duration. However, it does not provide an overall view of the measured values, which is apparent from average values of methane yield (Table 5) in 21 days for the respective variants and their subsequent statistical analyses. In the case of methane, its average yield ranged from 0.22 m^3/kg_{VS} in the variant of pure White s.c. (100%$_{w}$); up to 0.36 m^3/kg_{VS} in the variants with pure maize (100%$_{w}$) and Maize + White s.c. 9:1. The variants of pure maize and Maize + White s.c. (9:1–8.5:1.5) did not show any demonstrable difference; on the contrary, all other variants recorded a significant decrease in the methane yield with the increasing supplementation of White s.c. up to 0.22 m^3/kg_{VS}, which was measured in the pure White s.c. variant ($100\%_w$). As limiting for the inhibition of methane yield appeared a White s.c. supplementation higher than $20\%_w$. Because, from this limit, a significant methane yield decrease was recorded with the increasing content of White s.c. in relation with the pure maize silage variant, it can be considered as a certain form of control in the conducted experiment. The methane yield is presented only in relation to the amount of feedstock, not in relation to yield per hectare reason being the fact that we tested diverse model silages (Table 2), with the content of White s.c. ranging from 10 to $70\%_w$ that were prepared from the cultivated plant biomass.

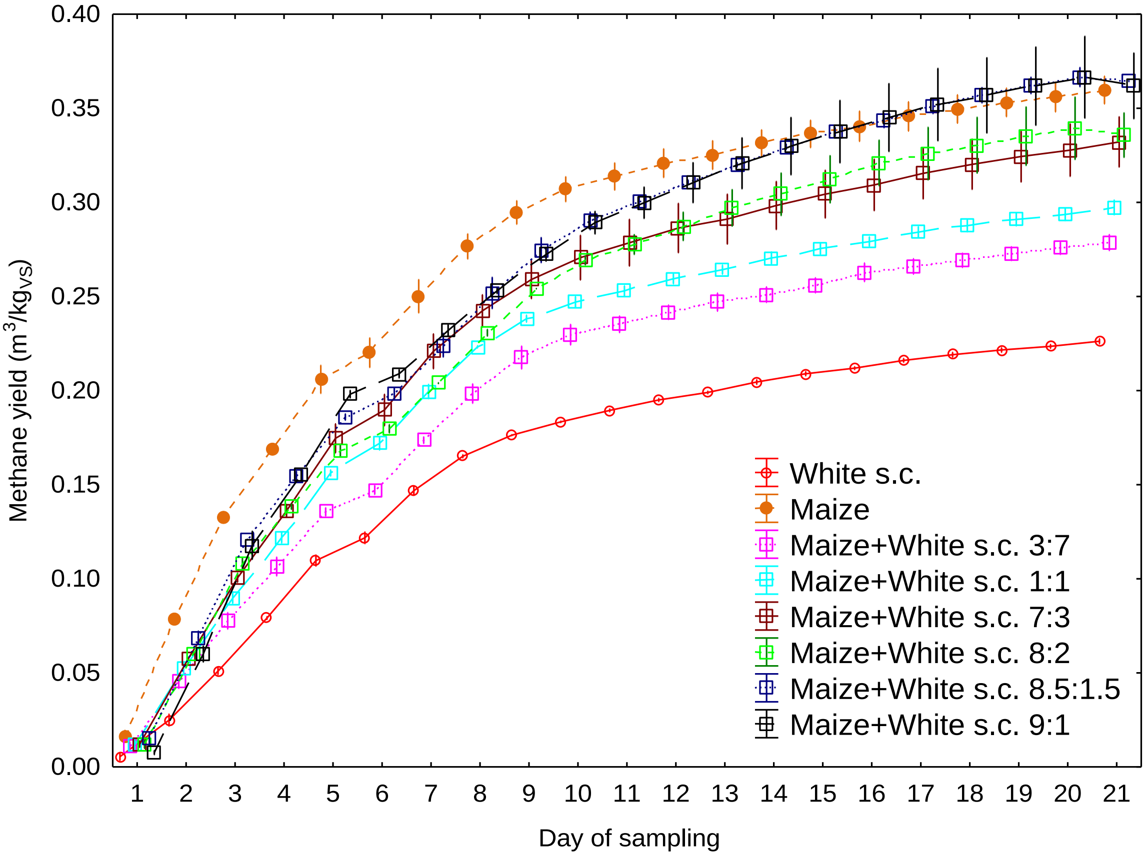

Figure 5. Methane yield during 21 days of the experiment (average values for every day of measurement, n = 3 for one measurement, ± SD).

3.3. Methane Content in Biogas

Figure 6 shows the course of methane concentration in biogas over 21 days. The course of the curves of individual variants shows similarity with the course of methane yield values (Figure 5), but the differences shown are less obvious. Maize silage without the addition of White s.c. exhibited the highest values of methane content in biogas up to 9 days of measurement. The differences between the silage monoculture and MC variants began to decrease significantly from Day 10 of the measurements. The only exception was the variant of White s.c. monoculture and the MC variant with the highest legume content, i.e., Maize + White s.c. 3:7. These two variants were exhibiting the lowest values of biogas content until the end of the experiment. The other MC variants showed a significant increase in the methane content of biogas from Day 10 of the measurements, and at the end of the experiment, the variants with the content of White s.c. up to $20\%_w$ reached similar values as the pure maize silage.

Table 5. Results of Tukey's HSD test for the parameter of average methane yield after 21 days of the experiment.

Variants	Maize	White s.c.	Maize + White s.c. 3:7	Maize + White s.c. 1:1	Maize + White s.c. 7:3	Maize + White s.c. 8:2	Maize + White s.c. 8.5:1.5	Maize + White s.c. 9:1
Average	**M = 0.271 m^3/kg_{VS}**	**M = 0.161 m^3/kg_{VS}**	**M = 0.199 m^3/kg_{VS}**	**M = 0.217 m^3/kg_{VS}**	**M = 0.240 m^3/kg_{VS}**	**M = 0.242 m^3/kg_{VS}**	**M = 0.263 m^3/kg_{VS}**	**M = 0.264 m^3/kg_{VS}**
Maize		0.000175	0.000177	0.000358	0.039756	0.060095	0.982983	0.988470
White s.c.	0.000175		0.005070	0.000255	0.000175	0.000175	0.000175	0.000175
Maize + White s.c. 3:7	0.000177	0.005070		0.456127	0.003545	0.002363	0.000189	0.000187
Maize + White s.c. 1:1	0.000358	0.000255	0.456127		0.176705	0.121646	0.001207	0.001094
Maize + White s.c. 7:3	0.039756	0.000175	0.003545	0.176705		0.999998	0.193271	0.175552
Maize + White s.c. 8:2	0.060095	0.000175	0.002363	0.121646	0.999998		0.272433	0.249201
Maize + White s.c. 8.5:1.5	0.982983	0.000175	0.000189	0.001207	0.193271	0.272433		1.000000
Maize + White s.c. 9:1	0.988470	0.000175	0.000187	0.001094	0.175552	0.249201	1.000000	

Legend to Table 5: Red color illustrates demonstrable difference between the respective experimental variants at a level of significance $p < 0.05$.

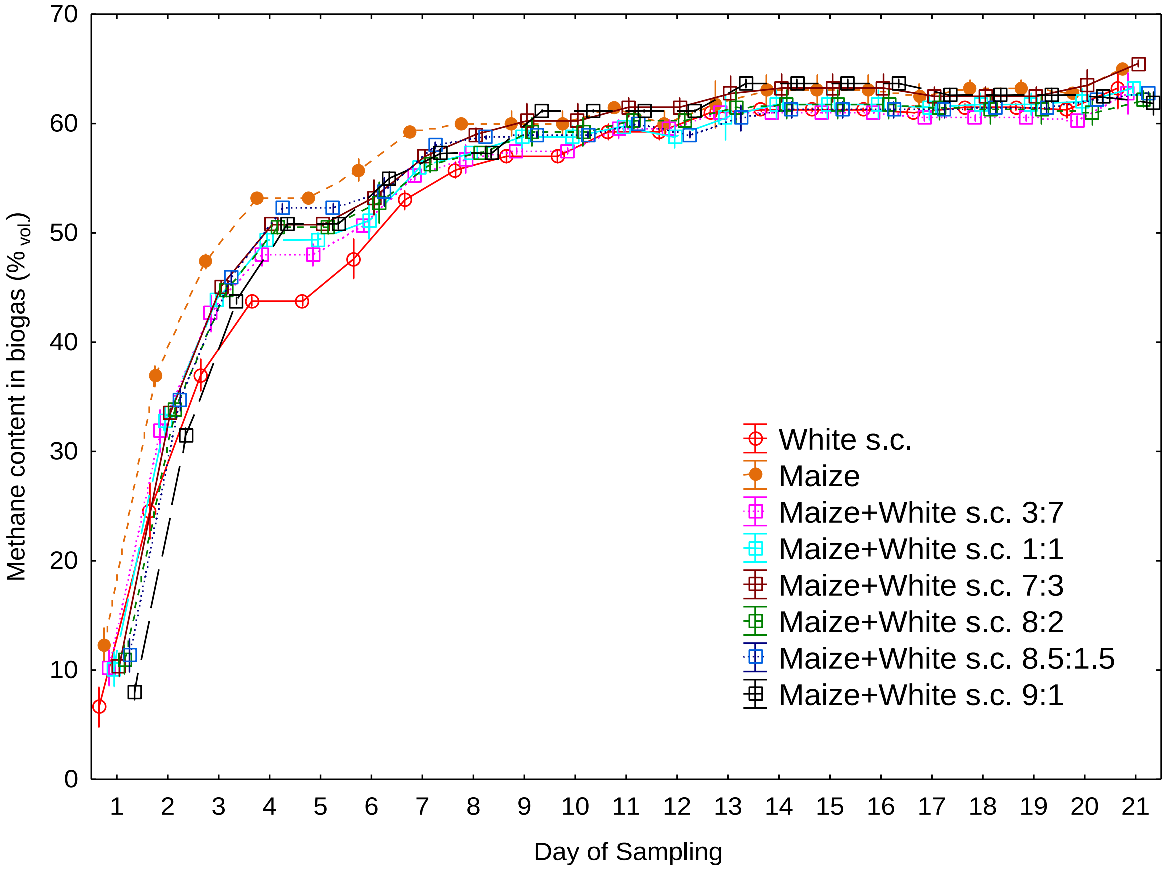

Figure 6. Methane content in biogas during 21 days of the experiment (average values for every day of measurement, n = 3 for one measurement, ± SD).

Significant differences between the respective variants are given in Table 6. The significant differences (Tukey's HSD test; $p < 0.05$) in the average values of methane content in biogas after 21 days of the experiment were found between the maize variant and the White s.c. variant. Maize silage showed the highest concentration of methane, not only in relation to silage consisting of White s.c. only, but also in relation to silage of MC (Maize + White s.c. 3:7). The methane content in the biogas increased with the decreasing White s.c. content in the silage (White s.c. < Maize + White s.c. 3:7 < Maize + White s.c. 1:1 < Maize + White s.c. 7:3 ≤ Maize + White s.c. 8:2 < Maize + White s.c. 8.5:1.5 < Maize + White s.c. 9:1.

However, it is necessary to point out that, although the increase in the methane content in biogas was evident in the variants with a lower White s.c. content in the silage, differences between the variant of White s.c. (100%$_{w}$) and the other variants of MC were only partially statistically significant. Significant differences as follows were found between White s.c. and Maize + White s.c. 7:3; Maize + White s.c. 8.5:1.5; Maize + White s.c. 9:1. It is also necessary to state that silages with a maize content higher than 50% had an average methane content in biogas at the same level as the pure maize silage (>55% of CH_4 in biogas).

Table 6. Results of Tukey's HSD test for the parameter of average methane content in biogas after 21 days of the experiment.

Variants	**Maize**	**White s.c.**	**Maize + White s.c. 3:7**	**Maize + White s.c. 1:1**	**Maize + White s.c. 7:3**	**Maize + White s.c. 8:2**	**Maize + White s.c. 8.5:1.5**	**Maize + White s.c. 9:1**
Average	**M = 56.52%**	**M = 52.27%**	**M = 53.61%**	**M = 54.37%**	**M = 55.82%**	**M = 54.75%**	**M = 55.18%**	**M = 55.51%**
Maize		0.0014	0.0335	0.1874	0.9831	0.3824	0.6430	0.8927
White s.c.	0.0014		0.6974	0.2104	0.0073	0.0932	0.0399	0.0155
Maize + White s.c. 3:7	0.0335	0.6974		0.9741	0.1665	0.8264	0.5701	0.3076
Maize + White s.c. 1:1	0.1874	0.2103	0.9741		0.6128	0.9996	0.9770	0.8299
Maize + White s.c. 7:3	0.9831	0.0073	0.1665	0.6128		0.8676	0.9831	0.9998
Maize + White s.c. 8:2	0.3824	0.0932	0.8264	0.9996	0.8676		0.9997	0.9752
Maize + White s.c. 8.5:1.5	0.6430	0.0399	0.5701	0.9770	0.9831	0.9997		0.9996
Maize + White s.c. 9:1	0.8927	0.0155	0.3076	0.8299	0.9998	0.9752	0.9996	

Legend to Table 6: Red color illustrates demonstrable difference between the respective experimental variants at a level of significance $p < 0.05$.

3.4. Interaction between Silage Parameters and Biomethane Yield

Interactions between the respective parameters were measured by using regression and PCA analyses (Appendix C, Table A1 and Figure A4). Relations between the parameters and their influencing by selected factors are illustrated in a diagram presented in Figure 7. It follows from the diagram, correlation matrix (Table 7) and appendix (Appendix C, Table A1) that the variability of measured values was most affected by two factors (more than 96%). Eigenvalues (Appendix C, Table A1) show a high percentage (>90%) of the overall explained variance of variables. Factor 1 can be considered crucial because it explains more than 90% of the variability of variables. This factor negatively correlated with the biogas and methane yield. On the other hand, it positively ($R > 0.5$) influenced the content of coumarin and proteins in the silage. The data confirm that the variability of measured parameters and their values were affected by the presence/absence of White s.c. in the silage, because the yield of biogas in the silage was decreasing with the increasing content of White s.c. (Figure 7), and the contents of coumarin, lipids and other substances in the silage were significantly increasing (Tables 3 and 4).

Part of the PCA analysis was also the calculation of the correlation matrix (Table 7), illustrating, in detail, relations among the individual parameters. It follows from the matrix that a strong dependence existed between the respective parameters—correlation. The correlation was of both negative and positive character. If we focus on the most important parameters of biogas yield and methane yield, we can see that they were negatively influenced by the content of coumarin in the silage; more precisely, the yield of these parameters was decreasing with its increasing content. Coumarin content was affected by the proportion of White s.c. in the silage, its concentration increasing with its increasing content (see Table 4). This significantly reflected in the biogas and methane yields, too, which were the lowest in variants with the highest content of White s.c. in the silage (Figure 5, Figure 6). The negative correlation between the biogas yield and the presence of CF in silage was at the same level as in the case of coumarin, where the CF content was decreasing with the decreasing presence of White s.c. in silage, and the yield of studied parameters (biogas and methane) was increasing.

Another relatively strong negative correlation ($R > -0.8$) was recorded between the content of biogas/methane and the content of protein in the used silage, where the protein content in silage was growing again with the increasing representation of White s.c., although not so conspicuously and clearly significantly as in the case of coumarin. Nevertheless, the content of proteins affected the yield of biogas and its changed (decreased/increased) concentrations in the silage correlated with the silage composition. It is important to note that the above qualitative parameters considerably affected the yield of biogas and methane. On the other hand, their influence on the methane concentration in biogas, although it was of the same character, i.e., negative, was markedly lower.

The other qualitative parameters of silage (content of starch, ADF, NDF, ADL) did not exhibit such strong dependences as the previous parameters. The group of ADF, NDF, and ADL parameters correlated negatively with the biogas yield. The relation was moderately strong ($R > -0.7$). By contrast, the content of starch in biomass exhibited a weak positive correlation ($R = 0.66$). Based on the conducted PCA analysis, it was apparent that these parameters were affected by silage composition, i.e., by the presence/absence of White s.c. in silage. The parameters of starch and lipids (which did not affect biogas yield $R = 0.24$) were apparently affected by another factor, rather than by the type of plant used for silage preparation.

Table 7. Correlation matrix from PCA—summary of correlations among the measured variables.

	Biogas Yield	Meth. Yield	Meth. in Biogas	TS	Proteins	Lipids	CF	Starch	ADF	NDF	ADL	Coumarin
Biogas yield	1.00	0.99	0.80	−0.29	−0.88	0.24	−0.91	0.66	−0.73	−0.65	−0.63	−0.91
Methane yield	0.99	1.00	0.86	−0.32	−0.85	0.28	−0.92	0.67	−0.72	−0.60	−0.61	−0.92
Meth. in biogas	0.80	0.86	1.00	−0.39	−0.62	0.40	−0.76	0.59	−0.55	−0.35	−0.38	−0.77
TS	−0.29	−0.32	−0.39	1.00	0.04	0.11	0.25	−0.31	−0.17	−0.24	0.02	0.17
Proteins	−0.88	−0.85	−0.62	0.04	1.00	−0.26	0.92	−0.65	0.91	0.85	0.73	0.89
Lipids	0.24	0.28	0.40	0.11	−0.26	1.00	−0.24	0.14	−0.42	−0.26	−0.53	−0.23
CF	−0.91	−0.92	−0.76	0.25	0.92	−0.24	1.00	−0.78	0.84	0.77	0.64	0.94
Starch	0.66	0.67	0.59	−0.31	−0.65	0.14	−0.78	1.00	−0.53	−0.56	−0.54	−0.64
ADF	−0.73	−0.72	−0.55	−0.17	0.91	−0.42	0.84	−0.53	1.00	0.92	0.67	0.84
NDF	−0.65	−0.60	−0.35	−0.24	0.85	−0.26	0.77	−0.56	0.92	1.00	0.64	0.71
ADL	−0.63	−0.61	−0.38	0.02	0.73	−0.53	0.64	−0.54	0.67	0.64	1.00	0.59
Coumarin	−0.91	−0.92	−0.77	0.17	0.89	−0.23	0.94	−0.64	0.84	0.71	0.59	1.00

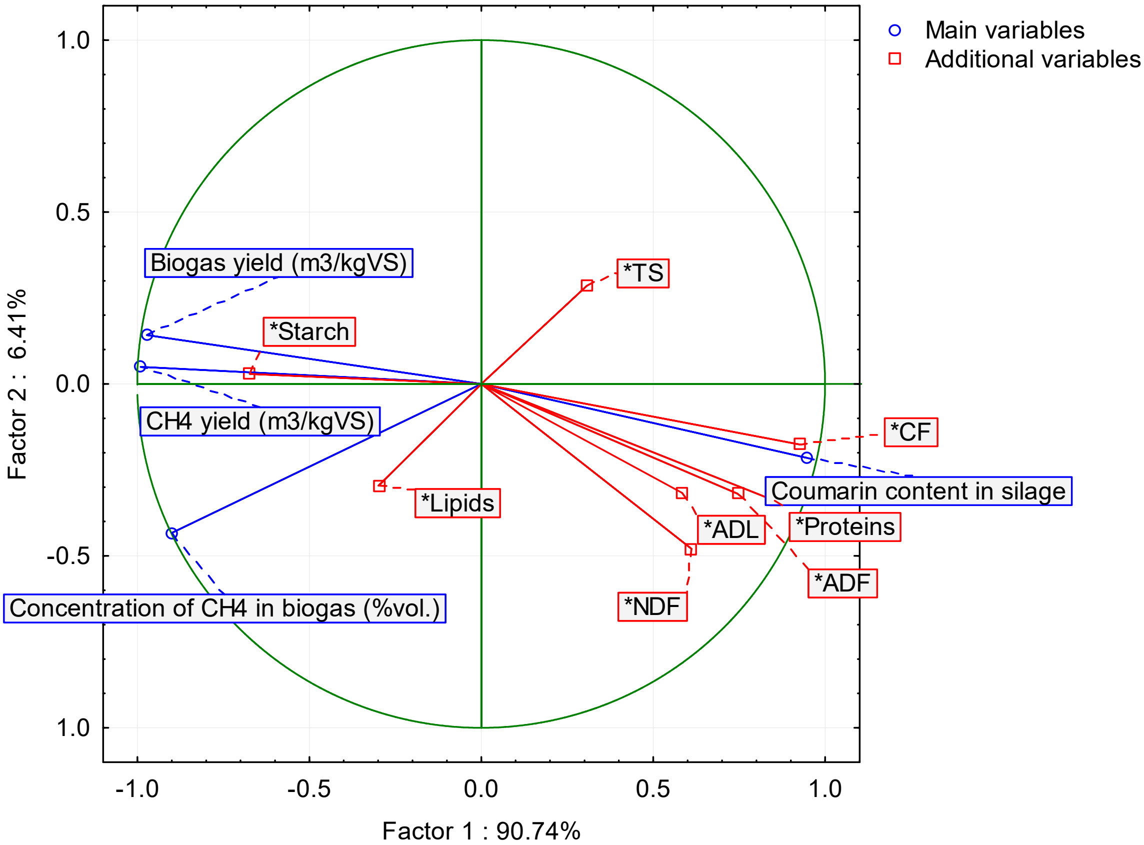

Figure 7. Projection of the variable on the factor plane (1 × 2). * Additional variables.

4. Discussion

4.1. Qualitative Parameters of Silage

Based on the measured values (Tables 3 and 4) and their statistical evaluation, a difference apparently existed among the respective silage types. Quite expectedly, a difference was found between the pure maize silage and the pure White s.c. The dry matter content (TS) of the pure Maize silage was 3% lower compared to pure White s.c. Pure maize silage contained 0.7% less organic dry matter (VS). Pure maize silage was poor in proteins (by 30%), CF (by 32%), ADF (by 35%), NDF (by 13%), ADL (by 57%) and coumarin (by 89%) when compared to pure White s.c. Pure maize silage was richer in starch (by 78%) and lipids (by 10%) when compared to pure White s.c. The difference between these silages resulted from different properties of the crops [34] each of which belonged in another group/family. Maize belongs in the family of *Poaceae*, which means that it is related to more popular cereals (wheat, barley, rye etc.). *Poaceae* crops have typically higher contents of starch and sugar, and lower contents of fiber and proteins in the grain, which was, in the case of maize, further augmented by breeding for the production of silage for BGP [35], compared with the other crops. White s.c. belongs in the family of *Fabaceae* with a typical higher content of grain proteins, in which the element accumulates in the period of peak vegetation. These crops are very often referred to as so-called high nitrogen or protein crops [34]. Silage quality was affected by the cultivated species [35,36]. It is exactly the difference in the content of grain and plant proteins that can be considered one of the main reasons for the detected differences [36], when the pure White s.c. silage exhibited high protein contents, which subsequently showed in the MC silages, too.

Relatively big differences were further observed in the experimental silages in the ADF, NDF, ADL and coumarin contents. Based on the studies published by Ferreira [35], it is possible to state that silage maize varieties have been bred for a long time to contain low contents of ADF, NDF and

ADL, and on the other hand a high content of polysaccharides (starch). This is in correlation with values determined in our experiment for maize silage. Legumes typically contain increased amounts of coumarin and other anti-nutritional or poorly digestible substances (various types of fiber), all this despite the effort to reduce the content of these substances by breeding [24,37]. Slepetiene et al. [38] reached similar conclusions concerning the composition of silage prepared of legumes. Exactly in the increased content of substances potentially inhibiting biomethane yield, they can see the greatest con of using legumes in BGP. Thus, the only solution is to monitor the MC silage composition and to find an optimum maize:legume ratio. The measured data indicate that the optimum maize vs. White s.c. ratio was 9:1–8:2 as in these variants, the indicators of silage quality were within acceptable intervals—more precisely, they corresponded with the composition of pure maize silage [24,35,37].

4.2. Biomethane Yield

According to Boe et al. [39], biomethane yield can be considered to be a qualitative indicator of the fermentation process in BGP. Biomethane yield can be affected particularly by the composition of input plant biomass entering the fermenter in the form of silage [35,40,41]. For this reason, biomethane yield was used in the presented research work as the main qualitative indicator of the effect of legume supplementation (White s.c.) to silage. Biomethane yield showed significant differences among silages consisting of White s.c. and maize in different ratios. Across the tested samples, the biomethane yield ranged from 0.22 to 0.36 m^3/kg_{VS}. The highest biomethane yield was observed from silage with a high share of maize (more than 85%). Biomethane yield from pure maize silage determined in our experiment (0.36 m^3/kg_{VS}) is in line with the meta-analysis made by Moritz von Cossel et al. [42], who mentioned the methane yield from the maize silage, ranging from 0.25–0.43 m^3/kg_{VS}. Maize silage (if prepared properly) reaches optimum values of biogas yield thanks to the high content of carbohydrates [35,41] and other digestible substances, as compared with alternative crops, thus representing the most important energy crop for BP [43]. In the silage prepared from pure White s.c., the lower starch content (4.51$\%_{TS}$ by 78% less compared to pure maize silage), higher lignin content (14.32$\%_{TS}$ by 57% more compared to pure maize silage) and higher coumarin content (17.51$\%_{TS}$ by 89% more compared to pure maize silage) had a negative effect on biomethane yield. This was confirmed also by Popp et al. [24], who point to a possibility of using White s.c. biomass for silage preparation, and for the subsequent biogas yield, which however cannot be used without the addition of maize silage. There was a follow up study [44] which confirms the biomethane yield determined during our fermentation test (Figure 5). The decreasing share of White s.c. and the increasing share of maize in silage was demonstrably increasing the yield of methane, with an optimum ratio being from 8:2 to 9:1 (Maize:White s.c.). A similar ratio was found in the experiment conducted by Kadáňková et al. [25], where the authors recommended max. 15%w of White s.c. in the silage to prevent methane yield inhibition. Moritz von Cossel et al. [42] claim that the methane yield for Yellow melilot (*Melilotus officinalis* L.) ranged from 0.26–0.29 m^3/kg_{VS}, which is a higher biomethane yield than the result obtained during our test 0.22 m^3/kg_{VS}.

The gauged data indicate that an MC silage can be prepared, which will be capable of competition with the pure monoculture silage in terms of methane yield and methane content in biogas. Nevertheless, its chemical composition and characteristics significantly influenced by the added legume crop have to be monitored [24,25,35,44]. The measured values demonstrate too that the representation of White s.c. in silage must not exceed 20$\%_{w}$, to prevent a negative influence on the biogas and methane yield. Similar values were also recorded in other studies [24,44]. However, there are results which—although corresponding with the submitted research—also offer another, opposing view. Popp et al. [24] found out, for example, that the presence of coumarin reduces the biogas yield, but only when the microbial community in the fermenter of biogas plant is not adapted for its use. Thus, an assumption exists that a silage with the higher content of coumarin containing legume can be used, too. This would, however, mean that microbiome in each biogas plant would have to be adapted to the content of anti-nutritional substances. According to Stinner et al. [19,37], legumes have been underestimated for a long time as to the use of their silage in biogas plants. The reason is that lower methane yield from unit area as compared with annual crops such as maize. At that, energy savings which, for example, in clover grass, can reach up to 20%, are not taken into account. As the input of mineral N forms is decreased when using legumes, these are replaced by biological N fixation. Moreover, the produced digestate can be applied to other crops, because legume crops do not need it. Based on studies published by Popp et al. [24] and Stinner et al. [37], we can state that the use of legumes for biogas yield in conventional biogas plants is feasible and has a potential to reduce negative the environmental impacts of the technology.

4.3. Methane Content in Biogas

Methane concentration in biogas can be affected by conspicuous differences in the chemical composition of input material [45,46]. The ratio of input material also plays a role [47]. Values obtained during our fermentation test (Figure 6 and Table 6) confirm that the addition of legume had no negative influence on the methane content in biogas. The final methane content in biogas during the fermentation test ranged from 61.8 to $65\%_{vol}$. Thus, the change in the composition of nutrients in the prepared silages did not reflect into differences in the concentrations of methane in biogas. The determined methane concentrations in biogas did not diverge from the literature data [48,49], where the methane content in biogas generated in agricultural biogas plants ranged from $55\%_{vol}$ to $70\%_{vol}$.

4.4. Interaction between Silage Parameters and Biogas Yield

The analysis of relations among the respective parameters revealed a strong negative dependence between the biomethane yield and the selected qualitative parameters of silage. The measured values confirm the negative influence of the increased CF, coumarin, proteins, ADF, NDF and ADL contents (R value decreasing in the following order CF ≥ coumarin > proteins > ADF > NDF > ADL) on the biogas yield.

It was also found that the above-mentioned qualitative parameters particularly negatively affected the yield of methane, but had no essential influence on the concentration of methane in biogas. Herrmann et al. [50] explain that methane yield depends primarily on the composition of silage, i.e., on the content of lipids, polysaccharides, proteins organic acids and alcohols, while the concentration of methane itself is affected mainly by the content of N substances in ensiled crops. The presence of N substances in the silage has a positive effect on the stability of anaerobic reactor [51]; this could be a space for the potential benefit of using legumes, which would increase the content of N substances in MC silages, and subsequently also the stability of the whole fermentation process in BP. N substances (proteins) are broken into amino-acids, and these are further broken by other organisms into products that can utilize the methanogenic *Archaea* [52]. On the other hand, a lot of studies are published dealing with the inhibiting effects of ammonia, which is released during the fermentation of materials with the high content of proteins [53–55]. This could explain the observed negative influence of protein content on the yield of biomethane. Pavlostathis et al. [56] arrived at a similar conclusion and confirm that, in the anaerobic environment, the hydrolysis of carbohydrates happens more rapidly as compared with proteins. Nevertheless, there are other opinions too. According to Wagner et al. [57], substrates rich in proteins exhibited a high potential for methane yield as compared for example with lipids or cellulose-containing substrates. Thus, a positive context should logically exist with a certain range of protein content in the substrate and with the methane yield. This was not confirmed in our case. By contrast, the presence of lipids affected the biomethane yield positively in our case. The situation was caused by the fact that the content of lipids in the prepared silages was low and hydrolysis of these substances then could not inhibit the fermentation process. Lipids were metabolized into free fatty acids and glycerol, and these products further metabolized into methane + CO_2/volatile fatty acids. Thus, in the lipid concentrations measured by us, the accumulation of long-chain fatty acids did not occur, which might have inhibited the anaerobic process [58].

The aim of breeding maize varieties suitable for having a silage of high quality is that the crop contains more than 82% of starch in the grain. Another requirement for maize varieties is the low content of ADF, NDF, and ADL [35]. This explains why the silage prepared from maize monoculture always reached demonstrably lower contents of these parameters (ADF, NDF, ADL) compared with White s.c. Furthermore, if there is a negative correlation between the content of these parameters in silage and the methane yield, then it is clear why the methane yield also grew with the increasing share of maize in silage. The negative influence of the presence of lignin (ADL) and fiber fraction (ADF, NDF) on the result of the fermentation of silage made from diverse crops, i.e., methane yield, was confirmed in an extensive experiment [50]. The authors tested 405 types of silages prepared from 43 different plant species and the mentioned negative influence of difficult-to-degrade C substances (lignin) was demonstrated across the plant species. On the other hand, the values measured in our experiment corroborate that the lower addition of White s.c. (up to 20%$_{w}$) did not negatively influence the increase of ADL, ADF, NDF contents in the silage and its impact on methane yield was of secondary character. This confirms the possibility of using silage from the mixed culture for methane yield, with no worries from the increased content of substances adversely affecting its yield.

Of polysaccharides, a demonstrably positive effect on methane yield was that of starch content in the silage. The content of starch in the maize silage significantly exceeded values measured in the White s.c. silage, as well as in the MC silage. This might have been caused according to Ferreira et al. [35] by the natural capacity of maize to synthetize carbohydrates in leaves, and subsequently transport them and store in the grain. It is exactly this property that makes from the maize an excellent crop for energy and feeding purposes [59], because C substances are necessary as a source of energy for (micro)organisms—be them a BGP fermenter or digestive tract of farm animals. The increased presence of starch in the silage resulted in the increased methane yield. Similar conclusions about the significance of carbohydrates were published from other studies and experiments [50,56,57]. The joint utilization of legume and maize for the methane yield can be viewed synergistically, too. A combined application of multiple substrate types may lead to the increased methane yield thanks to the joint fermentation of complex material of appropriate composition [60], with the resulting substrate composition (silage) being crucial for reaching the optimum methane yield [56,57].

5. Conclusions

The main aim of the presented study was to find an answer to the question of whether mixed cropping silages can be used in biogas plants. For this reason, various model silages were prepared, which varied in the content (from 10%$_{w}$ to 70%$_{w}$) of added legume. Results from the current study suggested that:

(a) The addition of legume (White s.c.) to the maize silage significantly affected silage composition with a direct proportion of the increasing legume content in the silage. The content of White s.c. in silage increased above 10%$_{w}$, significantly affecting the following qualitative parameters of silage: CF, ADF, NDF and coumarin content. ADF and NDF values in silage were proportional to the content of White s.c., and their content in the silage was increasing with the growing content of White s.c. The highest contents of ADF (38.84%$_{TS}$) and NDF (57.10%TS) were recorded in the model silage with the highest share of legume (70%$_{w}$ White s.c. and 30%w maize). Similarly, as in the case of ADF and NDF parameters in MC silages, the highest concentration of coumarin (16.33%$_{TS}$) was found in the model silage with the highest content of White s.c. (70%$_{w}$). The content of coumarin was decreasing with the decreasing share of White s.c. in silage.

(b) The addition of legume to the maize silage affected the methane yield. The methane yield was significantly decreasing, with a increasing share of White s.c. in silage. The share of White s.c. up to 15%$_{w}$ in silage decreased the methane yield by 3% compared with the pure maize silage. The addition of 20–30% decreased the methane yield by 11% compared with the pure maize silage. Thus, the addition of up to 20% had presumably no significant influence on decreasing the methane yield.

(c) The addition of legume to the maize silage did not negatively affect the biogas quality, i.e., did not reduce the concentration of methane in biogas if the share of legume in the silage did not exceed 50%$_w$. Silages with the content of White s.c. from 30%$_w$ to 10%$_w$ exhibited the average content of methane in biogas at a level of 55%. The average content of methane with using the maize silage alone and the silage made of White s.c. only were 56.5% and 52.27%, respectively.

Based on the measured data, it is possible to state that the hypothesis H_0 = Addition of the biomass of white sweetclover to the maize silage will affect neither biogas quality nor methane yield was disproved.

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Appendix A. Methods of Plant Sowing in the Mixed Cropping System Used from 2015 to 2018

Figure A1. Method of plant sowing in the first year (2017) of the experiment—developed in the period from 2015 to 2017.

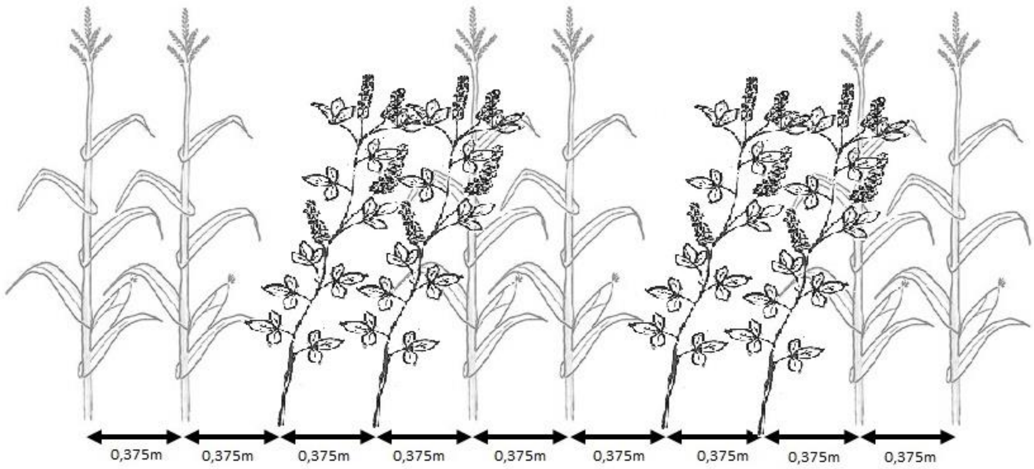

Figure A2. Method of plant sowing in the second year (2018) of the experiment.

Appendix B. Biogas Yield

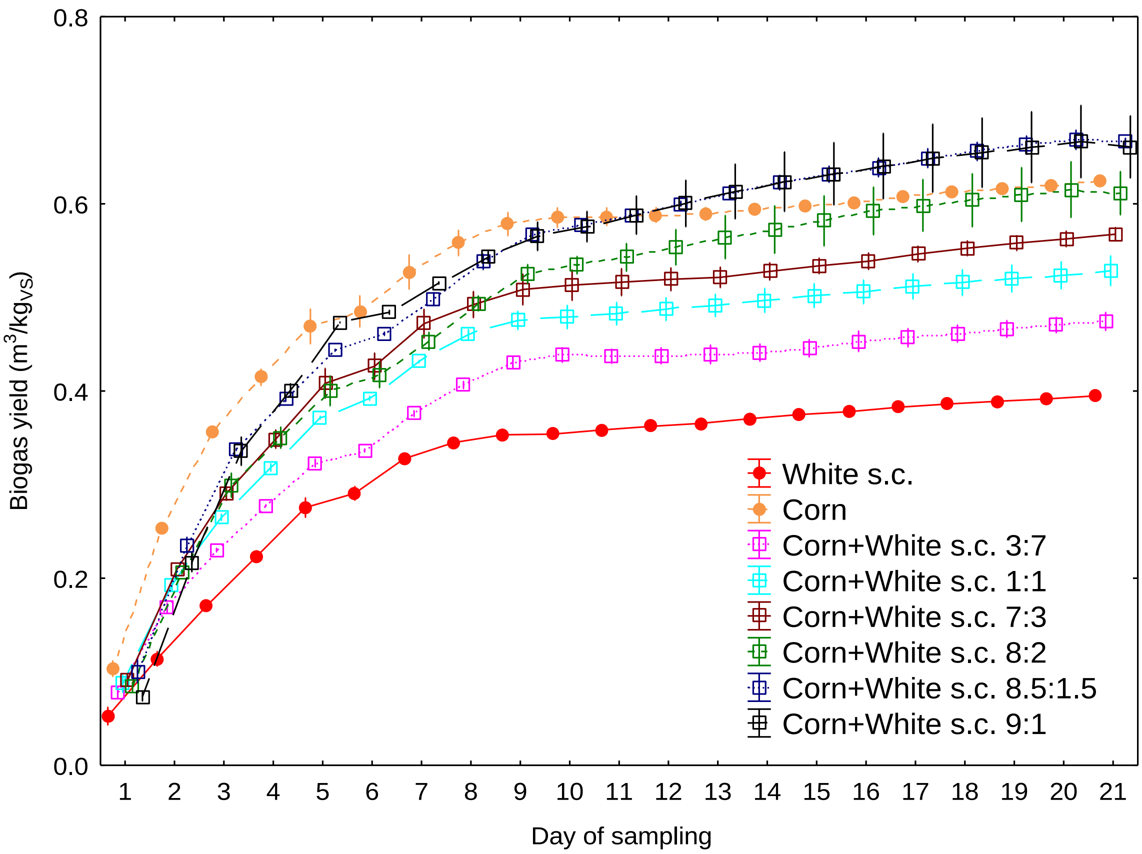

Figure A3. Biogas yield over 21 days of the experiment (average values for every day of measurement, n = 3 for one measurement, ±SD).

Appendix C. Results of PCA Analysis

Table A1. Factor coordinates of variables.

Variable	Factor Coordinates of Variables According to Correlations (Combination of All Parameters) Active and Additional Variables			
	Factor 1	**Factor 2**	**Factor 3**	**Factor 4**
Biogas yield (m^3/kg_{VS})	−0.97	0.14	−0.18	0.05
Methane yield (m^3/kg_{VS})	−0.99	0.05	−0.12	−0.04
Concentration of methane in biogas (vol. %)	−0.89	−0.43	0.05	0.01
Coumarin	0.94	−0.21	−0.29	−0.004
* TS	0.31	0.28	0.21	0.14
* Proteins	0.85	−0.34	−0.03	−0.09
* Lipids	−0.29	−0.29	0.05	−0.07
* CF	0.92	−0.18	−0.11	0.002
* Starch	−0.67	0.03	−0.02	0.05
* ADF	0.74	−0.32	−0.27	−0.14
* NDF	0.61	−0.48	−0.13	−0.18
* ADL	0.58	−0.32	0.01	0.01

* Additional Variable.

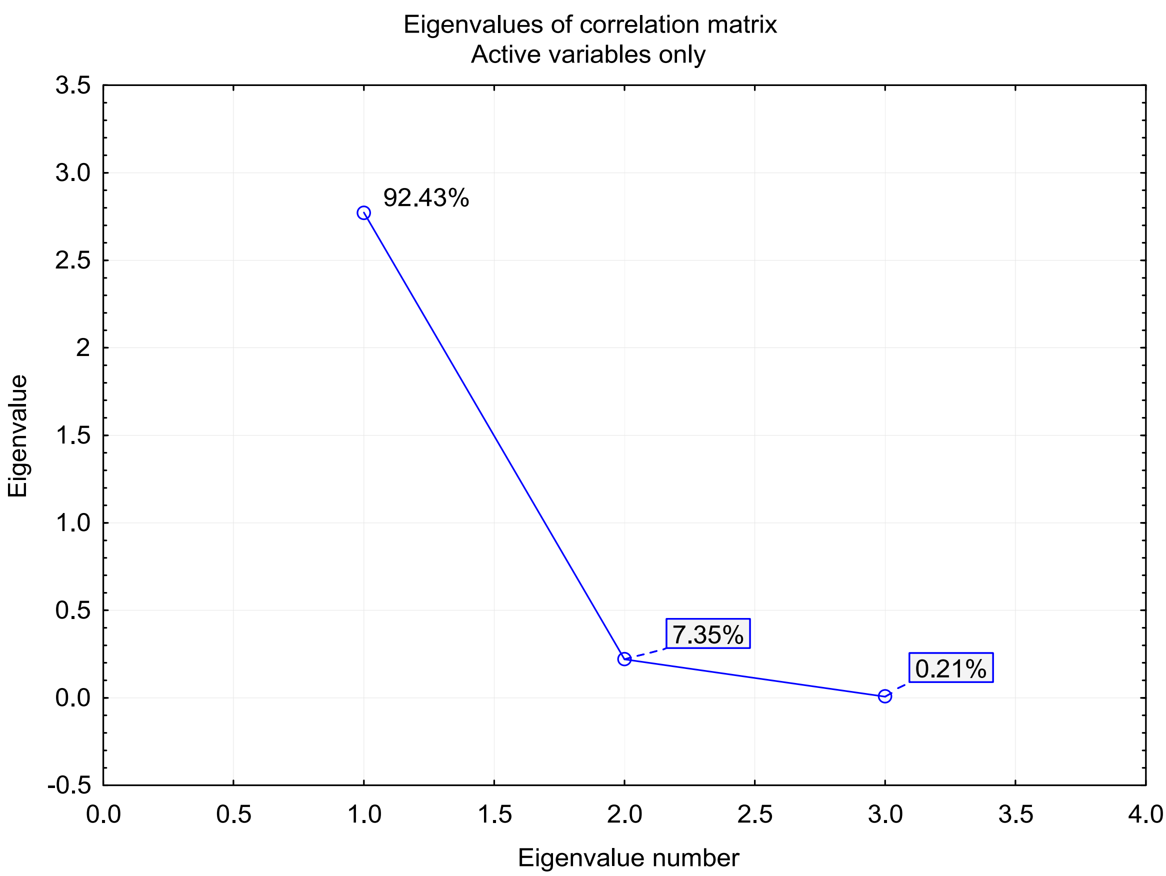

Figure A4. Eigenvalues of correlation matrix.

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