

Management of biomass of selected grape leaves varieties in the process of methane fermentation

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Abstract: Biogas plants are one of the most stable sources of renewable energy. Currently, there is a noticeable increase in the amount of post-production residues from agricultural production and agri-food processing (fruit and vegetable processing, fermentation, beet pulp, or lignocellulosic waste), which, can be used for biogas production after appropriate pretreatment. The aim of this study was to examine the possibility of using the biomass produced during the cultivation of grapes on a selected farm as a substrate for a biogas plant, taking into account the production process. The research was carried out in 2018–2020 in a vineyard located in the Sandomierz Upland in the south-eastern part of Poland. Own rooted vines were grown as a single continuous string with a trunk height of 40 cm and a length of one fixed arm approx. 0.9 m, on which six pivots were left every year after applying a short cut, from which 12–16 fruit shoots were derived, the so-called grapevines. Leaves were collected at random from three locations on the fruiting shoot, a total of 30 leaves in each replicate. Each sample consisted of 1/3 of the leaves collected at the bottom, 1/3 in the middle, and 1/3 at the top of the canopy. Leaf area was estimated with a model 3100 area meter on a sample of 30 leaves from each replicate. Both the quantity and quality of the obtained material as a substrate for methane fermentation were evaluated. Biogas yield tests in optimal conditions for mesophilic bacteria were conducted on three substrate samples referred to as ‘Regent’, ‘Seyval Blanc’, and ‘Solaris’. The yields of the tested material ranged from 51.0 to 59.0 Nm³ biogas per Mg of biomass.

Keywords: biogas plant, biomass utilisation, methane fermentation/biogasification, plant biomass, renewable energy

INTRODUCTION

The increase in energy demand and problems associated with current non-renewable energy resources have prompted researchers to take concrete actions related to, among others, the decarbonisation of the energy sector and the development of environmentally friendly technologies, including renewable energy devices [DAWID 2019]. Renewable energy sources are attracting attention worldwide because they are sustainable, improve environmental quality, and provide new job opportunities in rural areas [ISCI, DEMIRER 2007].

Biogas plants are one of the most stable sources of renewable energy. According to the European Union Biogas Barometer, Euroserv'er in 2017 [EurObserv'ER undated], there

were 17.4 thous. active anaerobic digestion plants in Europe with a total installed capacity of more than 8,700 MW_{el} and a total electricity production of 62.5 TWh (16.1 Mtoe). In addition, 367.0 upgraded biomethane units were in operation with a total refining capacity of 310,000 Nm³·h⁻¹ [EBA undated], enabling the production of biofuels together with electricity and heat.

More than 70% of biogas plants are farm-based as a result of important incentive policies (mainly applied in Germany, Austria, and Italy) and ambitious legal plans to increase the share of renewables to 20% of total energy consumed by 2020 [ERVINE 2015].

Biogas is one of the already well-established renewable energy carriers, and the technologies developed facilitate stable production thereof [WRZESIŃSKA-JĘDRUSIAK 2020]. Research con-

tinues to find innovative solutions to increase the efficiency of biogas production [WRZESIŃSKA-JĘDRUSIAK 2020], and reduce the energy consumption, environmental impact, or use of different raw materials in the methane fermentation process [VOYTOVYCH *et al.* 2020].

Each year, several million ha of agricultural waste are disposed of globally by various means such as incineration, land applications (mulching, composting), and landfilling. This global waste has great potential as a source of renewable energy and can be converted into high value by-products [ISCI, DEMIRER 2007]. The recovery of plant biomass and its potential for energy use is one of the most important innovations in the agricultural sector [GONZÁLEZ-DOMÍNGUEZ *et al.* 2014; MANZONE 2016; ROSÚA, PASADAS 2012].

Biogas production depends mainly on the type, quantity, and quality of the substrates fed. In agro-energy plants, different types of feedstocks are consumed in the meta-fermentation process [CHANDRA *et al.* 2012]. Sources of substrates for agricultural biogas plants include livestock manure, straw, beet leaves, grass, waste from agri-food processing, raw materials from such crops as corn, sorghum, and beet, and perennial crops (sugar miscanthus, hogweed, legumes, and their mixtures with grasses) [CHANDRA *et al.* 2012; GRALA *et al.* 2014]. The diverse and variable physical and chemical properties of substrates are the primary factors determining their applicability. The vast majority of alternative substrates for corn silage are wastes from the agri-food industry [MYCZKO *et al.* 2011]. The choice of the substrate is usually based on its availability to the biotechnology plant [WRZESIŃSKA-JĘDRUSIAK 2020]. Methane fermentation is considered an ideal way to manage organic waste and biomass from targeted energy crop plantations [GRALA *et al.* 2014]. Agricultural residues can become a potential source of biomass for energy production because they are available annually [BURG *et al.* 2017; VAN DAM *et al.* 2007].

The grapevine, which was cultivated over an area of 7.4 mln ha in 2018, is a globally widespread species with great economic importance [OIV 2019]. Vineyard biomass comprises unused by-products (such as vine pruning residues, leaves, and grape stalks) that can be managed by processing into compost and other products [BERES *et al.* 2017]. A study conducted by CORONA and NICOLETTI [2010] presented data showing that in Agrigento County, Italy, the production of pruned shoot biomass is $2.69 \text{ Mg} \cdot \text{ha}^{-1} \cdot \text{y}^{-1}$. Leaves and winter pruning wood are usually destroyed by burning in the fields or crushing into the soil, as are the herbaceous vine shoots. In commodity vineyards, they are mulched on site or stored outside the vineyard and burned [MANZONE 2016; SPINELLI *et al.* 2012]. Both solutions pose problems related to time consumption, economic issues, and environmental impact. Mulching contributes to the maintenance of organic matter, nutrients, and soil moisture, but it is also very dangerous in terms of disease spread [SCARLAT *et al.* 2013]. Winegrowers have so far burned the grape production waste raked into stacks directly at the edge of their field. This method contributes to the emission of significant amounts of particulate matter into the atmosphere [KESHTKAR, ASHBAUGH 2007]. Of particular note is the fact that vine pruning residues have particular quality characteristics compared to those of other lignocellulosic feedstocks, which may influence the choice and efficiency of conversion technologies [CHAU *et al.* 2009] and the potential for co-firing [MOLCAN *et al.* 2009].

Green mass production on the more weakly sunlit, fertile, and moist soils in the temperate climate is much greater than in lighter growing regions. The possibilities of using grapevine leaves for processing or drying are limited, as only leaves from the initial vegetation period are used for this purpose. It is economically viable to use the leaves as a raw material for renewable energy sources after harvesting the grapes; they are no longer needed at this stage of cultivation, because their role was to provide sugar to the grapes.

To date, studies on the use of wine production residues in methane fermentation have mainly focused on grape marc or wine sludge [DA ROS *et al.* 2016; EL ACHKAR *et al.* 2016; FIORE *et al.* 2016; MONTES, RICO 2020], while there are no literature reports on the biogasification-related management of other residues derived from maintenance treatments applied to grape crops.

The research presented in this paper is innovative, as the suitability of PIWI (PilzWiderstandsfähig) cultivars as biomass residues and their energy use potential are little known, compared to *Vitis vinifera* cultivars grown globally [BERES *et al.* 2017; MYCZKO *et al.* 2011; VAN DAM *et al.* 2007].

Therefore, the study was undertaken to verify the efficiency of methane production from vine leaves during the fermentation process in an agricultural biogas plant.

STUDY MATERIALS AND METHODS

RESEARCH MATERIAL

The study was conducted in 2018–2020 Nobilis Winery (50°39' N; 21°34' E) located in the Sandomierz Upland in the southeastern part of Poland.

Own rooted vines of the 'Seyval Blanc', 'Solaris', and 'Regent' cultivars commonly grown in Poland were planted in spring 2010 at a spacing of 2.0 × 1.0 m (5000 pcs·ha⁻¹) on loess soil. The plants were grown as a single fixed twine with a trunk height of 40 cm and one fixed arm length of ca. 0.9 m, on which six pivots were left each year after application of a short pruning cut, from which 12 to 16 fruit-bearing shoots, the so-called vines, were derived.

The experiment was conducted in a randomised block design and included three combinations with five repetitions. The repetitions were plots on which 10 plants were growing. All one-year shoots (epiphylls) were counted on the shrubs included in the experiment in autumn after the fruit harvest. In each combination, leaves were counted on 50 representative shoots, and then their weight was determined together with petioles and without petioles on an AXIS A250 electronic balance with an accuracy of 0.001 kg. Leaves were randomly collected from three locations on the fruiting shoot for a total of 30 leaves within each replicate. Each sampling consisted of 1/3 leaves taken at the lower, 1/3 at the middle, and 1/3 at the top of the canopy. Leaf area was estimated with the Area Meter model 3100 on a sample of 30 leaves from each replicate. Based on the results obtained, the number of annual shoots and leaves per plant, the area of 10 leaves and the area of all leaves per 1 ha, the mass of 10 leaves with petioles and without petioles, and the mass of 10 petioles were determined; the parameters determining the mass were also presented in terms of the area of 1 ha.

The results obtained in the experiment were statistically analysed using the two-factor analysis of variance method in SAS Enterprise Guide 5.1 software.

METEOROLOGICAL CONDITIONS

The average air temperature during the growing season i.e. from April to October in successive years of the study was higher than the multi-year average (Fig. 1). The measurements showed that the warmest year was 2018, this situation was influenced by higher average air temperatures in the following months i.e. April, May, July and September compared to 2019 and 2020. The growing season in 2020 was the coolest among those assessed.

Total precipitation during the 2018–2020 growing seasons ranged from 284.6 to 354.0 mm and was less than the multi-year average (Fig. 2). The driest growing season was in 2019, while 2020 recorded the highest precipitation total among the study years evaluated.

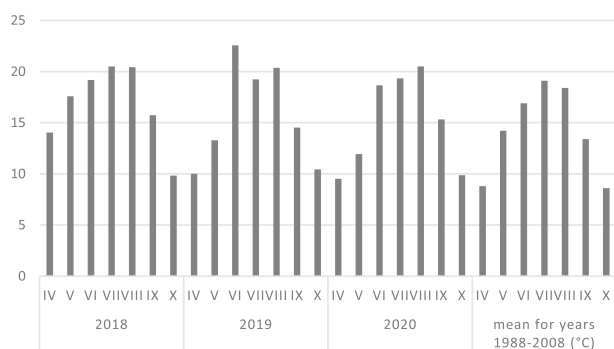


Fig. 1. Average monthly air temperatures to the Agrometeorological Station in Pęchów during the months of April to October in 2018–2020; source: own elaboration based on Procarn [undated]

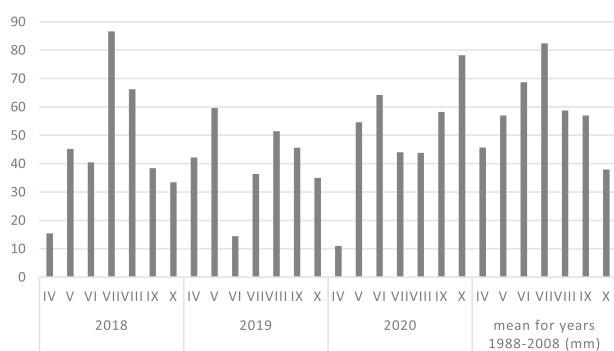


Fig. 2. Total precipitation according to the Agrometeorological Station in Pęchów during the months of April to October in 2018–2020; source: own elaboration based on Procarn [undated]

RESEARCH ON BIOGAS YIELD

In order to investigate the potential use of the grape production residues, i.e. the grape leaves, as a substrate for agricultural biogas plants, studies were conducted on their biogas yield and composition in terms of methane percentage. The studies were performed on the biogas production potential of grape leaves obtained after harvest as residues in optimal conditions for

mesophilic bacteria at 37°C. The research was performed using the standard methane fermentation test according to the modified method described in DIN 38414-8: 1985 on a test stand built with thermostatic eudiometric sets. The fermentation bottles of the eudiometric apparatus with a working volume of 0.5 dm³ were placed in a thermostatic water bath with forced circulation of the heating liquid. Biogas derivative studies were carried out in three fermentors in three repetitions for each material. The average mass of inoculum and the fermented mass of the substrate in the sample were determined (Tab. 1).

Table 1. Proportions of fermentation mixtures

Sample	Fermented substrate mass (mean) (g)	Mean inoculum mass (g)
'Regent'	12.7	473.4
'Seyval Blanc'	12.4	480.1
'Solaris'	12.9	479.9

Source: own elaboration.

The volume of biogas production was calculated in the Excel spreadsheet. On the basis of this graph, it was possible to determine whether the sample was working properly during the tests. The yield for a particular material was obtained and the average composition of the biogas was described. The pH of the substrate was determined according to the pH standard PN-EN 12176:2004. The dry mass was determined in accordance with the standard PN-EN 12880:2004, which consists in determining the loss of mass of the sediment sample while drying it to constant mass at a temperature of 105 ± 5°C. The contents of methane (CH₄), carbon dioxide (CO₂), oxygen (O₂) and hydrogen sulphide (H₂S) were determined using a Geotechnical Instruments GA 5000 analyser in the scope of CH₄ (0–100%) · CO₂ (0–100%) · O₂ (0–20%) · H₂S (0–1000 ppm). An inoculate prepared on the basis of the so-called post-ferment, which came from an agricultural biogas plant fed with plant substrates, was used to initiate the fermentation. Each fermentor was supplied with such a mass of the waste (grape leaves) that the initial load of the fermentor after conversion was equal to 5.0 kg ODM·m³ of its working volume (ODM is the dry matter content of organic matter expressed by the loss during roasting of the dry matter assessed by weight after a cycle of 3–5 h of dry matter roasting at temp. 550 ± 25°C). A control sample, in which only the inoculate was fermented, was also prepared. The test substrate was grape leaves with petioles.

Biogas yield tests were performed in optimal conditions for mesophilic bacteria on three substrate samples referred to as 'Regent', 'Seyval Blanc', and 'Solaris'.

Before the start of the research, samples of the tested materials were taken for physico-chemical analysis. As part of the basic analysis of the material, the pH, DM (dry matter content assessed by weight after 24 h of drying at temp. 105 ± 5°C), and ODM were determined (Tab. 2).

The total fermentation process of the 'Regent' substrate lasted 40 days, 90% of the biogas was formed after 13 days of fermentation (Fig. 1). After 40 days of fermentation, a relatively low degree of attenuation of organic dry matter (33%) was obtained, which corresponded to the yield of about 268 Nm³ of biogas from 1 Mg of organic dry matter. The poor fermentation

Table 2. Results of substrate baseline analysis

Substrate sample	pH	DM (%)	ODM (% DM)
'Regent'	3.6	22.4	87.7
'Seyval Blanc'	3.9	23.0	87.5
'Solaris'	4.2	22.8	84.2

Explanations: DM = dry matter, ODM = dry organic matter.
Source: own study.

was probably caused by the presence of organic compounds (e.g. lignin) in the medium and the significant lignification of the petioles. The presence of lignins makes the compounds contained in the fermented material resistant to decomposition in anaerobic conditions. The first portions of the produced biogas contained up to 270–290 ppm of hydrogen sulphide. The averaged biogas composition was as follows: 59% CH₄, 39% CO₂, 2.2% O₂, and 0.5% other gases. The other gases are a mixture of water in the form of water vapour (0–3.5%), ammonia NH₃, hydrogen sulphide H₂S, hydrogen H₂, nitrogen N₂, and other volatile compounds. The substrate contained about 1.83% DM of total nitrogen. However, with the degree of attenuation noted, it is unlikely that even high doses could generate fermentation-toxic concentrations of ammonium nitrogen in the digestate. The obtained biogas contained 59% methane and an average of approx. 193 ppm H₂S. The pH of the fermenting mix was 7.5 (Fig. 3).

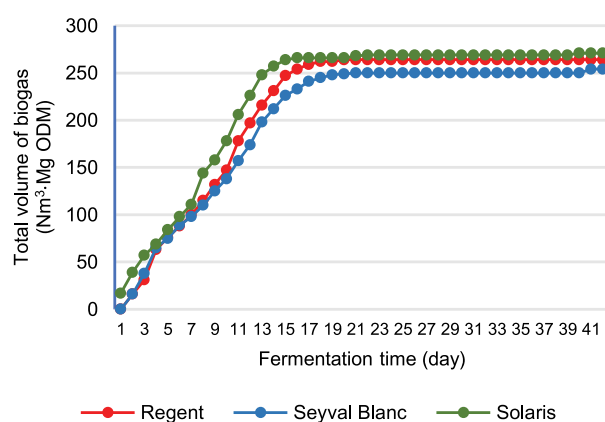


Fig. 3. Time profile generated during the research of dry organic matter (ODM) for the 'Regent', 'Seyval Blanc' and 'Solaris' varieties; source: own study

The biogas contained 64% of methane and an average of approx. 113 ppm H₂S. The first portions of the formed biogas contained up to 160–190 ppm of hydrogen sulphide. The averaged biogas composition was 64% CH₄, 29% CO₂, 2.3% O₂, and 4.4% other materials. The less fermenting pH 7 was 58, the total nitrogen content was 1.74% DM. Similarly to the other two samples, the 'Solaris' sample pH was on average 7.58. After 40 days of fermentation, low attenuation of equal dry matter (29%) was obtained, which was equivalent to a yield close to 274 Nm³ of biogas from 1 Mg of DM. 90% of the biogas was produced after 14 days of fermentation (Fig. 3).

The biogas contained 63% of methane and an average of approx. 82 ppm H₂S. The first portions of the formed biogas

contain up to 115–150 ppm of hydrogen sulphide. The averaged biogas composition was 63% CH₄, 30% CO₂, 1.7% O₂, and 5.6% other materials. The substrate contained about 1.45 DM of total nitrogen (Fig. 3).

The substrate contained about 3.3 kg of total nitrogen in 1 ha. However, with the recorded degree of attenuation, even its high doses will not generate high (toxic to fermentation) concentrations of ammonia nitrogen in the fermenting mass.

RESULTS

The number of fruiting shoots (vines) per one bush ranged from 15.2 to 18.8 and did not differ significantly between the assessed grapevine cultivars (Tab. 3). There was no significant effect of the study year on the evaluated parameter.

The number of leaves on one shoot ranged from 13.4 to 18.7 and did not differ significantly among the cultivars. The shrubs of the 'Regent' cultivar had significantly fewer leaves on one shoot than 'Seyval Blanc' and 'Solaris'. Significant differences were found between the years of the study. Regardless of the cultivar, the vines had a significantly greater number of leaves per shoot in 2018 than in 2020. Meteorological conditions had an influence on this state (2018 was the warmest year and 2020 was the coolest and the wettest year).

The number of leaves per plant ranged from 210.7 to 352.8, i.e. from 1,053,527.0 to 1,764,605.5 leaves·ha⁻¹, respectively. The statistical analysis did not show a significant effect of the cultivar on the assessed growth parameter. It was found that the number of leaves significantly depended on the year of the study. The vines in 2018 had a significantly higher number of leaves than in the other years, while this number in the last year of the study (the coolest and the wettest year) was significantly lower than in the previous years.

The area of 10 leaves ranged from 807.9 to 1,445.7 cm². It was shown that the evaluated growth parameter significantly depended on the cultivar. The 'Solaris' cultivar shrubs had significantly larger leaves than the other cultivars, while the 'Regent' cultivar had significantly smaller leaves. It was observed that the bushes of the examined cultivars had significantly smaller leaves in 2020 than in the other years. The leaf area per 1 ha unit ranged from 983,994.2 to 2,331,043.9 m² and largely depended on the cultivar. The 'Solaris' cultivar shrubs had a significantly larger leaf area per 1 ha unit than the shrubs of the other cultivars. An opposite tendency was observed in the case of 'Regent'. The examined parameter significantly depended on the analysed year of research. The bushes of the studied cultivars had a significantly larger leaf area per 1 ha in 2018 than in the other years; it was significantly smaller in 2020.

When considering the interactions between the cultivars and the years of the study, no significant differences were found for the evaluated number of shoots and number and area of leaves. The significant influence of the study year on the parameters considered in Table 3.

The weight of 10 leaves with petioles ranged from 59.0 to 95.3 g, while the weight of 10 leaves without petioles ranged from 41.2 to 73.6 g (Tab. 4). The statistical analysis of both evaluated parameters showed that the 'Solaris' cultivar bushes had significantly heavier leaves than the other cultivars, while the 'Regent' cultivar had significantly the lightest leaves. In both

Table 3. Evaluation of three grape varieties: 'Regent', 'Seyval Blanc' and 'Solaris' in terms of the number of shoots, number of leaves, and leaf area in 2018–2020

Factor		Number of shoots per bush	Number of leaves per shoot	Number of leaves on plant	Number of leaves on the top per 1 ha	Area of 10 leaves (cm ²)	Leaf surface per 1 ha area (m ²)
		pcs					
Cultivar (A)	'Regent'	17.8 ±2.2 ^A	13.8 ±1.2 ^B	247.0 ±38.5 ^A	1,235,000 ±192,613.6 ^A	807.9 ±43.7 ^C	9,87,880.0 ±4.3 ^C
	'Seyval Blanc'	15.2 ±1.8 ^A	16.3 ±1.4 ^A	248.3 ±39.7 ^A	1,241,666.6 ±198,611.8 ^A	971.4 ±29.7 ^B	1,205,658.3 ±2.9 ^B
	'Solaris'	16.6 ±1.7 ^A	16 ±0.6 ^A	267.0 ±32.3 ^A	1,335,000 ±161,709.6 ^A	1,445.7 ±44.1 ^A	1,930,410.0 ±4.4 ^A
	<i>p</i> -value	0.0912	0.0026	0.5893	0.5963	<0.0001	<0.0001
Year (B)	2018	18.8 ±1.4 ^A	18.7 ±1.3 ^A	352.8 ±41.5 ^A	1,764,605.5 ±140,784.7 ^A	1,300.7 ±146.1 ^A	2,331,043.9 ±18.4 ^A
	2019	17.9 ±3.5 ^A	16.8 ±3.6 ^{AB}	277.7 ±42.1 ^B	1,384,905.5 ±198,060.9 ^B	1,150.2 ±131.3 ^A	1,600,950.7 ±15.2 ^B
	2020	15.7 ±2.9 ^A	13.4 ±3.9 ^B	210.7 ±39.5 ^C	1,053,527 ±95,973.9 ^C	956.7 ±97.3 ^B	983,994.2 ±9.5 ^C
	<i>p</i> -value	0.0025	0.0214	0.0031	0.0029	0.0043	0.0051
A×B	<i>p</i> -value	0.7852	0.7532	0.4589	0.7453	0.6987	0.5465

Explanation: mean values marked with the same letters do not differ significantly at $\alpha = 0.05$, the bold value indicates a significant inter-value quality. Source: own study.

Table 4. Evaluation of three grape varieties: 'Regent', 'Seyval Blanc', and 'Solaris' in terms of leaf weight in 2018–2020

Factor		Mass of 10 leaves with petioles	Weight of 10 leaves without petioles	Weight of 10 petioles	Leaf mass with petioles from the surface	Leaf mass without petioles from the surface	Mass of petioles from the surface
		g			kg·ha ⁻¹		
Cultivar (A)	'Regent'	59.0 ±3.1 ^C	41.2 ±2.2 ^C	17.8 ±1.3 ^B	7,273.2 ±1111.5 ^B	5,072.9 ±769.2 ^B	2,200.2 ±362.5 ^A
	'Seyval Blanc'	63.6 ±2.3 ^B	49.5 ±1.5 ^B	14.2 ±1.3 ^C	7,930.6 ±1451.1 ^B	6,161.3 ±1090.1 ^B	1,769.3 ±384.1 ^B
	'Solaris'	95.3 ±2.7 ^A	73.6 ±2.2 ^A	21.6 ±1.4 ^A	12,749 ±1791.7 ^A	9,841.6 ±1306 ^A	2,907.3 ±506.3 ^A
	<i>p</i> -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Year (B)	2018	90 ±14.1 ^A	66.7 ±8.4 ^A	22.4 ±5.6 ^A	11,553.7 ±147.8 ^A	9,062.5 ±784.7 ^A	2,888.2 ±184.7 ^A
	2019	78.4 ±11.3 ^{AB}	58.7 ±7.9 ^{AB}	19.3 ±3.9 ^{AB}	10,062.9 ±1292.1 ^A	7,704.3 ±1177.2 ^A	2,490.9 ±345.0 ^A
	2020	62.4 ±9.5 ^B	44.3 ±7.3 ^B	14.9 ±3.7 ^B	2,422.5 ±95.9 ^B	5,690.4 ±395.4 ^B	1,925.4 ±154.8 ^B
	<i>p</i> -value	0.0057	0.0031	0.0222	<0.0001	0.0026	0.0030
A×B	<i>p</i> -value	0.6582	0.9621	0.7421	0.6587	0.8529	0.8269

Explanation: mean values marked with the same letters do not differ significantly at $\alpha = 0.05$, the bold value indicates a significant inter-value quality. Source: own study.

cases, it was shown that the bushes of the studied cultivars had significantly heavier leaves in 2018 than in 2020.

The weight of 10 petioles ranged from 14.2 to 22.4 g. The 'Solaris' cultivar shrubs had significantly heavier leaves than the other cultivars, while 'Seyval Blanc' petioles were significantly the lightest (Tab. 4). The evaluated parameter was characterised by a significantly higher value in 2018 (the warmest year) than in 2020 (the coolest and the wettest year).

The weight of all leaves with petioles in the area of 1 ha varied from 2,422.5 to 12,749.7 kg·ha⁻¹, while the weight of leaves without petioles ranged from 5,072.9 to 9,841.6 kg·ha⁻¹ and

differed significantly. The 'Solaris' cultivar shrubs had significantly heavier leaves (with and without petioles) than the other cultivars. The petioles weight per 1 ha in the 'Seyval Blanc' cultivar was significantly lower than in the other cultivars. The parameters of the green weight per 1 ha were significantly lower in 2020 than in the other years of the study. When considering interactions between the cultivars and the years of the study, no significant differences were found for the evaluated leaf weight. All the leaf weight parameters presented in Table 4 showed a significant influence of the research year.

The presented dendrogram (Fig. 4) allowed us to determine the similarity of leaf mass with and without petioles and petiole mass between the 'Regent', 'Seyval Blanc', and 'Solaris' cultivars in 2018. Based on the results obtained, three clusters showing clear similarities were identified. Based on the analysis, cluster 1 was found to represent the mass of leaves with and without petioles for the 'Solaris' cultivar. Cluster 2 was strongly grouped according to the petiole weight. A high similarity was observed between the 'Seyval Blanc' and 'Regent' cultivars, while 'Solaris' clearly stood out in the evaluation of this parameter. The largest last cluster consists of two groups grouped according to the evaluated parameters, i.e. leaf weight with petioles and without petioles. Both sub-clusters comprise 'Seyval Blanc' and 'Regent' cultivars.

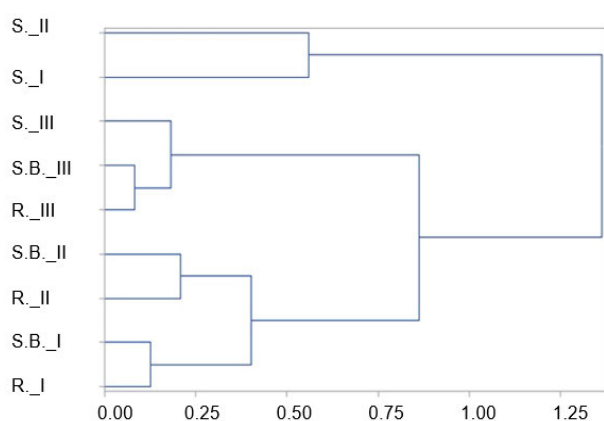


Fig. 4. Branching-tree diagram of leaf weight with and without petioles and petiole weight in 'Regent', 'Seyval Blanc', and 'Solaris' grapevines in 2018; I – area of 1 leaf (cm^2), II – weight of leaves per 1 ha with petioles (kg), III – weight of petioles, ($\text{ha}\cdot\text{kg}^{-1}$); source: own study

The fermentation process of the 'Regent' substrate was normal and undisturbed. After 40 days of fermentation, a relatively low degree of digestion of dry organic matter (33%) was obtained, which was equivalent to an output of approximately 53 Nm^3 biogas per Mg of biomass. There was 90% of the biogas produced after 13 days of fermentation. The poor fermentation was probably caused by the presence of organic compounds (lignins) in the substrate and the significant lignification of the petioles. Due to the presence of lignins, the compounds contained in the fermented material are resistant to decomposition in anaerobic conditions.

The first portions of the biogas produced contained up to 270–290 ppm of hydrogen sulphide.

The averaged biogas composition was as follows: 59% CH_4 , 39% CO_2 , 2.2% O_2 , and 0.5% other gases. The other gases were a mixture of water in the form of water vapour (0–3.5%), ammonia (NH_3), hydrogen sulphide (H_2S), hydrogen (H_2), nitrogen (N_2) and other volatile compounds. The substrate contained approximately 4.1 kg of total nitrogen per Mg. However, with the degree of digestion noted, it is unlikely that even high doses could generate fermentation-toxic concentrations of ammonia nitrogen in the digestate.

The biogas contained 59% methane and an average of approx. 193 ppm H_2S . The pH of the substrate was 4.2 and the dry matter content was about 23%.

Biogas yield tests were also performed in optimal conditions for mesophilic bacteria on the 'Seyval Blanc' substrate sample.

Here, no interference was observed in the ongoing fermentation process either.

After 40 days of fermentation, a low degree of digestion of dry organic matter (27%) was obtained, which was equivalent to a yield of approximately 51 Nm^3 biogas per ha of biomass. 90% of the biogas was produced after 13 days of fermentation. The poor attenuation of the substrate, as in the 'Solaris' sample, was related to the presence of organic compounds (lignins) in the substrate and the significant lignification of the leaf petioles. Such material does not easily decompose in anaerobic conditions. The biogas contained 64% methane and an average of approx. 113 ppm H_2S . The first portions of the biogas contained up to 160–190 ppm hydrogen sulphide. The averaged biogas composition was as follows: 64% CH_4 , 29% CO_2 , 2.3% O_2 , and 4.4% other gases. Similarly to the 'Regent' cultivar, the substrate contained about 4 kg of total nitrogen per 1 ha and the substrate pH was 3.9.

As in the previous two samples, the fermentation process in the 'Solaris' sample was normal and undisturbed. After 40 days of fermentation, a low degree of digestion of dry organic matter (29%) was achieved, which was equivalent to a yield of about 53 Nm^3 of biogas per Mg of biomass. 90% of the biogas was produced after 14 days of fermentation. The biogas contained 63% methane and an average of approx. 82 ppm H_2S . The first portions of the biogas contained up to 115–150 ppm hydrogen sulphide.

The averaged biogas composition was as follows: 63% CH_4 , 30% CO_2 , 1.7% O_2 , and 5.6% other gases.

Table 5 presents the results of the analysis of the biogas composition, pH, and dry matter content in the examined material from three grapevine cultivars. The statistical analysis showed a significant effect of the cultivar on the parameters studied. The dry matter ranged from 22.4 to 23.0%, while the percentage content of methane in the studied biogas ranged from 59.0 to 64.0%. These two parameters had significantly the highest value in 'Seyval Blanc' cultivar, and the lowest value was recorded in the 'Regent' cultivar. The level of carbon dioxide ranged from 29.0 to 39.0%, and significantly the highest level was recorded in the 'Regent' cultivar. The oxygen level ranging from 1.7 to 2.3% was similar in the 'Seyval Blanc' and 'Regent' cultivars. A significant difference in the level of the other gases ranging from 0.5 to 5.6% was observed between the 'Regent' cultivar and the other two cultivars. The substrate pH of the 'Seyval Blanc' cultivar differed significantly from that in the others, and the level of the evaluated parameter ranged from 3.9 to 4.2. The yield of the tested plant material was characterised by a large variation between the tested species from 253.0 in the 'Seyval Blanc' cultivar to 274.0 ($\text{Nm}^3\cdot\text{Mg}^{-1}$ ODM) in the 'Solaris' cultivar. There was no significant effect of the study year on the biogas composition, pH, and dry matter content in the grapevine cultivars. Considering the interactions between the cultivars and years, no significant differences in the dry matter content, pH and biogas composition were found.

The analysis of the multivariate correlations in Table 6 revealed a very strong significant correlation between dry matter and the methane level, carbon dioxide, other gases, and pH. The methane level correlated strongly significantly with carbon dioxide, other gases, and pH. The carbon dioxide content correlated strongly significantly with dry mass, methane, and other gases. The oxygen level correlated significantly negatively with the biogas yield. The other gases correlated strongly

Table 5. Evaluation of three grape cultivars ‘Regent’, ‘Seyval Blanc’, and ‘Solaris’ for substrate yield and biogas composition in 2018–2020

Factor		Dry matter	Methane	Carbon dioxide	Oxygen (O ₂)	Other gases	pH	Biogas yield (Nm ³ ·Mg ⁻¹ ODM)
		%						
Cultivar (A)	‘Regent’	22.4 ^C	59.0 ^C	39.0 ^A	2.2 ^A	0.5 ^C	4.2 ^A	268.0 ^B
	‘Seyval Blanc’	23.0 ^A	64.0 ^A	29.0 ^B	2.3 ^A	4.4 ^B	3.9 ^B	253.0 ^C
	‘Solaris’	22.8 ^B	63.0 ^B	30.0 ^B	1.7 ^B	5.6 ^A	4.2 ^A	274.0 ^A
	<i>p</i> -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Year (B)	2018	23.1 ^A	63.2 ^A	33.3 ^A	2.1 ^A	4.2 ^A	4.1 ^A	271.0 ^A
	2019	22.7 ^A	62.0 ^A	32.6 ^A	2.0 ^A	3.7 ^A	4.0 ^A	260.0 ^A
	2020	22.3 ^A	61.1 ^A	31.7 ^A	1.9 ^A	3.3 ^A	3.9 ^A	249.0 ^A
	<i>p</i> -value	0.3258	0.5879	0.9517	0.5471	0.3698	0.45689	0.7514
A×B	<i>p</i> -value	0.5321	0.7532	0.9512	0.7582	0.4564	0.5454	0.7321

Explanation: mean values marked with the same letters do not differ significantly at $\alpha = 0.05$, the bold value indicates a significant inter-value quality; ODM = dry organic matter.

Source: own study.

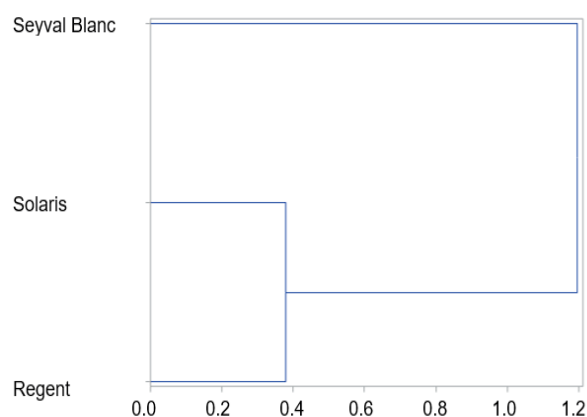
Table 6. Correlation coefficient of the substrate yield and biogas composition, regardless of the variety and year of research

Substrate and composition of biogas	Dry matter	Methane (CH ₄)	Carbon dioxide (CO ₂)	Oxygen (O ₂)	Other gases	pH	Biogas yield (Nm ³ ·Mg ⁻¹ ODM)
	%						
Dry matter (%)	1						
Methane (CH ₄ , %)	0.9897	1					
	<0.0001						
Carbon dioxide (CO ₂ , %)	-0.9707	<0.0001	1				
	<0.0001						
Oxygen (O ₂ , %)	-0.0339	-0.1764	0.2731	1			
	0.9309	0.6499	0.4772				
Other gases (%)	0.8472	0.9142	-0.9499	-0.5611	1		
	0.0041	0.0006	<0.0001	0.1169			
pH	-0.7559	-0.6547	0.5765	-0.6286	-0.2923	1	
	0.0185	0.0055	0.1041	0.0698	0.4453		
Biogas yield (Nm ³ ·Mg ⁻¹ ODM)	-0.5447	-0.4193	0.32733	-0.8197	-0.0156	0.9607	1
	0.1294	0.2612	0.3899	0.0068	0.9682	<0.0001	

Source: own study.

significantly with dry matter, methane, and carbon dioxide levels. The pH value correlated significantly with the biogas yield, which correlated significantly with the dry matter and methane level. The last parameter considered, the biogas yield correlated significantly with oxygen and pH.

Figure 5 shows the results of the analysis of the biogas composition as well as pH and dry matter of the investigated research material against the considered three grapevine species. The presented dendrogram (Fig. 5) allowed the determination of similarity between the ‘Regent’, ‘Seyval Blanc’, and ‘Solaris’ cultivars regardless of the investigated study year. Based on the results obtained, two clusters showing clear similarities were identified. Cluster 1 represents ‘Seyval Blanc’ while cluster 2 shows similarity between ‘Regen’ and ‘Solaris’, which have similar parameters.

**Fig. 5.** Branching plot for the ‘Regent’, ‘Seyval Blanc’, and ‘Solaris’ cultivars regardless of the biogas composition; source: own study

DISCUSSION

In this study, the efficiency of biomass produced during the cultivation of grapes as a raw material for biogas plants was assessed. Both the quantity and quality of the obtained material as a substrate for methane fermentation were assessed.

Viticulture and wine production is a time-consuming, multi-step process producing a large amount of organic and inorganic waste. It has been calculated that grape cultivation and harvesting produces about 5 ha of solid waste per hectare per year, while the amount of wastewater from wineries varies depending on the volume of production from 650,000 m³ (Greece) to more than 18,000,000 m³ (Spain) per year [ARVANITOYANNIS *et al.* 2006a, b]. Therefore, the need to reuse bio-waste and wine waste becomes evident. In the current low-carbon economy promoting the zero waste policy and with the growing environmental awareness of climate change and depletion of natural resources, the need for recycling and reuse seems most advisable.

In Poland, grapevine is of little economic importance; nevertheless, the grapes and the wines produced here are of very good quality [KAPLAN, NAJDA 2014; LISEK 2004; 2008; 2009; 2011; DOBROWOLSKA-IWANEK 2016]. In the 2020/2021 marketing year, producers registered with the National Support Centre for Agriculture (Pol. Krajowy Ośrodek Wsparcia Rolnictwa – KOWR) [KOWR undated] cultivated grapevines on 547.4 ha. The high interest in grapevine cultivation in temperate climates results e.g. from the introduction of new interspecific hybrids of grapevines into cultivation, which are quite popular, especially in organic vineyards, due to their high resistance to fungal diseases. The botanical species *Vitis vinifera* includes PIWI cultivars often grown in northern European countries [DE LA FUENTE LLOREDA 2018; JANA *et al.* 2016]. For example, the cultivars ‘Hibernal’, ‘Regent’, and ‘Solaris’ are grown in Germany, while ‘Malverina’, ‘Savilon’, or ‘Laurot’ are popular in the Czech Republic [RADOVA *et al.* 2016]. According to SINOQUET [2021], the 10 most prevalent PIWI strains grown in Switzerland in the 2015/2016 season included ‘Cabernet Jura’, ‘Johanniter’, ‘Solaris’, ‘Maréchal Foch’, ‘Regent’, ‘Seyval Blanc’, ‘Muscat Bleu’, ‘Divico’, ‘Sovignier Gris’, and ‘Leon Millot’. For example, sparkling wines in Brazil are often based on *V. labrusca* and hybrids [CALIARI *et al.* 2014]. In Rio Grande do Sul (Brazil), only 7% of the vineyards are planted with European grape cultivars, and the rest are American or hybrid grapes such as ‘Cabernet Cortis’, ‘Cabernet Carbon’, ‘Bronner’, or ‘Regent’ [DE BEM *et al.* 2016].

It may seem that vine plant wastes, broadly defined, can be used as feedstocks for biogas plants [PULVIRENTI *et al.* 2015, RONGA *et al.* 2018; 2019] and pyrolysis [ALLESINA *et al.* 2018], thus increasing vineyard efficiency and productivity by providing green energy and fertilisers such as digestate and biocarbon [RONGA *et al.* 2019]. A species with high energy potential is grapevine. In the present study, it was shown that grapevine can produce significant amounts of biomass that can be used in biogas plants, among others. The number of leaves per hectare ranged from 1,241,666.6 to 1,335,000.0 pcs·ha⁻¹ depending on the cultivar, covering an area of 997,880.0 to 1,930,410.0 m². For leaf area, this parameter was shown to be significantly dependent on cultivar. The weight of all leaves with petioles in an area of 1 ha ranged from 7,273.2 to 12,749.7 kg·ha⁻¹, while without petioles

from 5,072.9 to 9,841.6 kg·ha⁻¹ and differed significantly among the grapevine cultivars evaluated.

There is scattered information in the literature about the biomethane potential of many different organic biomasses, including energy crops [WEILAND 2003], fruits and vegetables [SCANO *et al.* 2014], and food processing waste [LABATUT *et al.* 2011; ROATI *et al.* 2012]. In addition, some authors have collected and summarised methane production values in a large dataset for practical comparison [DEUBLEIN, STEINHAUSER 2008; FIORE *et al.* 2016; RAPOSO *et al.* 2012; ROATI *et al.* 2012]. In the available literature, there is no information on the yield of methane production from grape leaves during the fermentation process in an agricultural biogas plant.

Crop production produces not only crops, but also plant biomass such as leaves, stems, roots, and biomass from the annual cutting of bushes and orchards, among others. Much of the organic waste from agriculture can be utilised for energy purposes as substrate or co-substrate in a biogas plant. Depending on the substrate to be utilised, biogas plant technologies should be considered.

The quality of grapes is influenced not only by environmental conditions but also by plant pruning or leaf removal treatments, which promotes exposure of these fruits to sunlight [FENG *et al.* 2015] and provides a method to reduce the occurrence of grapevine diseases [LISEK 2004]. Plant parts removed from cultivation can constitute significant amounts of biomass that should be managed. One way to do this may be to use the biomass in the biogasification process.

Plant residues from vine pruning consist of lignocellulose with a negligible concentration of soluble sugars; hence, a pretreatment step may be required for efficient methane production. To date, a study on methane fermentation of biomass from grapevine pruning (stems and branches) without pretreatment, with a methane yield of 53.8 ± 0.4 cm³ CH₄·g⁻¹ VS (VS – volatile solids) has been conducted. The low methane yield is related to the low solubility of complex carbohydrates (cellulose and hemicellulose) of lignocellulosic biomass. In contrast, when the material samples were subjected to steam explosion in this study, the methane yield was almost doubled, reaching 104.1 ± 1.0 cm³ CH₄·g⁻¹ VS [NITSOS *et al.* 2015]. The leaves examined as a residue from grape production have a low degree of attenuation. This is probably a result of the presence of organic compounds such as lignins and significant lignification of petioles in the substrate. During the study, from the obtained material, after 40 days of fermentation, a relatively low degree of attenuation of dry organic matter from 27% to 33% was obtained. The biogas yield ranged from 253 to 274 m³·Mg ODM where, for example, corn with DM 20–35% has a yield of 450 to 700 m³·Mg ODM. This indicates the possibility of using grape leaves as a substrate for biogas plants. However, it is advisable to develop a substrate-specific technology including substrate pretreatment and hydrolysis. Due to the high availability of research material, the research is being continued with a view to developing such a technology [MYCZKO *et al.* 2011].

Anaerobic fermentation may be particularly suitable for the treatment of wine waste due to its high content of nutrient-rich organic matter and noticeable energy potential. In a study on different by-products of wine production, i.e. grape pomace, grape stalks, and wine sludge, subjected to biogasification, it was found that the potential methane production in batch trials of

grape pomace and wine sludge in thermophilic conditions was high: 0.34 and 0.37 Nm³ CH₄·kg⁻¹ VS_{fed}, respectively, compared to grape stalks, where it was only 0.13 Nm³ CH₄·kg⁻¹ VS_{fed} [DA ROS *et al.* 2016]. Similarly, the work of JASKO *et al.* [2012] showed that grape sludge is a suitable substrate for methane fermentation, with a biogas yield of 855.5 dm³·kg⁻¹ VS. Studies on anaerobic digestion of wine sludge with determination of the influence of electro-oxidation as a pretreatment process showed that this process has a significant positive effect on biogas production increasing its value to 330 dm³·kg⁻¹ VS after 1.5 ha of treatment, compared to wine sludge without pretreatment – 180 dm³·kg⁻¹ VS [ARENAS SEVILLANO *et al.* 2020]. In order to maintain proper operating parameters of a biogas plant, it is necessary to constantly control the quality of the raw material feeding the fermentation mixture. The efficiency of a biogas plant, the content of methane in the biogas, and the fertilising quality of the digestate depend on the physical state and the chemical quality of the raw materials delivered. Therefore, it is necessary to monitor the process constantly. A number of physical and chemical parameters affect the course and efficiency of methane fermentation. Not only the pH, temperature, and quality of the input to the fermentation chamber, but also the mode of fermentation process implementation (one-, two-, or multi-stage, mesophilic, thermophilic, psychrophilic), the type of fermenter used, etc. are important.

Biogas yield testing can be performed on a single substrate or a mixture of substrates. The use of a mixed form fed into digesters results in a new quality. The use of a mixture may result in an increase or a decrease in the biogas yield in relation to the results achieved with the use of single substrates. It is associated with e.g. a change in the C:N ratio, changes in the concentration of micro- and macro-elements, and the possibility of changing the reactivity of substances present in individual substrates. This may result in the formation of more or less available compounds for processing into biogas by bacteria.

Currently, there is a noticeable increase in post-production residues from agricultural production and from agri-food processing (fruit and vegetable processing, post-boiler slop, beet pulp, or lignocellulosic waste), which can be used for biogas after appropriate pretreatment. This is related to the need for waste management. The yields of the material tested ranged from 51.0 to 59.0 Nm³ biogas per Mg of biomass, which is not high compared to, for example, 70–300 Nm³ biogas per Mg of biomass from herb production residues [WRZEŃSKA-JĘDRUSIAK 2020]. However, biogas plants can contribute to solving the problems associated with the management of troublesome residues from the production of grapes (shoots and leaves). After pretreatment, this material can be used as a co-substrate in the biogas process.

CONCLUSIONS

1. During a three-year study it was shown that grapevine cultivar had a significant effect on the number of leaves per shoot, leaf area and leaf weight.
2. The cultivar 'Solaris' was characterised by the significantly highest leaf area and leaf weight among the evaluated cultivars.
3. It was shown that the year of study had a significant effect on most of the evaluated parameters of the amount and size of biomass produced. 2018 was the warmest year among the

assessed research years and had a significant impact on the assessed biomass parameters.

4. During the biogas yield studies, similar results were obtained for biogas yield for all three grape varieties tested.
5. The biogas yields of 'Regent' and 'Solaris' were on the same level, while lower values were recorded for 'Seyval Blanc', and the methane content in the obtained biogas was the highest for 'Seyval Blanc' and 'Solaris'.
6. Analysing the results obtained from the field experiment and biogas yield studies, it can be concluded that the 'Solaris' cultivar is the most efficient and effective.

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