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## **Estimation of biomethane yield from silage fermented biomass of oilseed radish (*Raphanus sativus* L. var. *oleiformis* Pers.) for different sowing and harvesting dates**

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**Abstract.** The potential possibility of using oilseed radish biomass of different sowing dates (technological interval from spring to summer (post-harvest) sowing) and phenological harvesting (budding-green pod) after the silage fermentation procedure for the production of biogas and biomethane using the methodology of anaerobic digestion with the addition of inoculum was investigated. Comprehensive methods for assessing the kinetics and dynamics of production of both total biogas volumes and biomethane production levels were applied, with an assessment of the levels of approximation of the curves of daily volume fixation.

Based on a systematic analysis with an assessment of the individual characteristics of the kinetics of the process of anaerobic digestion of silage substrate for each variant of the experiment, the high potential of this crop at different terms of its sowing and harvesting with a level of bioproductivity at the level of 0.54–3.62 t ha<sup>-1</sup> DM (depending on the phase and timing of sowing) at the level of biomethane productivity in the range of SMY 201.03–319.66 L<sub>N</sub> kg<sup>-1</sup><sub>ODM</sub> at the level of biomethane concentration in the range of 49.92–59.11%.

The maximum level of biomethane production was achieved when using silage mass (inoculum subtracted) obtained by silage fermentation from fresh biomass harvested in the flowering phase during the first sowing period with a level of biochemical methane potential (BMP<sub>Gomp</sub>) of 344.13 L<sub>N</sub> kg<sup>-1</sup><sub>ODM</sub>, specific methane yield (SMY) of 319.66 L<sub>N</sub> kg<sup>-1</sup><sub>ODM</sub> at a maximum specific methane production rate (R<sub>m</sub>) of 33.74 L<sub>N</sub> kg<sup>-1</sup><sub>ODM</sub> d<sup>-1</sup>.

**Key words:** oilseed radish, silage, anaerobic fermentation, biogas, specific methane yield.

### **INTRODUCTION**

In 2009, the EU began to develop policies to ensure a ‘sustainable energy union and long-term climate change policy’. As part of this project, the Renewable Energy Directive (RED) was created, which initially set a binding target of 20% of final energy consumption from renewable sources by 2020 (Kaletnik et al., 2020a, 2020b; Tokarchuk et al., 2020). In Europe, as of 2022, 22% of the total primary energy supply comes from renewable sources. Of this share, more than half - 58% is biomass energy. In total, at the beginning of 2022, the European Union had 880 biomethane plants concentrated in 20 countries based on the use of biomass from various crops. The European Union plans

to produce 35 billion cubic meters of biomethane by 2030 to replace 10% of conventional gas consumption. And by 2050, it wants to increase this share to 60 percent. The total potential for biomethane production in the world is 1.15 trillion cubic meters per year (Maurus et al., 2021; EBA Statistical Report, 2023; Honcharuk et al., 2023a, 2023b). According to various estimates, 43–60% of this production potential is still generated from crop biomass (EBA Statistical Report, 2023).

At the same time, it is understood that achieving the noted potential and biogas production in terms of the use of biomass from specific crops requires a constant expansion of their range with a focus on the most adapted species with low resource capacity of their cultivation technologies for each country (Herrmann et al., 2016, 2016a; Komasilovs et al., 2021). Such monitoring is carried out on an ongoing basis, both from the point of view of in-depth study of traditional crops for biogas production in the total number of 25–30 species to the study of new crops, including wild species and even weeds (Zhang, 2021). Among these studied species, a group of cruciferous crops (spring and winter rape, white mustard, biomass waste from cabbage vegetable crops, and turnip) have proven to be effective candidates for biogas (biomethane) production both in the world and in Ukraine (Morozova et al., 2020; Jacob et al., 2022; Słomka & Oliveira, 2021; Gioulounta et al., 2023). At the same time, it is noted that a number of common crop species are not fully involved in the study of the efficiency and productivity of biomethane fermentation processes and the assessment of their possible and effective use in the system of alternative biofuel production (Molinuevo-Salces et al., 2013, 2013a; Zhang, 2021).

From this point of view, the classical oilseed radish (*Raphanus sativus* L. var. *oleiformis* Pers. (synonymously known as *Raphanus sativus* var. *oleifera* Metzg.)), a typical representative of the Cruciferous family with a wide range of adaptive properties and valuable biological traits, which is adapted to cultivation at different cultivation periods from early spring to late autumn with the possibility of full-species intermediate cultivation in crop rotations of different rotation (Tsytsiura, 2019, 2020a, 2021a, 2021b, 2022a) is an attractive candidate for studying in the processes of biomethane production from its biomass. It is also noted that oilseed radish, from the point of view of forming a significant mass in a short period against the background of a wide range of adaptive properties and taking into account the appropriate levels of C/N ratio, can be considered as a potential candidate for the implementation of the goals of the green course and alternative bioenergy, since it combines the valuable properties of phytoremediation and phytomeliorant and the energy intensity of its biomass determined by the biomethanization process (Herrmann et al., 2014, 2016, 2016a; Hu et al., 2018; Qiao et al., 2018). At the same time, it should also be noted its importance as a green fertilizer with intensive development of the root system, providing intensive soil drainage and replenishing the soil profile with a significant amount of organic matter against the background of the proven effect of anti-nematode activity and soil biofumigation (Rinnofner et al., 2008; Thiessen & EntzMartin, 2011; Jabnoun-Khiareddine et al., 2016; Hemayati et al., 2017; Jaskulska et al., 2017; Technical Note, 2020; Hansen et al., 2022). In addition, its positive effect on agrophysical properties and soil regimes, reduction of the level of total weeds under systematic cultivation as a predecessor and intermediate crop in crop rotations of different rotation intensities has been established (Lehrschb & Gallian, 2010; Chapagain et al., 2020; Tsytsiura, 2020b; Hudek et al., 2022). Its importance has also been proven from the point of view of using seed oil as a component

of mixed biofuels (Ávila & Sodr , 2012; Chammoun et al., 2013; Faria et al., 2018; Tsytsiura, 2022b).

The noted agro-technological properties of oilseed radish can be attributed to a potential candidate for biomethane production, given that classical crops for biogas processing according to the basic principles of methane co-fermentation should be characterized by multifunctionality with the possibility of intermediate cultivation in crop rotation (MolinuevoSalcesi et al., 2014). In this regard, it is important to study such indicators as the net energy efficiency per hectare and biomass efficiency per hectare ( $\text{m}^3 \text{CH}_4 \text{ha}^{-1}$ ) as important parameters for the application of intercrops as substrates in methane fermentation (Sepp l , 2013; Launay et al., 2022). Plant biomass, due to the high content of lignocellulosic substances, comprising mainly cellulose, hemicellulose, and lignin, requires pretreatment in order to improve susceptibility to biodegradation (Gioulounta et al., 2023). On the other hand, in recent years, interest in this crop has tended to grow, but its use as a component of biomethane production has focused on studying the efficiency of the cake after processing its seeds into oil for biomethane fermentation (Hu et al., 2018; Wendel et al., 2020; Junges et al., 2020). This significantly narrows its assessment in the areas of multi-purpose application directly in mobile biogas plants and stationary biofuel production directly within rural areas to ensure their bioenergy autonomy.

Taking into account the above-mentioned and the poorly studied biogas productivity of crude biomass of oil radish, the aim of the work was to investigate the biogas and methanogenic potential of oilseed radish (*Raphanus sativus* L. var. *oleiformis* Pers.) and to estimate the biogas efficiency of its biomass.

## MATERIALS AND METHODS

**Agrotechnological conditions of oilseed radish biomass syruing.** This research was carried out in 2020–2022 at Vinnytsia National Agrarian University (49°11' N, 28°22' E), during the potentially possible oilseed radish growing season of April–September (178 days). Height above sea level: 325 m. The area has a temperate continental climate. During the study period, the maximum and minimum average daily temperatures were 18.3 °C in July and 15.8 °C in May, respectively. Mean annual relative humidity was 77% and mean annual precipitation was 480–596 mm.

The soil cover of the research field was represented by gray forest soils (Luvic Greyic Phaeozem soils) (IUSS Working Group, 2015) of medium loamy texture (according to the State Standard of Ukraine (SSU) ISO 11277:2005). According to the results of the agrochemical survey of the soil and the determination of basic indicators in accordance with national standards, it had the following agrochemical parameters (for the period of crop rotation) humus 2.02–3.20% (according to SSU 4289:2004), mobile forms of nitrogen 67–92  $\text{mg kg}^{-1}$  (according to SSU ISO 11261:2001), phosphorus 149–220  $\text{mg kg}^{-1}$  (according to SSU 4115-2002) and potassium 92–126  $\text{mg kg}^{-1}$  (according to SSU 4115-2002) with metabolic acidity of the soil solution ( $\text{pH}_{\text{KCl}}$ ) 5.5–6.0 (according to SSU ISO 10390:2001).

The research was conducted using the variety 'Zhuravka', which is widespread in the region, using the method of pre-sowing design of its agrocenosis, which is used for the least costly variant of growing the crop in the research area with a sowing rate of

2.0 million seeds ha<sup>-1</sup> using the conventional row method on an unfertilized background. The general scheme of the experiment is presented in Table 1.

**Table 1.** Factors of the experiment (at four times replication (N = 48))

Factor in the timing of leaf mass harvesting	Factor of sowing time	Interaction of the factors
A <sub>1</sub> (budding stage, BBCH 50-53)	B <sub>1</sub> (I) (second decade of April - early spring)	A <sub>1</sub> B <sub>1</sub> , A <sub>1</sub> B <sub>2</sub> , A <sub>1</sub> B <sub>3</sub> ,
A <sub>2</sub> (flowering stage, BBCH 64-67)	B <sub>2</sub> (II) (first decade of May - spring)	A <sub>1</sub> B <sub>4</sub> A <sub>2</sub> B <sub>1</sub> , A <sub>2</sub> B <sub>2</sub> ,
A <sub>3</sub> (green pod stage, BBCH 73-75)	B <sub>3</sub> (III) (third decade of May - late spring)	A <sub>2</sub> B <sub>3</sub> , A <sub>2</sub> B <sub>4</sub> , A <sub>3</sub> B <sub>1</sub> ,
	B <sub>4</sub> (IV) second decade of June - summer)	A <sub>3</sub> B <sub>2</sub> , A <sub>3</sub> B <sub>3</sub> , A <sub>3</sub> B <sub>4</sub>

The hydrothermal regime of the period preceding sowing and the period of active vegetation was estimated by the hydrothermal coefficient (HTC, Eq. (1)):

$$HTC = \frac{\sum R}{0.1 \times \sum t_{>10}}, \quad (1)$$

where is the sum of precipitation ( $\sum R$ ) in mm for the period with temperatures above 10 °C, the sum of effective temperatures ( $\sum t > 10$ ) for the same period, reduced by a factor of 10. Ranking of HTC values (Evarde-Bundere & Evarde-Bunders, 2012): HTC > 1.6 - excessive humidity, HTC 1.3–1.6 - humid conditions, HTC 1.0–1.3 - moderately dry conditions, HTC 0.7–1.0 - dry conditions, HTC 0.4–0.7 - very dry conditions.

The analysis of weather conditions and the level of their variability for the period 2013–2022 was carried out on the basis of the coefficient of significance of deviations ( $C_{sd}$ ) of the elements of the agrometeorological regime of each of the studied years from the average long-term one in accordance with Eq. 2:

$$C_{sd} = \frac{(X_i - X_{av})}{S}, \quad (2)$$

where  $C_{sd}$  – coefficient of significance of deviations; current weather element;  $X_{av}$  – indicator of the long-term average (at least ten-year period);  $S$  – standard deviation;  $i$  – year number.  $C_{sd}$  level:  $0 \div 1$  – conditions are close to normal;  $1 \div 2$  – conditions differ significantly from the long-term average;  $> 2$  – conditions are close to extreme.

**Table 2.** Estimation of HTC values during the active vegetation of oilseed radish, 2020–2022

Year of study	Months of the growing season								On average for the period V–IX
	V		VI		VII		VIII		
	$X_i$	$C_{sd}$	$X_i$	$C_{sd}$	$X_i$	$C_{sd}$	$X_i$	$C_{sd}$	
2020	5.489	4.637	1.474	-5.683	0.649	-1.344	0.474	-0.468	-0.715
2021	0.530	-0.322	1.077	-6.080	1.589	-0.404	1.513	0.571	-1.559
2022	1.388	0.536	1.483	-5.674	0.854	-1.139	1.77	0.828	-1.362
$X_{av}$ 2013–2022	1.905		1.385		0.868		0.725		–
$S_{2020–2022}$	2.236		0.193		0.435		0.770		–

According to the significance of deviations of the mean monthly value of the HTC from the average long-term data, the years of the study period by the value of  $C_{sd}$  (Table 2, Fig. 1) are classified as 2021 - dynamically variable with extremes of excessive moisture and extra-dry conditions, 2020 and 2022 - conditions close to typical for the long-term hydrothermal regime of the study area. The years of research in the order of

increasing stress on the growth processes of oilseed radish plants from the standpoint of the dynamics of the amplitude of changes in average daily temperatures are as follows: 2022–2021–2020. The timing of leaf mass selection was set in accordance with the international scale of plant development BBCH (Growth stages of mono- and dicotyledonous plants) (Test Guidelines..., 2017).

In each phase of accounting, plants were cut at a height of 5 cm in the morning to minimize direct exposure to the sun on the tissues of cut plants and transported immediately to the laboratory for further analysis. Plant samples were collected from 1 m<sup>2</sup> plots at 4 randomly selected locations.

The characteristics of the plant mass were determined using a laboratory scale YP50002 (5 kg) with a discretion of 0.01 g. The resulting dried samples were re-weighed and ground using a Vilitek VLM-16 800 g 2,200 mL laboratory mill.

**Preparation of silage prototypes.** Crop materials were chopped to a particle length of < 20–30 mm and preserved by ensiling. The laboratory silage process was carried out in 1.5-liter glass silos (J. WECK GmbH u. Co. KG, Wehr, Germany) immediately after grinding with pressing and full filling of the containers and subsequent hermetic sealing according to the methodology described in detail by Seale & Knap (2011), taking into account the methodology used in the studies of Herrmann et al. The closure system provided for the free release of gases generated during anaerobic fermentation. The temperature regime during 90 days of silage was constant at 25 °C ± 0.5. The silage was a single component without preservatives in quadruplicate for each experimental variant.

**Chemical analysis of leaf biomass.** All laboratory chemical analyses applied to the obtained crushed samples of oilseed radish (moisture, ash, fat, protein and fibre content according to AOAC (2005) expressed on an absolutely dry weight basis) were carried out in quadruplicate in a specialized certified and accredited laboratory of Vinnytsia branch of the state institution 'Institute for Soil Protection of Ukraine' which is certified according to ISO 22000 international certification.

Crop materials of oilseed rape obtained from experimental plots of different sowing dates were wilted to a target DM content of 30–35% before the laboratory silage procedure (according to the recommendations of Herrmann et al. (2016)) before chopping and ensiling.

**Chemical analysis of the silage mass.** The obtained silage samples of different variants were subjected to low-temperature storage in a CRO/400/40 refrigerator (equipped with a temperature field control system) at 18 °C ± 0.5 after their removal from glass experimental silos according to the recommendation of Herrmann et al. (2016) before further processing for chemical composition analysis and biomethane production. The dry matter (DM) and organic dry matter (ODM) contents were measured by drying in an oven at 105 °C and then ashing the dried sample at 550 °C according to standard methods (AOAC, 2005). The dry matter content was adjusted to account for the loss of organic acids and alcohols during oven drying according to the recommendations of Herrmann et al. (2016).

The silage pH was determined using a high-precision combined pH electrode for continuous pH monitoring Sen Tix 41 (WTW, Weilheim, Germany, 2018 modification). For the pH analyses 5 g of the ensiled samples were previously diluted with 100 mL of distilled water and homogenized by blending for 15 min. Additional chemical components (lactic acid, volatile fatty acids, alcohols) were analyzed in cold silage extracts in the certified and accredited laboratory of the quality of crop products of Vinnitsa Oil Seeds



Crushing Factory (private joint stock company) taking into account the recommendations of Apelt (2016). In particular, lactic acid was analyzed using a liquid chromatograph HPLC-1100 BIOEVOPEAK, taking into account the methodological recommendations of Xiao-song (2012) and Herrmann et al. (2016).

The analysis of volatile fatty acids (acetic, propionic, n-butyric, iso-butyric, n-valeric, iso-valeric, and n-caproic) and alcohols (ethanol, propanol, 1,2-propanediol, 2,3-butanediol) was performed using a Shimadzu GC-2014 gas chromatograph (modified with a capillary column and flame ionization detector) (Shimadzu Corporation, Japan). Crude fat and crude fiber were determined according to the guidelines of Herrmann et al. (2014, 2016, 2016a) using standard laboratory methods (SSU (State standard of Ukraine) 8844:2019, SSU 8128:2015, SSU ISO 16472:2013, SSU ISO 7982:2015).

Determination of nitrogen content and calculation of crude protein content by the Kjeldahl method in dry soil biomass was performed using the KjeLROC Kd-310 analyzer (ISO 17025) (in accordance with the State standard of Ukraine ISO 5983:2003, 2003 and SSU (State standard of Ukraine) 8108:2015). The content of total organic carbon (TOC) in the dry weight of plants after mechanical grinding was determined using a laboratory analyzer of total organic carbon of the TOC-LCPH series according to the standard protocol for low-temperature thermocatalytic oxidation of plant material.

Crude protein was calculated as the elemental nitrogen content multiplied by the standard conversion factor of 6.25, which is accepted with certain reservations for fresh and silage biomass of a whole group of crops of different species and families (Sáez-Plaza et al., 2013). The C/N ratio in silages was calculated as the ratio of Total Organic Carbon (TOC) to Total Kjeldahl Nitrogen (TKN) (according to Słomka & Oliveira (2021)) to assess the intensification of anaerobic digestion. Nitrogen-free extracts (NfE) were calculated as the difference between the content of 100% dry matter and the corresponding content of crude protein, crude fiber, crude fat and crude ash.

**Batch anaerobic digestion test.** The assessment of the potential methane yield from laboratory silage samples with the determination of general parameters of its quality was investigated during anaerobic fermentation according to the general methodology proposed by Herrmann et al. (2016a) and Tang et al. (2020). For anaerobic fermentation, we used 1.0-liter glass vessels filled (filling level no more than 0.75 of the total volume of the vessel) with inoculum and an amount of plant material that the ratio of  $ODM_{\text{substrate}}$  to  $ODM_{\text{inoculum}}$  was 0.5, taking into account the recommendations of Field et al. (1988) and Satyanarayana et al. (2008). Further actions to prepare the substrate for anaerobic digestion were carried out in accordance with the guidelines of Carvalho et al. (2011), which provided for bringing the final volume of the vessel to 700 mL with distilled water and normalizing the pH of the inoculum medium and silage substrate in the resulting mixture (based on the studies of Jayaraj et al. (2014) and Putra et al. (2023)) to 7.0–7.2 by corrective addition (if necessary, according to the preliminary control determination) of a 10M NaOH solution. As a control, a reactor containing only inoculum was used in order to discount that volume from the final volume of experimental variants. Anaerobic conditions in the laboratory reactors were created by flushing with nitrogen for 2/3 minutes immediately before closing the reactor.

Digestive enzyme obtained as a result of fermentation of pig manure for 14 days and obtained from the commercial enterprise 'Organic-D', which has a well-established biogas fermentation system of the European level of certification, was used as an

inoculant. The resulting substrate called ‘Efluent’ is certified in 2018 (Technical conditions of Ukraine 20.1-38731462-001:2018) and patented in Ukraine (Kaletnik et al., 2022b; Lohosha et al., 2023). Based on the analysis in the Prime Lab Tech laboratory (according to the ISO 22000 international certification), this digestate had the following average chemical characteristics: pH  $8.2 \pm 0.3$ ; DM  $2.5 \pm 0.7\%$ ; TDS  $62.7 \pm 3.3\%$  of DM; N  $2.9 \pm 1.2 \text{ g kg}^{-1}$ ;  $\text{NH}_4\text{-N}$   $2.3 \pm 0.7 \text{ g kg}^{-1}$ ; organic acids  $1.7 \pm 0.5 \text{ g kg}^{-1}$ ; Total organic carbon (TOC)  $32.8 \pm 2.7\%_{\text{DM}}$ ; Total Nitrogen (TN)  $1.64 \pm 0.39\%_{\text{DM}}$ ; C/N ratio  $20 \pm 2.5$ ). The inoculant was selected taking into account the level of C/N ratio in the variants of silage-fermented mass of oil radish (average C/N = 19.64-20.91). In order to avoid influencing the resulting indicators of biomethane productivity, given the significantly lower level of biomethane productivity due to the inoculum passing the anaerobic fermentation stage before the start of this experiment, the level of this ratio should be at the level of the value of this indicator for the added substrate, which corresponds to the basic methodological recommendations (according to Richter (2010) and Hidalgo-Sánchez et al. (2023)). The vessels were placed in a water bath with a constant temperature of  $35 \text{ }^\circ\text{C} \pm 0.5$  with prolonged incubation for 60 days. Active shaking of the vessels was performed once a day at the same time. The biogas generated during the incubation period was collected in wet gas meters and recorded using a standard procedure for displacement of acidified saturated barrier solution with NaCl (VDI, 2006). The biogas volume was determined daily, corrected for the volume of biogas produced by the inoculum without substrate and normalized to standard conditions (dry gas,  $0 \text{ }^\circ\text{C}$ , 1,013 hPa). For this correction, the inoculum without substrate was subjected to the same anaerobic digestion procedure and used in the analysis of biogas production kinetics as a correction basis (according to Herrmann et al., 2016). Biogas composition for methane content was measured using a portable gas analyzer equipped with infrared sensors (HONEYWELL BW Flex, USA). The specific methane yield was calculated as the sum of methane produced during the test period per ODM of the silage sample in the corresponding experimental variant.

**Evaluation of process kinetics.** Kinetic analysis to determine the rate of biodegradation of silage samples during anaerobic digestion. For this purpose, standardized approaches were applied using the first-order differential equation (Eq. (3)) and the modified Gompertz equation (Eq. (4)) with fitting to the cumulative methane production curves (Lay et al., 1997; Mittweg et al., 2012) obtained during anaerobic digestion tests using the CurveExpert Professional v. 2.7.3 software package (Hyams Development) and Matlab R2016a software (TheMathWorks Inc., Natick, MA, USA).

$$y(t) = y_m (1 - e^{-k_1 t}) \quad (3)$$

where,  $y(t)$  is the cumulative specific methane yield at time  $t$  ( $\text{L}_\text{N} \text{ kg}^{-1}_{\text{ODM}}$ ),  $y_m$  is the maximum specific methane yield at theoretically infinite digestion time ( $\text{L}_\text{N} \text{ kg}^{-1}_{\text{ODM}}$ ),  $t$  is the time (days) and  $k$  is the first order decay constant ( $\text{day}^{-1}$ ).

Specific methane production rate ( $R_m$ ) was determined by Eq. 4:

$$y(t) = y_m \exp \left[ -\exp \left( \frac{R_m e}{y_m} (\lambda - t) + 1 \right) \right] \quad (4)$$

(after the transformation by logarithmization:  $R_m = \frac{y(t) \left[ \ln \left( \ln \frac{y(t)}{y_m} \right) - 1 \right]}{e[\lambda - t]}$ )

where,  $y(t)$  is the cumulative specific methane yield at time  $t$  ( $\text{L}_\text{N} \text{ kg}^{-1}_{\text{ODM}}$ ),  $y_m$  is the maximum specific methane yield at theoretically infinite digestion time ( $\text{L}_\text{N} \text{ kg}^{-1}_{\text{ODM}}$ ),

$R_m$  is the maximum specific methane production rate ( $L_N \text{ kg}^{-1}_{\text{ODM}} \text{ d} (\text{day})^{-1}$ ),  $t$  is the time (days) and  $\lambda$  is the lag phase (days),  $e$  = Euler's number.

The lag period ( $\lambda$ ) was defined as the period from the beginning of the anaerobic digestion test to the onset of methane emission, recorded in hours and subsequently converted to days with a conversion to a day length of 24 hours with the onset of gas emission recorded (based on the recommendations of Kim & Kim (2020)) using the MQ-4 methane gas sensor with the Arduino AVR Pic board.

The half-life ( $t_{50}$ ) was determined based on the interpretation of the modified Gompertz equations as the time when 50% of the maximum specific methane yield is reached (days). The degree of integrated connection of the biochemical composition of oilseed radish silage biomass with the main indicators of the kinetics of the productive process of biomethane production was estimated by the value of the coefficient of determination of the connection (Equation 5) and the use of the method of weighting the correlation graph in two interpretations according to Equations 6 and 7:

$$d_{yx} = r_{ij}^2 \times 100 \quad (5)$$

$$G = \sum_{|r_{ij}| \geq \alpha} |r_{ij}| \quad (6)$$

where  $r_{ij}$  is the correlation coefficient between the  $i$ -th and  $j$ -th indicator. Only reliable correlation coefficients were used in the calculation.

$$G' = \left( \sum_{|r_{ij}| \geq \alpha} |r_{ij}| \right) / n \quad (7)$$

$n$  is the number of statistically significant correlation coefficients.

**Statistical analysis of research results.** It was conducted using Past 4.13 software (Øyvind Hammer, Norway) with the calculation of Pearson's correlation coefficients and multiple regression analysis according to the standard procedure (Wong, 2018). To compare the regression models, we used the coefficient of determination ( $R^2$ ), adjusted coefficient of determination ( $R^2_{\text{adj}}$ ), root mean square error (RMSE), and relative root mean square error (RRMSE).

The data obtained were analysed using analysis of variance (ANOVA) with determination of the share of influence of factors in the dispersion scheme. Tukey's HSD test in R (version R statistic i386 3.5.3) with multiple comparisons of the parameter means at the 99.9%, 99% and 95% family-wise confidence levels were used. In evaluating the obtained array of multiple values, standard indicators were used for analysing variable data (multi-year and genotypic components:  $\bar{x}$  – average, SD – standard deviation,  $C_v$  – coefficient of variation).

## RESULTS AND DISCUSSION

According to Herout et al. (2011) and Ahlberg & Nilsson (2015) and Mathur & Singh (2023) the assessment of the potential of a particular crop for biogas production should necessarily include an assessment of both its bioproductivity in terms of the formation of an appropriate level of biomass yield and a general assessment according to the generally accepted chemical analysis structure. Taking into account these indicators allows us to draw a conclusion about the bioenergy potential of the species and the feasibility of its cultivation for biogas production. Evaluation of different sowing

dates of oilseed radish, which are traditionally practiced in relation to this crop in the research area, proved a relatively high level of its bioproductivity both in early spring and summer with significant differences between the variants (Table 3).

**Table 3.** Oilseed radish biomass yield depending on the sowing date (average for 2020–2022), t ha<sup>-1</sup> ( $\bar{x} \pm SD$ )

Sowing date	Phenological stages					
	budding stage (BBCH 50–53)		flowering stage (BBCH 64–67)		green pod stage (BBCH 73–75)	
	1	2	1	2	1	1
I	9.18 ± 1.15	0.93 ± 0.10	17.29 ± 2.62	2.18 ± 0.33	24.77 ± 3.18	3.67 ± 0.45
II	8.11 ± 1.42	0.80 ± 0.14	15.17 ± 2.08	1.85 ± 0.22	21.60 ± 2.78	3.37 ± 0.38
III	6.59 ± 1.40	0.71 ± 0.16	11.70 ± 2.73	1.62 ± 0.32	12.47 ± 3.50	2.20 ± 0.53
IV	5.87 ± 1.59	0.54 ± 0.15	8.55 ± 3.11	1.28 ± 0.34	10.57 ± 2.90	1.71 ± 0.40
Tukey's test ( $R_{\min}$ for $p_{\text{adj}} < 0.05$ )	0.23–0.57	0.07–0.11	0.85–1.57	0.12–0.21	1.09–1.78	0.18–0.27

1 – in raw weight, t ha<sup>-1</sup>; 2 – in dry matter (DM), t ha<sup>-1</sup>.

Taking into account the findings on the comparable productivity of cruciferous crops for multiple uses (Maier et al., 2017; Sánchez et al., 2023), oilseed radish can be attributed to crops with high productive potential in terms of agrobiomass yield both in total weight and dry matter. There is also a steady tendency to decrease the level of biomass yield with a shift in the sowing dates of the crop.

Thus, the overall decrease in biomass yield with a sequential shift in sowing dates compared to the most productive first term was 12.41%, 39.97%, 51.23%, respectively, for the second to fourth applied terms. In terms of biomass yield in terms of dry matter, similar indicators amounted to 11.21%, 33.19% and 47.94%, respectively.

The chemical analysis of oilseed radish leaf mass also had significant differences with changes in the timing of sowing of oilseed radish (Table 4). The analysis of the presented indicators made it possible to estimate the rate of the so-called physiological aging of oilseed radish plants and the consideration of this factor in the suitability of its biomass for biogas production both in terms of changes in phenological phase s and changes in sowing dates. Comparing the phenological phase s of budding and green pod for the average sowing time, it was found that there was a steady tendency to decrease the content of crude protein by 37.52% and crude ash by 32.09%, increase the content of crude fat by 6.20%, crude fiber by 49.33%, with a relatively stable NfE (increase by 1.89%). At the same time, due to an increase in the content of total organic carbon by 7.96% against the background of an adequate decrease in the content of crude protein, the C/N ratio increased by 1.72 times. Certain regularities were also noted for the shift in sowing dates in the fourth-first comparison within the accounting phenological phases. For the budding phase, there was an increase in crude protein (and Total Kjeldahl Nitrogen (TKN)) by 22.56%, organic carbon by 0.34%, and a decrease in the C/N ratio by 13.97%. For other side, the content of crude fat, crude fiber and crude ash increased by 37.60%, 24.68% and 34.22%, respectively, with a decrease in NfE by 23.48%. For the flowering phase, in the same variant of comparing the first and fourth terms, an increase in TKN by 6.76%, organic carbon by 1.64% with a decrease in the C/N ratio by 3.79% was noted.

**Table 4.** Chemical composition of oilseed radish leaf mass by stages of growth and development depending on the sowing date and phenological phase of plants (average for 2020–2022)

Sowing date	Crude protein (CP) (% <sub>DM</sub> )			Crude fat (CF) (% <sub>DM</sub> )			Crude fibre (CFb) (% <sub>DM</sub> )			Crude ash (CA) (% <sub>DM</sub> )			NfE (% <sub>DM</sub> )		
	$\bar{x}$	SD*	C <sub>v</sub> **	$\bar{x}$	SD	C <sub>v</sub>	$\bar{x}$	SD	C <sub>v</sub>	$\bar{x}$	SD	C <sub>v</sub>	$\bar{x}$	SD	C <sub>v</sub>
	Budding stage (BBCH 50–53)														
I	13.12	2.40	18.29	2.58	0.53	20.54	17.22	3.83	22.24	13.12	1.12	8.54	53.96	4.76	8.82
II	14.13 <sup>c</sup>	2.17	15.36	2.22 <sup>c</sup>	0.46	20.73	25.14 <sup>a</sup>	5.11	20.33	13.24 <sup>d</sup>	1.89	14.27	45.40 <sup>b</sup>	4.69	10.33
III	19.27 <sup>a</sup>	2.61	13.54	2.14 <sup>c</sup>	0.52	24.30	21.52 <sup>b</sup>	4.72	21.93	18.07 <sup>a</sup>	3.90	21.58	39.00 <sup>a</sup>	5.44	13.95
IV	16.08 <sup>b</sup>	0.94	5.85	3.55 <sup>b</sup>	0.77	21.69	21.47 <sup>b</sup>	2.58	12.02	17.61 <sup>a</sup>	4.41	25.04	41.29 <sup>a</sup>	6.12	14.82
	Flowering stage (BBCH 64–67)														
I	13.89	1.11	7.99	3.04	0.27	8.88	21.77	4.62	21.22	12.58	2.55	20.27	48.72	4.56	9.36
II	13.63 <sup>d</sup>	0.62	4.55	2.73 <sup>c</sup>	0.32	11.72	26.91 <sup>a</sup>	5.89	21.89	12.53 <sup>d</sup>	1.42	11.33	44.20 <sup>c</sup>	4.71	10.66
III	17.48 <sup>a</sup>	1.65	9.44	2.75 <sup>c</sup>	0.52	18.91	23.12 <sup>c</sup>	5.34	23.10	14.17 <sup>b</sup>	2.83	19.97	42.48 <sup>b</sup>	7.38	17.37
IV	14.23 <sup>c</sup>	1.03	7.24	2.52 <sup>c</sup>	0.71	28.17	24.78 <sup>c</sup>	1.72	6.94	14.54 <sup>b</sup>	3.08	21.18	43.93 <sup>c</sup>	3.25	7.40
	Green pod stage (BBCH 73–75)														
I	8.08	1.12	13.86	3.14	0.98	31.21	32.31	6.73	20.83	8.39	0.68	8.10	48.08	8.18	17.01
II	8.92 <sup>c</sup>	1.66	18.61	3.61 <sup>c</sup>	0.78	21.61	32.34 <sup>d</sup>	7.13	22.05	9.04 <sup>c</sup>	1.99	22.01	46.09 <sup>c</sup>	2.35	5.10
III	11.44 <sup>b</sup>	2.85	24.91	2.04 <sup>b</sup>	0.20	9.80	31.88 <sup>c</sup>	5.27	16.53	11.27 <sup>a</sup>	1.32	11.71	43.37 <sup>b</sup>	3.29	7.59
IV	10.67 <sup>b</sup>	4.35	40.77	2.35 <sup>b</sup>	0.65	27.66	30.92 <sup>b</sup>	5.34	17.27	10.55 <sup>b</sup>	2.46	23.32	45.51 <sup>b</sup>	5.59	12.28
Sowing date	Total Kjeldahl Nitrogen (TKN) (% <sub>DM</sub> )						Total organic carbon (TOC) (% <sub>DM</sub> )			Ratio C/N					
	$\bar{x}$	SD*	R <sub>v</sub> ***			$\bar{x}$	SD	R <sub>v</sub>	$\bar{x}$	R <sub>v</sub>					
	Budding stage (BBCH 50–53)														
I	2.10	0.38	1.72–2.48			37.47	2.08	35.39–39.55			17.84	15.95–20.60			
II	2.26 <sup>c</sup>	0.35	1.91–2.61			38.12 <sup>b</sup>	2.29	35.83–40.41			16.87 <sup>b</sup>	15.48–20.33			
III	3.08 <sup>a</sup>	0.42	2.67–3.50			37.63 <sup>c</sup>	2.82	34.81–40.45			12.22 <sup>a</sup>	11.56–13.04			
IV	2.57 <sup>b</sup>	0.15	2.42–2.72			37.17 <sup>b</sup>	3.09	34.08–40.26			14.46 <sup>a</sup>	14.08–14.80			
	Flowering stage (BBCH 64–67)														
I	2.22	0.18	2.04–2.40			38.67	3.57	35.10–42.24			17.42	17.21–17.60			
II	2.18 <sup>d</sup>	0.10	2.08–2.28			40.04 <sup>a</sup>	3.89	36.15–43.93			18.37 <sup>b</sup>	17.38–19.27			
III	2.80 <sup>a</sup>	0.26	2.53–3.06			39.14 <sup>b</sup>	4.28	34.86–43.42			13.98 <sup>a</sup>	13.78–14.19			
IV	2.28 <sup>c</sup>	0.16	2.11–2.44			39.37 <sup>b</sup>	4.63	34.74–44.00			17.27 <sup>c</sup>	16.46–18.03			
	Green pod stage (BBCH 73–75)														
I	1.29	0.18	1.11–1.47			41.03	3.89	37.14–44.92			31.81	30.55–33.45			
II	1.43 <sup>c</sup>	0.27	1.16–1.69			40.92 <sup>c</sup>	4.17	36.75–45.09			28.62 <sup>a</sup>	26.69–31.68			
III	1.83 <sup>b</sup>	0.46	1.37–2.29			40.38 <sup>b</sup>	4.55	35.83–44.93			22.07 <sup>a</sup>	19.63–26.15			
IV	1.71 <sup>b</sup>	0.70	1.01–2.40			40.03 <sup>b</sup>	5.02	35.01–45.05			23.41 <sup>a</sup>	18.78–34.66			

\*SD – standard deviation; \*\*C<sub>v</sub> – coefficient of variation, R<sub>v</sub> – range of values. Significance levels in comparison to the first (I) sowing term: a – 0.1%; b – 1%; c – 5%; d – no significant difference.

The crude fat content decreased by 17.11%, while the crude fiber and crude ash content increased by 13.83% and 15.88%, respectively, with a decrease in NfE by 9.83%.

For the green pod phase, the elemental nitrogen and organic carbon content increased by 6.76% and 1.64%, respectively, with a decrease in the C/N ratio by 3.79%. Under the same conditions, crude fat, crude fiber, crude ash and NfE content decreased by 22.85%, 1.48%, 6.54% and 0.71%, respectively. According to the generalizations of

Heiermann et al. (2009), Al Seadi et al. (2013), Tauš et al. (2020) and the presented data, the leaf mass of oilseed radish should be attributed to plants of potential candidates for biogas production through anaerobic digestion, with the optimum phenological phase use corresponding to the flowering phase of oilseed radish. It is for this phase that the necessary balance between the accumulation of fiber, nitrogenous compounds and nitrogen-free extractives has been established against the background of a variable hydrothermal regime. Such conclusions are also confirmed in the studies of Carvalho et al. (2011) and Molinuevo-Salces et al. (2013).

In the study by Herrmann et al. (2016), the period of possible biogas application of oilseed radish leaf mass covers the interval of BBCH 51–69 (end of budding and beginning of flowering phases). According to Belle et al. (2015), this phenological period also covers the beginning of fruit formation of oilseed radish plants, provided that it is combined with various wastes and manure.

The possibility of optimizing the suitability of plant biomass for anaerobic fermentation with methane production by preliminary silage has been investigated (Kreuger et al., 2011; López-Aguilar et al., 2023), which is most relevant for crops with a low C/N ratio and low dry organic matter content (Opurum, 2021; Hülsemann et al., 2023). Similar conclusions were confirmed in our research by applying the preliminary process of anaerobic silage fermentation to the options for harvesting oilseed radish leaf mass (Table 5).

It should be noted that previous studies (Ammann et al., 2009; Villalobos & Brummer, 2013; Zhou et al., 2021; Sánchez et al., 2023) emphasizes the value of oilseed radish as a silage crop during the phenological period of budding–flowering, but most researchers noted that due to the high moisture content of the mass and low values of the sugar minimum, its silage is difficult in a single-species composition, so preference should be given to combined silage of oilseed radish with cereals, cereal-legume mixtures and straw. Silage of oilseed radish in its pure form did not contribute to the long-term (traditional period of 4-6 months) preservation of the preserved mass before use. Depressurization of the silage mass at the beginning of its feeding led to a rapid deterioration in the quality of silage and a significant deterioration in its combinability. The appearance of a sharp unpleasant odor and rapid oxidation with blackening of the leaf-stem chopped mass was also characteristic of pure oilseed radish silage. It is for these reasons that the silage mass of oilseed radish can be considered as one of the components of biogas production, as emphasized in the studies of Carvalho et al. (2011), Molinuevo-Salces et al. (2013a) and Herrmann et al. (2016). The analysis of the chemical composition of silage mass of different terms and phases confirmed the technological reservations regarding the silageability of oilseed radish. Based on the level of alcohols and a number of acids (i-butyric, butyric, i-valeric, valeric and caproic acid) in the silage-fermented mass, a gradual increase in chemical stability and quality of silage was noted in the green pod phase. Thus, the concentration of these acids, when comparing the average time between the flowering and green pod phases, decreases by 2.58 times in favor of the latter phenological phase. A similar decrease with an index of 2.03 was observed for the concentration of alcohols in the silage mass. At the same time, the total acidity of the mass decreases by 0.4–0.6 pH units due to an adequate decrease in the content of a number of acids (lactic, acetic and propionic).

**Table 5.** Dry matter, organic dry matter and chemical parameters of silage fermentation of oilseed radish depending on the sowing date and phenological stage of plants (average for 2020–2022)

Sowing date	Dry matter (DM), %		Organic dry matter (ODM) (% <sub>DM</sub> )		Crude protein (CP) (% <sub>DM</sub> )		Crude fat (CF) (% <sub>DM</sub> )		Crude fibre (CFb) (% <sub>DM</sub> )		Crude ash (CA) (% <sub>DM</sub> )		Ratio C/N	
	$\bar{x}$	SD*	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Budding stage (BBCH 50–53)														
I	11.51	1.17	73.62	0.21	11.92	0.23	2.89	0.15	18.27	0.21	14.63	0.14	18.42	0.18
II	10.84 <sup>b</sup>	1.56	73.84 <sup>d</sup>	0.33	12.87 <sup>b</sup>	0.25	2.42 <sup>c</sup>	0.17	26.27 <sup>a</sup>	0.15	14.55 <sup>d</sup>	0.18	17.96 <sup>c</sup>	0.22
III	11.92 <sup>c</sup>	1.41	74.41 <sup>b</sup>	0.27	17.54 <sup>a</sup>	0.65	2.51 <sup>c</sup>	0.25	22.81 <sup>a</sup>	0.17	19.52 <sup>a</sup>	0.23	12.63 <sup>a</sup>	0.36
IV	9.93 <sup>b</sup>	1.74	72.92 <sup>b</sup>	0.25	14.68 <sup>a</sup>	0.36	3.79 <sup>b</sup>	0.18	22.59 <sup>a</sup>	0.09	18.69 <sup>a</sup>	0.27	14.85 <sup>a</sup>	0.29
Flowering stage (BBCH 64–67)														
I	13.82	1.08	82.57	0.18	12.55	0.15	3.41	0.17	22.93	0.18	13.74	0.28	18.39	0.23
II	14.55 <sup>b</sup>	0.93	83.24 <sup>c</sup>	0.15	12.37 <sup>c</sup>	0.25	3.18 <sup>c</sup>	0.19	28.17 <sup>a</sup>	0.23	13.92 <sup>c</sup>	0.13	19.57 <sup>b</sup>	0.29
III	15.52 <sup>a</sup>	2.29	83.78 <sup>b</sup>	0.11	16.47 <sup>a</sup>	0.42	3.22 <sup>c</sup>	0.29	25.24 <sup>a</sup>	0.27	15.79 <sup>b</sup>	0.26	14.38 <sup>a</sup>	0.31
IV	12.74 <sup>b</sup>	1.57	81.77 <sup>b</sup>	0.22	13.11 <sup>b</sup>	0.64	2.97 <sup>b</sup>	0.45	26.51 <sup>a</sup>	0.10	15.84 <sup>b</sup>	0.30	18.24 <sup>a</sup>	0.38
Green pod stage (BBCH 73–75)														
I	16.85	2.14	88.27	0.12	7.04	0.69	3.61	0.42	33.27	0.22	10.17	0.12	34.37	0.38
II	17.73 <sup>b</sup>	1.89	86.55 <sup>b</sup>	0.15	7.49 <sup>c</sup>	0.41	3.97 <sup>c</sup>	0.26	34.11 <sup>c</sup>	0.27	10.88 <sup>c</sup>	0.30	32.64 <sup>b</sup>	0.41
III	16.17 <sup>c</sup>	1.74	85.17 <sup>a</sup>	0.17	10.22 <sup>a</sup>	0.25	2.58 <sup>b</sup>	0.12	32.91 <sup>c</sup>	0.17	12.51 <sup>a</sup>	0.14	24.19 <sup>a</sup>	0.27
IV	15.72 <sup>b</sup>	2.27	84.38 <sup>a</sup>	0.11	9.58 <sup>a</sup>	0.55	2.92 <sup>c</sup>	0.36	32.29 <sup>c</sup>	0.19	12.08 <sup>a</sup>	0.37	25.27 <sup>a</sup>	0.30
Sowing date	Sum of i-butyric, butyric, i-valeric, valeric and caproic acid (% <sub>DM</sub> )				Sum of ethanol, propanol, 1,2-butanediol, 2,3-propanediol (% <sub>DM</sub> )		pH		Lactic acid (% <sub>DM</sub> )		Sum of acetic and propionic acid (% <sub>DM</sub> )			
	$\bar{x}$	SD*	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD		
Budding stage (BBCH 50–53)														
I	0.73	0.05	0.88	0.03	4.4	0.1	10.31	0.12	3.60	0.09				
II	0.84 <sup>c</sup>	0.09	0.98 <sup>c</sup>	0.05	4.2 <sup>c</sup>	0.1	11.12 <sup>b</sup>	0.14	3.82 <sup>c</sup>	0.07				
III	0.62 <sup>c</sup>	0.09	0.67 <sup>b</sup>	0.08	4.1 <sup>c</sup>	0.0	10.09 <sup>c</sup>	0.18	2.74 <sup>b</sup>	0.10				
IV	0.96 <sup>b</sup>	0.11	1.12 <sup>a</sup>	0.07	4.2 <sup>c</sup>	0.3	11.74 <sup>b</sup>	0.11	3.55 <sup>d</sup>	0.08				
Flowering stage (BBCH 64–67)														
I	0.54	0.07	0.69	0.03	4.6	0.1	9.51	0.18	3.11	0.10				
II	0.47 <sup>d</sup>	0.05	0.58 <sup>c</sup>	0.05	4.6 <sup>d</sup>	0.0	8.07 <sup>a</sup>	0.14	2.71 <sup>b</sup>	0.12				
III	0.41 <sup>c</sup>	0.14	0.55 <sup>b</sup>	0.07	4.8 <sup>b</sup>	0.0	7.11 <sup>a</sup>	0.16	2.24 <sup>b</sup>	0.07				
IV	0.72 <sup>b</sup>	0.09	0.78 <sup>c</sup>	0.09	4.5 <sup>c</sup>	0.2	9.98 <sup>c</sup>	0.09	2.92 <sup>c</sup>	0.07				
Green pod stage (BBCH 73–75)														
I	0.29	0.09	0.47	0.05	4.8	0.1	6.92	0.10	2.34	0.05				
II	0.11 <sup>a</sup>	0.08	0.32 <sup>b</sup>	0.05	5.0 <sup>b</sup>	0.2	6.45 <sup>c</sup>	0.08	2.17 <sup>c</sup>	0.05				
III	0.38 <sup>c</sup>	0.05	0.45 <sup>d</sup>	0.07	4.8 <sup>d</sup>	0.2	7.09 <sup>c</sup>	0.07	2.07 <sup>c</sup>	0.08				
IV	0.44 <sup>b</sup>	0.12	0.56 <sup>c</sup>	0.11	4.7 <sup>c</sup>	0.1	7.55 <sup>c</sup>	0.12	2.39 <sup>d</sup>	0.03				

\* SD – standard deviation; Significance levels in comparison to the first (I) sowing date: a – 0.1%; b – 1%; c – 5%; d – no significant difference.

This dynamics is maintained with a similar character in the comparison of the first to the fourth sowing term). At the same time, the ratio of these acids (based on the studies of Rezende et al., 2015; Raboanatahiry et al., 2021; Chen et al., 2023; Wang et al., 2023) indicates a normal course of lactic acid fermentation and good preservation of leaf mass. Such features of biochemical changes are associated with the fact that in the fruiting

phase (from the beginning of intensive green pod formation), the green mass of oilseed radish undergoes significant biochemical transformations, which opens up opportunities for independent silage of the leaf-stem mass of oilseed radish, due to a decrease in the buffering capacity of cell sap due to a decrease in the content of protein substances (Table 5) in plants during the approach and course of the generative phases of growth and development.

Due to these biochemical transformations in the anaerobic process of silage fermentation, natural changes in the properties of all potential plant species occur (Herrmann et al. (2016): decrease in protein content against the background of an increase in crude fat, fiber and ash content. The well-known process of compaction of the chemical formula of the mass during anaerobic silage is also characteristic of cruciferous plant species (Borreani et al., 2018; Serhat et al., 2022). Due to the identified features, the stability of silage mass preservation of oilseed radish increased consistently from silage samples collected in the budding phase to the same samples collected in the green pod phase. The system of shifting the sowing dates from early spring (I) to summer (IV) changes this stability by creating conditions for active fermentation with the formation of a larger proportion of organic acids (an increase of 9.87% in the systems of the first to fourth sowing date) as a result of slow anaerobic decay processes. Such processes, according to studies (Satyanarayana et al., 2008; Kim & Kim, 2020; Tang et al., 2020; Oporum, 2021), indicate an increase in the share of associated gases in the total composition of biogas, in particular the share of hydrogen sulfide, nitrite derivatives, which ultimately leads to a predicted shortening of the lag period of the anaerobic fermentation process (López-Aguilar et al., 2023) and also leads to a general reduction in the duration of the period with biomethane release in the interval of the overall anaerobic fermentation process (Kalyuzhnyi et al., 2009; Kintl et al., 2022). That is, taking into account the chemical composition of oilseed radish silos of different studied variants, a more intensive biomethane production process with a greater variable component at the points of daily fixation should be expected with an increase in the proportion of fermentation associated gases for the mass harvested during the budding phase of later sowing dates. Gradually, with the physiological aging of oilseed radish plants, with the achievement of the flowering phase and the green pod phase, the process of biogas production will have a longer fixation interval, a more pronounced lag phase, with a decrease in the variability of the gas structure in the total volume of biogas productivity at the corresponding fixation dates. These features, taking into account the chemical composition of oilseed radish silage, are based on the conclusions of a number of studies (Mähnert et al., 2005; Abu-Dahrieh et al., 2011; Rodriguez et al., 2017; Darimani & Pant, 2020; Beausang et al., 2021; Jauhiainen, 2022; Szwarc et al., 2022).

Attention should also be paid to such a criterion as the C/N ratio, which, according to many researchers, determines the intensity of the biogas anaerobic fermentation process. The C/N ratios of cover crops generally vary from 9 to 40 (Zheng, 2009; Justes et al., 2012; Ma et al., 2018; Hansen et al. 2021; Yang et al., 2022). The optimal C/N ratio for anaerobic degradation depends on the substrate used, but a value between 20–30 is recommended (Kwietniewska & Tys, 2014; Wang et al., 2015). When a substrate has low C/N ratios, it is considered to contain relatively high ammonium concentrations, inhibiting microbial growth and anaerobic digestion (Cerón-Vivas et al. (2019); Choi et al., 2020). According to Choi et al. (2020), carbon to nitrogen (C/N ratio) has effects on methane production yield and it is a very important factor for stable

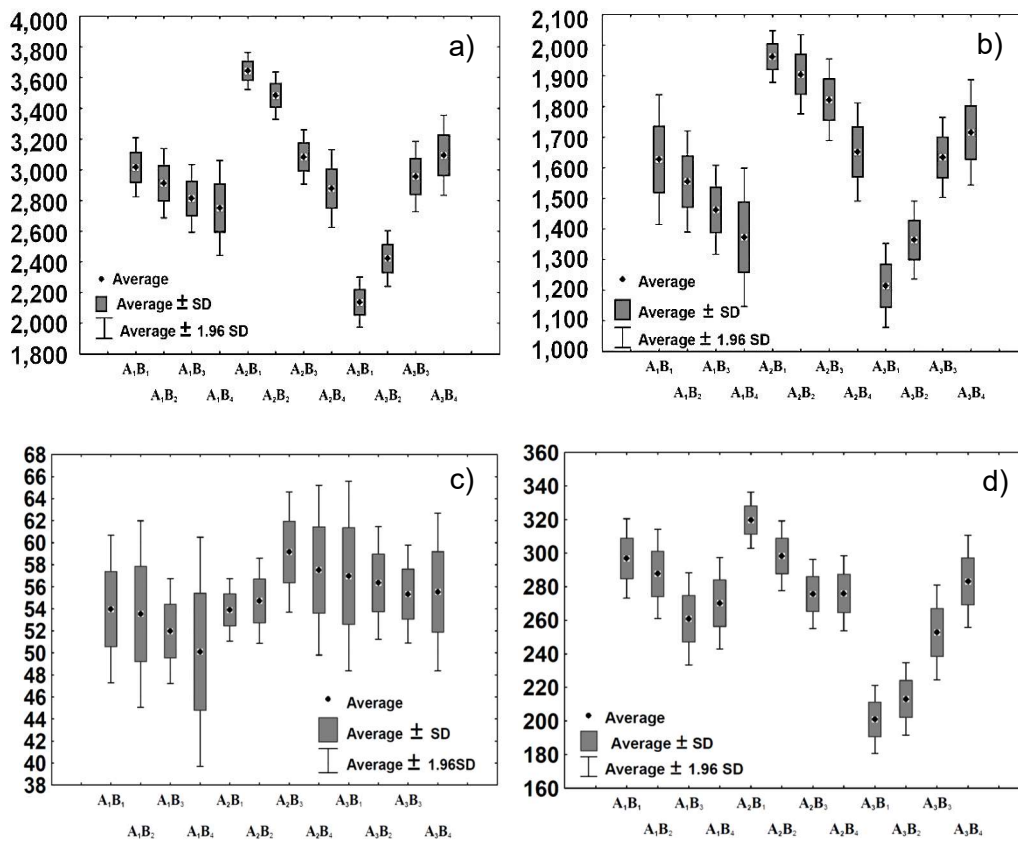


operation. When the C/N ratio is too high, biogas yield does not show the optimum due to acidogenic bacteria rapidly consuming nitrogen compared to methanogenic bacteria. When the C/N ratio is too low, most microbes rapidly consume nitrogen for growth. Although this has a positive effect on methane production rate. However, the lack of carbon type causes that decrease in acid formation, nitrogen accumulates in the form of ammonium ions (NH<sub>4</sub>) that increase the pH (Yen & Brune, 2007) which adversely affects biogas production. At the same time, despite the established optimal C/N interval (20–30), it is emphasized (Guarino et al., 2016) that this indicator has certain species-specific reservations based on the chemical characteristics of a particular agrobiomass and biogas production technology. Thus, according to the conclusions of the same Guarino et al. (2016), depending on the mode of biogas production, this interval had wider limits from 9 to 50, and in the studies of Dębowski et al. (2022) and Manyi-Loh & Lues (2023), the optimal value of this ratio had a complex complementary nature of interaction and biomethane productivity of the anaerobic digestion substrate in the range from 10 to 30. Similar conclusions regarding the specificity of the formation of the C/N ratio were made in these studies. Thus, for fresh leaf mass (Table 5), the C/N ratio was in the range of 12.22–31.81 and had a steady upward trend as the plants aged phenologically. Thus, averaged over the sowing dates for the budding phase, this indicator was 15.35. At the flowering and green pod stages, it was at the level of 16.76 and 26.48, respectively. In view of the established optimal interval of 20–30 with a possible species deviation of 10-30 and the available value of the standard deviation in the assessment of annual variation, oilseed radish should be attributed to crops with a suitable capacitive ratio of biochemical composition, which determines a certain potential for biogas yield. Given that the first and second sowing dates have a long-term average of above 30 for the green pod phase, the optimal predicted period of use of crude plant biomass without preliminary silage fermentation is in the range of phenological development of the BBCH 64–70. On the other hand, this level of C/N ratio allowed us to note the value of oilseed radish leaf mass for green fertilizer, which is especially valuable, given a number of studies (Li et al., 2019; Liu et al., 2020; Hansen et al., 2021; Wang et al., 2021), when used in the budding-flowering phase of BBCH 50-64 with a C/N ratio of 12.22–18.37. The use of laboratory silage fermentation of oilseed radish leaf mass, which is consistent with the findings of Mähnert et al. (2005) and Abu-Dahrieh et al. (2011), ensured an increase in the C/N ratio for all experimental variants with an interval of 12.63-34.37, while maintaining the same trend within the phenological phases as in the variant before the use of silage fermentation. That is, the very process of silage of oilseed radish, which is confirmed by the peculiarities of the chemistry of silage fermentation of cruciferous crops (Kamalak et al., 2005; Raboanatahiry et al., 2021; Chen et al., 2023) allowed to optimize the variants by the C/N ratio.

The previous generalizations were confirmed in the assessment of the cumulative biogas and biomethane productivity of inoculated oilseed radish silage obtained from variants of different sowing dates at different phenological phases of harvesting (Fig. 1).

The total volume of biogas production in the interval of harvesting phases (Fig. 1, a) of leaf-stem mass was 2,137–3,643 mL with a maximum when using for preliminary silage fermentation the leaf-stem mass harvested in the flowering phase at the first sowing date of oilseed radish. The minimum value of this indicator was noted in the variant of the first sowing date for harvesting in the green pod phase. The level of variation of this indicator had different values within the combinatorics of the

experimental variants. The maximum values of variation were determined for the fourth (Cv 4.48–5.74%), and the minimum – for the first sowing dates (Cv 1.69–3.88%).



**Figure 1.** Statistical evaluation of biogas productivity of oilseed radish silages (inoculum subtracted) obtained from plant mass of different sowing and harvesting periods, 2020–2022 (a – cumulative biogas production during the experimental period (mL); b – cumulative biomethane production during the experimental period (mL); c – methane content (%); d – specific methane yields (SMY) of oilseed radish silages from different variants (L<sub>N</sub> kg<sup>-1</sup> ODM); A<sub>n</sub>B<sub>n</sub> – indicates of the variants).

It should be noted that the achieved level of total biogas production is consistent with studies on biogas fermentation of oilseed radish agrobiomass in other studies (2,700–3,100 mL according to Belle et al. (2015), 2,200–3,200 mL according to Molinuevo-Salces et al. (2013, 2014, 2014a), 3,500–3,700 mL according to Carvalho et al. (2011)). In comparison to other representatives of the cruciferous family (white mustard, spring and winter rape, and some wild species of cruciferous plants, according to Herrmann et al. (2016), Maier et al. (2017), Liebetau et al. (2021), Słomka & Oliveira (2021) and Lallement et al. (2023)) silage-fermented biomass of oilseed radish can be attributed to a crop with high biogas potential. At the same time, the maximum level of cumulative biomethane accumulation due to digestate inoculum was 656 mL over a period of 60 days of anaerobic fermentation, which corresponds to a sufficient level of cofermentation (Jankowska et al., 2017).

The index of cumulative biomethane productivity (different variants of the experiment (Fig. 1, b) was determined in the range of 1,215–1,963 mL with a similar distribution within the variants of phenological development and sowing dates with an increase in the total variation to the average long-term value to the level of Cv 4.08–8.40%. At the same time, for all terms of biomass harvesting, the fourth term had the highest level of variation with an index of 1.73 before the first sowing dates. This confirms the previously made conclusions about the peculiarities of the chemical composition of oilseed radish silages of summer sowing in terms of the presence of organic acids and the content of the main components, which, according to the study by Molinuevo-Salces et al. (2013), caused fluctuations in the share of methane in the total biogas composition and an increase in the share of other biogas components.

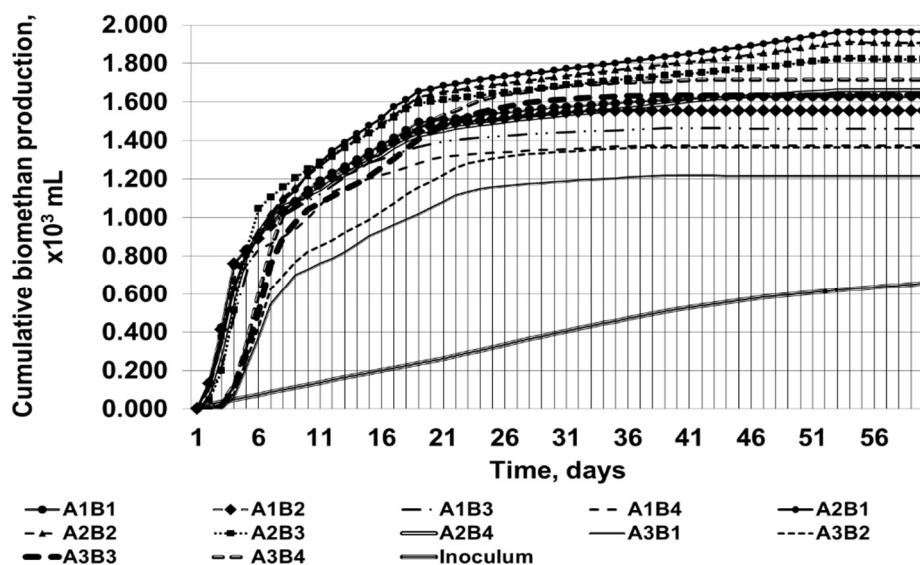
The difference in the variation of the indicator value can be explained by differences in the chemical composition of the leaf-stem mass determined for different phenological phases and harvesting dates, which led to differences in the chemical composition of the silage in the corresponding variant of the experiment. These are the reasons noted in the studies by Kalyuzhnyi et al. (2009), Borreani et al. (2018) and Lallement et al. (2023) from the point of view of the main factors that determine the potential biogas productivity of pretreated agrobiomass through silage fermentation.

These conclusions are also confirmed by estimating the share of methane in the generated biogas volume (Fig. 1, c), where, in fact, for the fourth sowing term, an average minimum level of this indicator was noted at 54.34% (with Cv 8.27–10.96%). The highest proportion of methane was noted on average for the phenological phase s of selection for the third sowing term with an average of 55.45% in the overall combination of experimental variants (at Cv 4.27–6.76%). At the same time, it should be noted, based on the interannual variability of indicators, a certain constancy of the intensity of anaerobic fermentation of oilseed radish silage, which is consistent with the findings of Rabemanolontsoa & Saka (2013), Bumbiere et al. (2021) on the relative species stability of agrobiomass of potential candidates for biogas production and the findings of McDonald et al. (1991), Herrmann et al. (2011, 2012) and Auxenfans et al. (2017) on a certain leveling of the basic structure of chemical composition indicators under certain pretreatment procedures for biogas fermentation, including preliminary silage. According to Herrmann et al. (2016), for cruciferous crops, including fodder radish, the cumulative biomethane production for autumn sowing (intermediate cultivation) ranges (in terms of volume) from 900–1,400 mL with a methane share of 53.9–57.3%. A number of other studies provide data on close values of the noted intervals (Carvalho et al., 2011, Suominen et al., 2012; Molinuevo-Salces et al., 2013, 2014, 2014a; AL-Huqail et al., 2022).

Based on the comparison of the results of the cumulative level of biogas productivity and the cumulative level of biomethane production, the specific methane yields of oilseed radish silages (Fig. 1, d) from different variants were in the range 201.03–319.66 LN kg<sup>-1</sup> ODM (group Cv 12.95%) (the minimum was noted in the variant of the first sowing date for the green pod phase, and the maximum - in the variant of the first sowing date for the flowering phase). The average value of this indicator for the budding phase was 278.92 LN kg<sup>-1</sup> ODM, for the flowering phase 292.45 LN kg<sup>-1</sup> ODM, and for the green pod phase 237.54 LN kg<sup>-1</sup> ODM.

Among the sowing dates, the maximum level of specific methane yields (SMY) was achieved during the years of research at the fourth sowing date of 276.47 LN kg<sup>-1</sup> ODM, and the minimum - at the third sowing date of 263.12 LN kg<sup>-1</sup> ODM. This confirms the conclusions made earlier about the influence of changes in the biochemical composition of oilseed radish leaf biomass due to the shift in terms from early spring to summer on the features of the overall biogas productivity of the silage mass. In particular, this is an increased content of crude protein with a reduced content of crude fiber against the background of an increased content of nitrogen-free extractives, which forms optimal conditions for the intensity of biogas fermentation (Dandikas et al., 2014).

The determined interval for specific methane yields falls within the interval established in a number of studies specifically for the radish genus 170–350 LN kg<sup>-1</sup> ODM (Carvalho et al., 2011; Herrmann et al., 2016; Molinuevo-Salces et al., 2013, 2014, 2014a) although there is an upper limit of up to 350–380 LN kg<sup>-1</sup> ODM and a lower limit of 120–150 LN kg<sup>-1</sup> ODM (Amon et al., 2007; Belle et al., 2015).



**Figure 2.** Cumulative biomethane production during the experimental period of oilseed rape silages (inoculum subtracted) obtained from plant mass of different sowing and harvesting dates, mL (average adjusted for biomethane productivity of inoculum and standardized dry gas indicators for 2020–2022 for each variant) (A<sub>n</sub>B<sub>n</sub> – indication of combinatorial variants according to Table 1).

The cumulative curves (Fig. 2) corresponded to the nature of the Gompertz growth curves (Lay et al., 1997) correlated adequately with the specified interval of values in the context of experimental variants, and had a complex shape mainly in the period of 3–21 days of fermentation. Taking into account the statement of Wang (2010), this character indicates the oscillatory nature of the formation of the indicator at the points of fixation with different ordinal placement of indicators with a pronounced downward structure of values in the dynamics. This is confirmed by the results of approximating the actual curve points to the model functions with the estimation of the correlation and prognostic components (RMSE and RRMSE) (Table 6). This mathematical relation is fully consistent with the mathematical models of biogas production from both raw plant

material of bioenergy crops and subjected to various preliminary procedures of preparation for the final biofermentation process and is reflected in a number of publications (Parker et al., 2005; Carvalho et al., 2011; Triolo et al., 2011; Podkówka, 2012; Thomsen et al., 2014; Batstone et al., 2015; Herrmann et al., 2016; Einarsson & Persson, 2017; Pabón-Pereira et al., 2020; von Cossel et al., 2021; Tasnim et al., 2022; Fajobi et al., 2023).

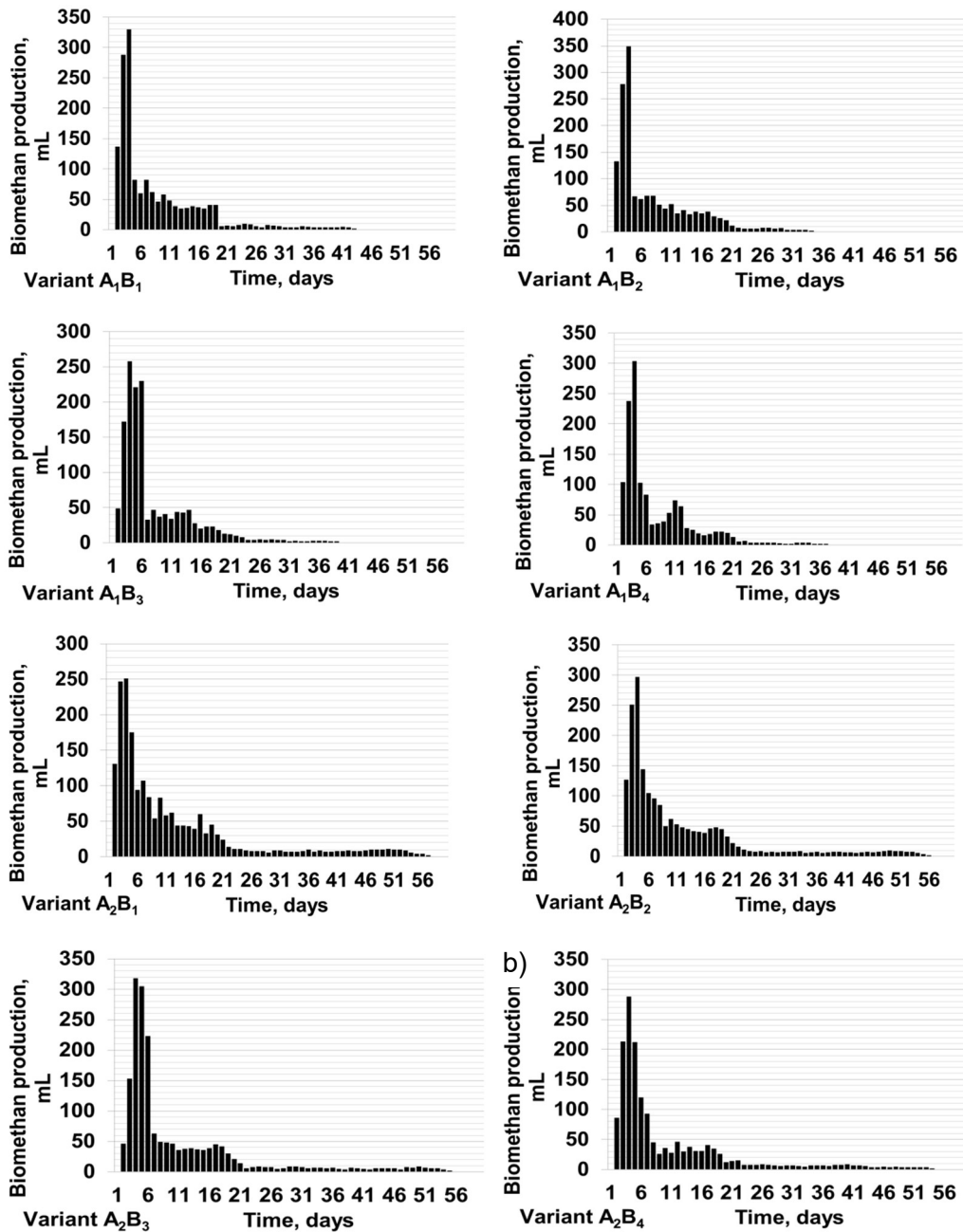
**Table 6.** Functional expression of the cumulative biomethane and biomethane productivity of oilseed radish silages (inoculum subtracted) obtained from plant mass of different sowing and harvesting periods (average data for 2020–2022)

Variant*	Equation of dependence on the time of anaerobic fermentation (t)	Parameters of the equation			Statistical parameters for assessing the reliability of fitting the actual dynamics to the theoretical expression of the equation						
		$y_m$	$k_1$	S	$R^2_{(adj.)}$	RMSE	RRMSE	$p$			
Cumulative biomethan production (y) at time (t) (mL)											
A <sub>1</sub> B <sub>1</sub>	$y(t) = y_m(1 - e^{-k_1 t})$	1,625.533	0.122	45.700	0.985	128.91	18.96	< 0.001			
A <sub>1</sub> B <sub>2</sub>		1,564.910	0.129	46.828	0.980	129.47	19.15	< 0.001			
A <sub>1</sub> B <sub>3</sub>		1,473.787	0.129	62.617	0.961	132.28	19.58	< 0.001			
A <sub>1</sub> B <sub>4</sub>		1,380.248	0.134	44.167	0.975	130.93	19.21	< 0.001			
A <sub>2</sub> B <sub>1</sub>		1,917.568	0.097	54.127	0.982	129.11	19.07	< 0.001			
A <sub>2</sub> B <sub>2</sub>		1,862.261	0.102	54.770	0.980	129.61	19.18	< 0.001			
A <sub>2</sub> B <sub>3</sub>		1,784.985	0.111	78.609	0.961	132.37	19.64	< 0.001			
A <sub>2</sub> B <sub>4</sub>		1,626.935	0.110	60.126	0.969	131.68	19.59	< 0.001			
A <sub>3</sub> B <sub>1</sub>		1,265.749	0.080	73.296	0.950	134.94	19.97	< 0.001			
A <sub>3</sub> B <sub>2</sub>		1,422.200	0.080	81.548	0.951	134.21	19.88	< 0.001			
A <sub>3</sub> B <sub>3</sub>		1,635.270	0.068	96.530	0.950	135.11	20.05	< 0.001			
A <sub>3</sub> B <sub>4</sub>		1,767.454	0.087	101.153	0.955	133.89	19.57	< 0.001			
Biomethan production (y) at time (t) (mL)											
Variant*		Equation of dependence on the time of anaerobic fermentation (t)	Parameters of the equation				Statistical parameters for assessing the reliability of the fitting				
			a	b	c	d	S	$R^2_{(adj.)}$	RMSE	RRMSE	$p$
A <sub>1</sub> B <sub>1</sub>		$y(t) = \frac{a + bt}{1 + ct + dt^2}$	-5.721	5.070	-0.322	0.028	12.476	0.952	78.96	8.69	< 0.001
A <sub>1</sub> B <sub>2</sub>	-4.237		6.525	-0.417	0.049	13.569	0.943	79.39	8.87	< 0.001	
A <sub>1</sub> B <sub>3</sub>	-2.131		7.891	-0.496	0.068	13.968	0.936	81.12	9.14	< 0.001	
A <sub>1</sub> B <sub>4</sub>	-1.148		9.446	-0.539	0.080	14.107	0.927	83.56	9.75	< 0.001	
A <sub>2</sub> B <sub>1</sub>	-40.878		41.926	-0.547	-0.108	9.951	0.961	76.97	8.35	< 0.001	
A <sub>2</sub> B <sub>2</sub>	-27.221		30.650	-0.543	0.097	11.468	0.954	78.27	8.47	< 0.001	
A <sub>2</sub> B <sub>3</sub>	-2.497		8.973	-0.433	0.052	12.319	0.960	77.18	8.59	< 0.001	
A <sub>2</sub> B <sub>4</sub>	-6.290		14.139	-0.491	0.071	8.099	0.974	73.27	7.52	< 0.001	
A <sub>3</sub> B <sub>1</sub>	-3.877		3.804	-0.275	0.019	14.563	0.905	90.49	11.18	< 0.001	
A <sub>3</sub> B <sub>2</sub>	-13.283		5.989	-0.336	0.031	12.186	0.917	87.29	10.57	< 0.001	
A <sub>3</sub> B <sub>3</sub>	-4.278		4.741	-0.309	0.024	13.741	0.914	88.14	10.91	< 0.001	
A <sub>3</sub> B <sub>4</sub>	-5.721		5.070	-0.322	0.028	12.477	0.950	78.81	8.69	< 0.001	

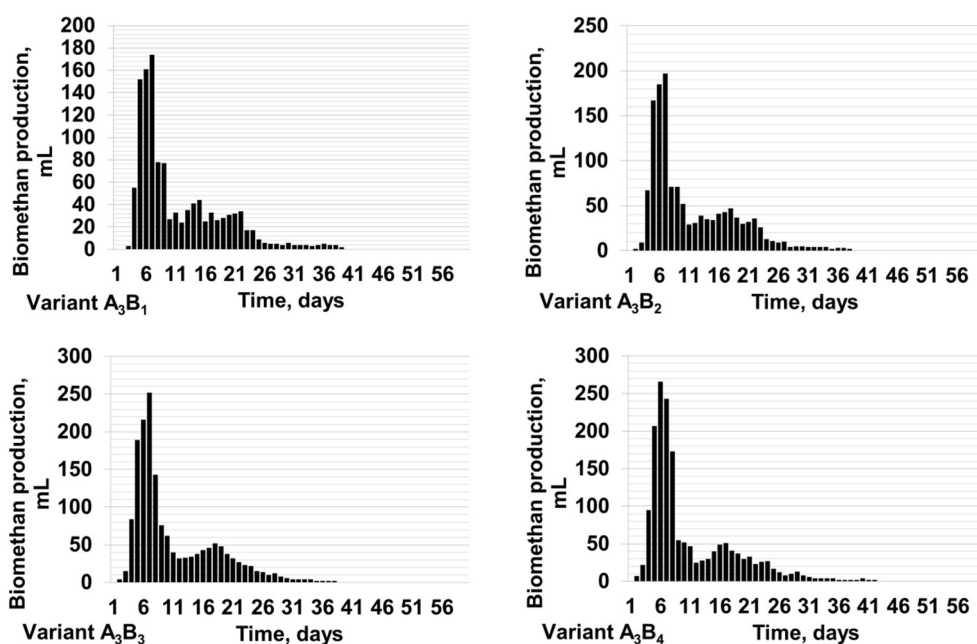
\* – in accordance with the combinatorics of options according to Table 1.

The cumulative curves of biomethane yield for different variants of research (Fig. 3) confirm the conclusions made about the specifics of biomethane productivity from different variants of oilseed radish silage. The dynamics, while generally similar in

the ordinal height of the graphs, has certain differences. Within the variant for the budding phase (indexing of variants A1), an intensive increase in methane emission was noted on day 3–5 and dominated by oscillatory maxima in the interval of 7–10 days.



**Figure 3.** Biogas production during the experimental period of oilseed rape silages (inoculum subtracted) obtained from plant mass of different sowing and harvesting dates (average adjusted for standardized dry gas indicators for 2020–2022).



Continued Figure 3. Biomethane production during the experimental period of oilseed rape silages (inoculum subtracted) obtained from plant mass of different sowing and harvesting dates (average adjusted for standardized dry gas indicators for 2020–2022).

The period of minimal biomethane activity was observed from day 23, and the complete cessation of the process from day 35–43. The most complicated idiogram of the graph for the budding phenological phase was observed for the variant of the fourth sowing date. A rather short lag period was established (average  $1.32 \pm 0.15$  days). For the phenological phase of flowering (indexing of variants A2), a more smoothed character of the graph was noted. An intensive increase in methane emission was observed on day 3–4, followed by oscillatory maxima in the interval of 7–13 days.

The period of minimal biomethane activity was observed from 26–29 days, and the complete cessation of the process from 39–42 days. The most complicated idiogram of the graph for the flowering phenological phase was noted for the variant of the first and fourth sowing dates of oilseed radish. The duration of the lag period increased and averaged  $1.49 \pm 0.13$  days.

For the phenological phase of the green pod (indexing of variants A<sub>3</sub>), a complex oscillatory nature of the graph (three peak values) was noted. There was an intensive increase in methane emission on day 5–7 followed by oscillatory maxima in the interval of 13–19 days depending on the sowing date (indexing of variants B<sub>1</sub>–B<sub>4</sub>). The period of minimal biomethane activity was observed from 22–25 days, and the complete cessation of the process from 55–58 days. The most complicated idiogram of the graph for the flowering phenological phase was observed for the variant of the first and fourth sowing dates of oilseed radish. The duration of the lag period was maximum for this phenological phase for all sowing dates and averaged  $2.09 \pm 0.65$  days with a maximum for the first sowing date. At the same time, a number of features were noted that distinguish oilseed radish as a potential candidate for biogas production. In particular,

the value of the coefficient  $k_1$  (the first order decay constant ( $\text{day}^{-1}$ )) in the equation of dependence on the anaerobic fermentation time (Table 6) determined for fodder radish in the studies of Herrmann et al. (2016) was in the range of 0.190–0.273, although among cruciferous crops in his studies, the value of this indicator was the lower limit of 0.144.

In Słomka & Oliveira's (2021) research for white mustard, based on the analysis of the presented curves, this coefficient had a lower limit of 0.090–0.100. It is also reported (Ahlberg & Nilsson, 2015) that the nature of cumulative curves of biogas production can have a complex oscillatory character due to the specific biochemical composition of the biomass taken for fermentation, which can increase the value of this coefficient  $k$  at the starting sections of the curve and reduce its value for the overall analysis of the graph on the general abscissa of the graph.

The same conclusions regarding the wide-interval value of the  $k_1$  coefficient were noted in the studies of Herout et al. (2011) and Maier et al. (2017). At the same time, Fajobi et al. (2023) and Pabón-Pereira et al. (2020) noted that greater intensity of ordinal growth of the cumulative biogas production curve is characteristic of biomass with a lower dry matter content, rich in proteins and nitrogen-free extractives.

This is confirmed by the results presented in the case of the budding phase, especially of the fourth sowing term, where a similar character of the biochemical composition of the plant mass is noted with a value of  $k_1$  in the range of 0.122–0.134.

Conversely, for the green pod phase, with a decrease in crude protein content, an increase in crude fiber content, and a decrease in the content of nitrogen-free extractives, its value was in the range of 0.068–0.087. Due to this, the standard error of the graphs approximation naturally increases consistently when comparing the budding and flowering phenophases and confirms the conclusions drawn from the analysis of individual graphs of biomethane production for each experimental variant.

As for the mathematical analysis of the dynamics of biomethane production, taking into account the steady downward oscillatory dynamics, the presence of several peaks on the general downward curve, and the graphical dependence which was selected on the basis of the approximation process using the CurveExpert Professional software package is a linear and quadratic relationship that forms a complex curve configuration with a consistent peak increase at the 3–7<sup>th</sup> fixation point and a consistent steady decrease of hyperbolic character ( $1/(x(t)^{-1})$ ) (according to Gavril & Schönheim (1982)). This character of the curves, the level of approximation of which for all variants of the experiment was confirmed for the level of significance of 0.1% ( $p < 0.001$ ) indicates that the process of biomethane production from oilseed radish silage is an intensively decaying process, the main productive component of which is concentrated in the fixation cycle of 4–7 days, and the final process of productive biomethane production does not reach the full cycle of the selected experimental duration of anaerobic digestion (in our case, 60 days) (as indicated by the ratio of the coefficients  $a$  and  $b$  against the background of low values of the coefficients  $d$  according to Bardsley & Childs (1975)). Additional indicators of the kinetics of biomethane production from oilseed radish silage (inoculum subtracted) (Table 7) allowed us to determine the features characteristic of oilseed radish in comparison with other traditional bioenergy crops from the cruciferous family. Thus, the previously analyzed indicator of methane concentration in the total biogas volume produced averaged 54.91% (with a range of 49.92–59.11%), which corresponds to the range of 53.9–57.3 for fodder radish determined by Herrmann et al.



(2016) and determined by (Carvalho et al., 2011; Belle et al., 2015) at the level of 50–60% for oilseed radish in single-component or multicomponent anaerobic digestion.

**Table 7.** Biomethane production characteristics of oilseed rape silages (inoculum subtracted) depending on the sowing date and phenological phase of plants (average for 2020–2022 for the total number of observations for each variant)

Sowing date	Methane content (%)		BMP <sub>Gomp</sub> (L <sub>N</sub> kg <sup>-1</sup> ODM)		R <sub>m</sub> (L <sub>N</sub> kg <sup>-1</sup> ODM d <sup>-1</sup> )		t <sub>50</sub> (d)*		Lag period (λ) (d)***	
	$\bar{x}$	SD**	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Budding stage (BBCH 50–53)										
I	53.95	3.54	304.94	11.55	27.88	1.17	3.57	0.32	1.08	0.17
II	53.42 <sup>d</sup>	4.53	298.97 <sup>c</sup>	13.87	26.63 <sup>b</sup>	1.29	2.96 <sup>b</sup>	0.40	1.25 <sup>c</sup>	0.13
III	51.96 <sup>b</sup>	2.53	274.74 <sup>b</sup>	14.17	24.98 <sup>b</sup>	1.58	3.89 <sup>c</sup>	0.25	1.51 <sup>b</sup>	0.11
IV	49.92 <sup>b</sup>	5.49	263.39 <sup>b</sup>	14.35	23.40 <sup>a</sup>	1.67	3.00 <sup>c</sup>	0.25	1.47 <sup>b</sup>	0.19
Flowering stage (BBCH 64–67)										
I	53.90	1.32	344.13	7.06	33.74	1.25	4.50	0.50	1.18	0.12
II	54.69 <sup>c</sup>	1.98	314.19 <sup>b</sup>	8.38	32.69 <sup>b</sup>	1.39	4.35 <sup>b</sup>	0.42	1.46 <sup>c</sup>	0.15
III	59.11 <sup>a</sup>	2.50	280.30 <sup>a</sup>	9.68	31.58 <sup>a</sup>	1.47	3.61 <sup>a</sup>	0.38	1.75 <sup>a</sup>	0.10
IV	57.69 <sup>a</sup>	4.11	289.75 <sup>a</sup>	11.90	28.67 <sup>a</sup>	1.61	3.91 <sup>a</sup>	0.44	1.58 <sup>b</sup>	0.14
Green pod stage (BBCH 73–75)										
I	56.86	4.60	204.01	9.85	21.30	1.12	4.85	0.48	2.88	0.21
II	56.33 <sup>c</sup>	2.72	222.29 <sup>b</sup>	10.84	23.89 <sup>b</sup>	1.21	5.38 <sup>b</sup>	0.52	1.92 <sup>b</sup>	0.17
III	55.27 <sup>b</sup>	2.34	263.62 <sup>a</sup>	13.84	28.55 <sup>a</sup>	1.55	4.37 <sup>c</sup>	0.63	1.83 <sup>b</sup>	0.15
IV	55.42 <sup>b</sup>	3.72	287.26 <sup>a</sup>	14.98	29.46 <sup>a</sup>	1.49	4.45 <sup>c</sup>	0.58	1.71 <sup>b</sup>	0.12

BMP<sub>Gomp</sub>: biochemical methane potential derived from the modified Gompertz equation; R<sub>m</sub>: maximum specific methane production rate; \*t<sub>50</sub>: half-life (counting from the beginning of methane emission); \*\*SD: standard deviation; \*\*\*: in terms of days from hourly accounting in the ratio of 1 day = 24 hours; Significance levels in comparison with the first (I) sowing term: a – 0.1%; b – 1%; c – 5%; d – no significant difference.

Taking into account that the controlled anaerobic process leads primarily to a gas mixture of 51–75% methane (Badger et al., 1979; Zubr, 1986; Mohanty et al., 2022) and for traditional cruciferous crops this figure ranges from 51.3–62.8% (Herrmann et al., 2016; Rahman et al., 2018; Kılıç et al., 2021; Țîței, 2021, 2022) oilseed radish biomass in the variant of preliminary sludge fermentation can be effectively utilized as a component of biogas production.

It has been established that from the point of view of the performance indicators of the kinetics of biogas production and the actual proportion of methane, it is optimal to harvest oilseed radish biomass with subsequent silage for biogas fermentation in the flowering phase (BBCH 64–67). For this phase, the average methane content in the total biogas production was 56.35%, with biochemical methane potential (BMP<sub>Gomp</sub>) at 307.09 L<sub>N</sub> kg<sup>-1</sup>ODM, specific methane yields (SMY) (Fig. 1, d) of 292.45 L<sub>N</sub> kg<sup>-1</sup>ODM (share of SMY/BMP<sub>Gomp</sub> 95.23%) and maximum specific methane production rate (R<sub>m</sub>) at 31.67 L<sub>N</sub> kg<sup>-1</sup>ODM d<sup>-1</sup>. The values of these indicators for the budding phase (BBCH 50–53) were 52.31%, 278.92 L<sub>N</sub> kg<sup>-1</sup>ODM, 285.51 L<sub>N</sub> kg<sup>-1</sup>ODM (the share of SMY/BMP<sub>Gomp</sub> 97.69%) and 25.72 L<sub>N</sub> kg<sup>-1</sup>ODM d<sup>-1</sup>, respectively. For the phenological phase of the green pod (BBCH 73–75) was at the level of 55.97%, 237.54 L<sub>N</sub> kg<sup>-1</sup>ODM, 244.30 L<sub>N</sub> kg<sup>-1</sup>ODM (share of SMY/ BMP<sub>Gomp</sub> 97.24%) and 25.80 L<sub>N</sub> kg<sup>-1</sup>ODM d<sup>-1</sup>, respectively.

The efficiency of biogas productivity varied within the sowing dates. Averaged over the phenological phases budding-green pod (BBCH 50–75) for the first sowing period, the parameters of biogas production performance were as follows: methane content 54.90% with biochemical methane potential ( $BMP_{Gomp}$ ) at the level of  $284.36 \text{ L}_N \text{ kg}^{-1}_{ODM}$ , specific methane yields (SMY) (Fig. 3, d)  $272.53 \text{ L}_N \text{ kg}^{-1}_{ODM}$  (the share of  $SMY/BMP_{Gomp}$  95.83%) and the maximum specific methane production rate ( $R_m$ ) at the level of  $27.64 \text{ L}_N \text{ kg}^{-1}_{ODM} \text{ d}^{-1}$ .

The ratio index for SMY and  $R_m$  in comparison to the first sowing term for the second to fourth sowing term, depending on the phenological phase of biomass selection, was at the level of 0.81–1.41 and 0.84–1.38, respectively. At the same time, taking into account the level of SMY ( $> 200 \text{ L}_N \text{ kg}^{-1}_{ODM}$ ) according to the assessment of the potential of bioenergy crops (Amon et al., 2007; Vindis et al., 2009; Herrmann et al., 2016; Kılıç et al., 2021), all sowing dates of oilseed radish have a fairly high level of productive potential for biogas processing.

According to the assessment of the maximum achievable potential for the phenological phase of budding and flowering, the first and second sowing dates had the optimum, and the third and fourth – for the green pod phase. This distribution pattern is primarily confirmed by the peculiarities of the biochemical composition of both raw biomass and biomass after silage fermentation. In particular, during the green pod phase, an intensive increase in the proportion of crude fiber was observed with a corresponding decrease in nitrogen-free extractives (NfE) (Tables 4, 5). Another possible factor in the advantage of later sowing dates for harvesting at the green pod stage is the appearance of pods with seed germs in the structure of the leaf-stem mass. It is during the fruiting period that intensive synthesis of fatty acids begins in oilseed radish (Tsytsiura, 2022) and an intensive increase in the concentration of glycosaminolates from  $50$  to  $160 \mu\text{mol g}^{-1}$  is noted (Carlson et al., 1985; Prieto et al., 2019). Given the possible influence (inhibition) of glucosinolates on the intensity of biofermentation of the crude mass of cruciferous plant species (Barba et al. (2016); Sun et al. (2020); Bichsaem et al. (2021)). This issue may also be an additional factor in the reduction of SMY for oilseed radish at the fruiting stages (BBCH  $> 70$ ) and requires further study as it is an important factor in the effective use of traditional and less common cruciferous plant species for biofermentation for bioenergy needs.

Certain peculiarities were also noted for the indicator of anaerobic fermentation half-life ( $t_{50}$ ). A number of studies have found that its value varies for different bioenergy crops in the range of 1.5–9.5 (Pettersson et al., 2007; Vindis et al., 2009), and for cruciferous plants it ranges from 2.62–5.11, and in particular for fodder radish 2.68–3.69 (Carvalho et al., 2011; Herrmann et al., 2016). For oilseed radish, within the experimental variants, this indicator differed significantly (2.96–5.38) both in terms of biomass harvesting and sowing dates with a pronounced tendency of its growth (on average, in comparison with the budding phase (BBCH 50–53), the growth index was 1.22, and by the green pod phase (BBCH 73–75) it was 1.42) during the maturation of oilseed radish plants. Within the sowing dates, the trend is the opposite (the index of decrease in comparison with the first sowing date for the second term was 0.98, for the third term 0.92, for the fourth term 0.88). Based on this, a conclusion was made that confirms the analysis of the analysis of biomethane production graphs in terms of variants (Fig. 4, b) regarding the peak period in the anaerobic fermentation of oilseed radish silage on the 3<sup>rd</sup>–7<sup>th</sup> day of fixation, as well as the belonging of the kinetics of biomethane production

from oilseed radish biomass to a high-amplitude pronounced damping process, which is consistent with the findings of Petersson et al. (2007) and Yen et al. (2017). This specificity of the process kinetics is also confirmed by the lag period ( $\lambda$ ) in the range of 1.08–2.88 for different oilseed radish variants. Such a short lag period was obtained in the study of Carvalho et al. (2011), where the process of biogas formation was observed on the second day after the laying of silage from oilseed radish biomass for anaerobic fermentation. This nature of the formation of the lag phase duration is explained by the results of Kim & Kim (2020), who found that the lag phase could be caused by the acidification by volatile fatty acid (VFA) accumulation and the initial VFA to volatile solid ratio. To reduce the lag-phase, the VFA/Alk ratio should be maintained below 0.4.

The initial VFA/VS ratio below 10% enhanced anaerobic digestion performance and digestion time reduction at a high protein loading rate. Taking into account the previous conclusions about the increase in both crude fat content and total fatty acid content due to the strengthening of fruit and seed formation subphases with a rich amino acid composition (Bell et al., 2000; Barthet & Daun, 2002; Ávila & Sodr e, 2012) against the background of an increase in dry matter content during the green pod phase (BBCH 73–75), a longer lag period of 2.09 days (for comparison, the same indicator averaged by sowing dates for the flowering phase (BBCH 64–67) was 1.49 and for the budding phase (BBCH 73–75) - 1.33). The variable dynamics of the lag phase duration ( $\lambda$ ) within the sowing dates is explained by the same reasons (according to Kim & Kim (2020)) for the ratio of biochemical components of silage-fermented oilseed radish leaf-stem mass noted in the assessment of the effect of sowing dates on the chemical composition of the silage substrate.

Based on the preliminary analysis, it should be noted that the biochemical composition of the substrate undergoing anaerobic fermentation will affect both the chemistry and the kinetics of the biogas formation process under appropriate temperature conditions. Such conclusions are consistent with the basic publications on this issue (Weißbach & Kuhla, 1995; Ofori & Becker, 2008; Carvalho et al. (2011); Rath et al., 2013; Dandikas et al., 2014; Herrmann et al., 2016; Lamba et al., 2016; Jankowska et al. 2017; Mart nez-Guti rrez 2018; Kılıç et al., 2021; Czubaszek et al., 2022; Lallement et al., 2023). The results of research in this area in the application to the anaerobic fermentation of oilseed radish silage are to some extent similar to the results of these scientists (Table 8). However, it should be noted that there are certain features that distinguish this system of indicators from the point of view of assessing the impact on the kinetics of the biomethane production process. Taking into account the values of the determined correlation coefficients, the SMY (specific methane yield) for oilseed radish silages (inoculum subtracted) was tendentially higher (the level of 'Moderate correlation' was taken into account (according to the recommendations of Dandikas et al., 2014)) with lower crude fiber content (CFb, coefficient of determination ( $d_{yx}$ ) 53.29%), higher organic dry matter (ODM,  $d_{yx}$  19.36%), organic acid derivatives (SBVa,  $d_{yx}$  20.25%; SAPa,  $d_{yx}$  30.25%), alcohols (Alc,  $d_{yx}$  23.04%), lactic acid (LA,  $d_{yx}$  18.49%), but despite the higher concentration of these acids at a lower acidity of the substrate (at a higher numerical pH value) (pH A,  $d_{yx}$  10.89%).

**Table 8.** Pearson's correlation coefficients of chemical components and methane production characteristics of all crop silage samples (for a joint system of matching variants-repetitions-years (N = 144))

	1. DM*	2. ODM	3. CP	4. CF	5. CFb	6. CA	7. NfE	8. C/N	9. SBVa	10. Alc	11. pH	12. La	13. SAPa	14. y <sub>m</sub>	15. k <sub>1</sub>	16. MC	17. SMY	18. R <sub>m</sub>	19. t <sub>50</sub>	20. λ
1		0.59	-0.68	0.36	0.66	-0.60	0.12	0.54	-0.79	-0.77	0.78	-0.79	-0.70	0.08	-0.52	0.50	-0.43	0.04	0.66	0.60
2			-0.68	0.33	0.69	-0.61	0.63	0.73	-0.77	-0.74	0.75	-0.78	-0.68	0.26	-0.61	0.69	0.44	0.22	0.54	0.66
3				0.26	0.71	0.93	0.66	0.99	0.62	0.56	-0.59	0.64	-0.68	0.44	0.80	-0.34	0.24	0.17	0.70	0.66
4					0.24	-0.30	0.08	0.31	-0.36	-0.26	0.39	-0.38	-0.28	0.44	-0.26	0.28	-0.37	-0.16	0.48	0.66
5						-0.80	0.12	0.77	-0.80	-0.79	0.78	-0.83	-0.73	-0.38	-0.84	0.59	0.22	-0.64	0.73	0.66
6							-0.61	0.77	0.74	0.67	-0.72	0.76	0.52	-0.01	0.86	-0.40	0.43	-0.78	0.73	0.66
7								0.59	-0.06	0.04	0.08	-0.08	0.27	-0.01	-0.32	-0.03	0.22	-0.64	0.73	0.66
8									-0.66	-0.60	0.64	-0.69	-0.48	-0.06	-0.84	0.41	0.36	-0.32	0.76	0.66
9										0.79	-0.78	0.75	0.72	-0.06	0.86	-0.66	0.45	-0.02	-0.82	0.76
10											-0.76	0.77	0.72	-0.06	0.84	-0.63	0.48	-0.01	-0.76	0.76
11												-0.69	0.73	-0.06	-0.67	0.75	0.33	0.28	-0.81	0.77
12													-0.61	0.16	0.69	-0.63	0.43	0.28	-0.81	0.77
13														-0.18	0.76	-0.64	0.55	0.28	-0.81	0.77
14																-0.58	0.75	0.28	-0.81	0.77
15																	0.25	0.28	-0.81	0.77
16																		0.20	-0.81	0.77
17																			0.45	0.66
18																				0.66
19																				0.57

\*\*10.2 11.2 10.8 5.6 12.6 11.4 4.7 11.3 11.5 11.1 11.1 10.7 9.9 5.0 12.1 9.3 8.4 5.8 11.4 11.0  
 \*\*\*0.64 0.59 0.57 0.31 0.70 0.63 0.58 0.59 0.72 0.69 0.65 0.63 0.58 0.42 0.64 0.51 0.44 0.49 0.63 0.57

$r = |0| - |0.4|$  No or weak correlation;  $r = |0.4| - |0.7|$  Moderate correlation;  $r = |0.7| - |1.0|$  Strong correlation.  
 \*DM: dry matter; ODM: organic dry matter; CF: crude fat; CP: crude protein; CFb: crude fibre; CA: crude ash;  
 NfE: nitrogen-free extracts; C/N: carbon to nitrogen ratio; SBVa: sum of i-butyric, butyric, i-valeric, valeric and caproic acid; Alc: Sum of ethanol, propanol, 1,2-butanediol, 2,3-propanediol; pH: silage acidity; LA: lactic acid; SAPa: sum of acetic and propionic acid; y<sub>m</sub>: is the maximum specific methane yield at theoretically infinite digestion time; k<sub>1</sub>: first order decay constant; MC: methane content; SMY: specific methane yield; R<sub>m</sub>: maximum methane production rate; t<sub>50</sub>: half-life period; λ: lag phase period. \*\* Graf G; \*\*\* Graf G'. For a significance level of  $p < 0.05$ , the interval  $r = 0.16 - 0.20$ , for  $p < 0.01$   $r = 0.21 - 0.26$ , for  $p < 0.001$   $r > 0.26$ .

The  $R_m$  (maximum methane production rate) was significantly higher with lower crude fiber content (CFb, coefficient of determination ( $d_{yx}$ ) 40.96%), higher crude ash content (CA,  $d_{yx}$  18.49%), higher  $k_1$  (first order decay constant,  $d_{yx}$  54.76%) and lower silage substrate acidity (pH A,  $d_{yx}$  7.80%). The  $t_{50}$  (half-life period) was tendentially higher due to higher crude fiber content (CFb,  $d_{yx}$  53.29%), higher dry and organic dry matter content (DM,  $d_{yx}$  43.56%; ODM,  $d_{yx}$  29.16%), higher C/N ratio ( $d_{yx}$  57.76%), lower content of a number of organic acid derivatives and silage fermentation alcohols (SBVa, La, SAPa, Alc:  $d_{yx}$  28.09–67.24%), lower crude protein content (CP,  $d_{yx}$  49.00%), lower crude ash content (CA,  $d_{yx}$  60.84%), higher pH (lower acidity of the silage substrate) ( $d_{yx}$  62.41%) with a lower value of  $k_1$  (first order decay constant,  $d_{yx}$  77.44%). The duration of the lag phase ( $\lambda$ ) had the same dependence as the  $t_{50}$  (half-life period) due to the identity of its formation mechanism in the anaerobic fermentation system (Kim & Kim (2020)).

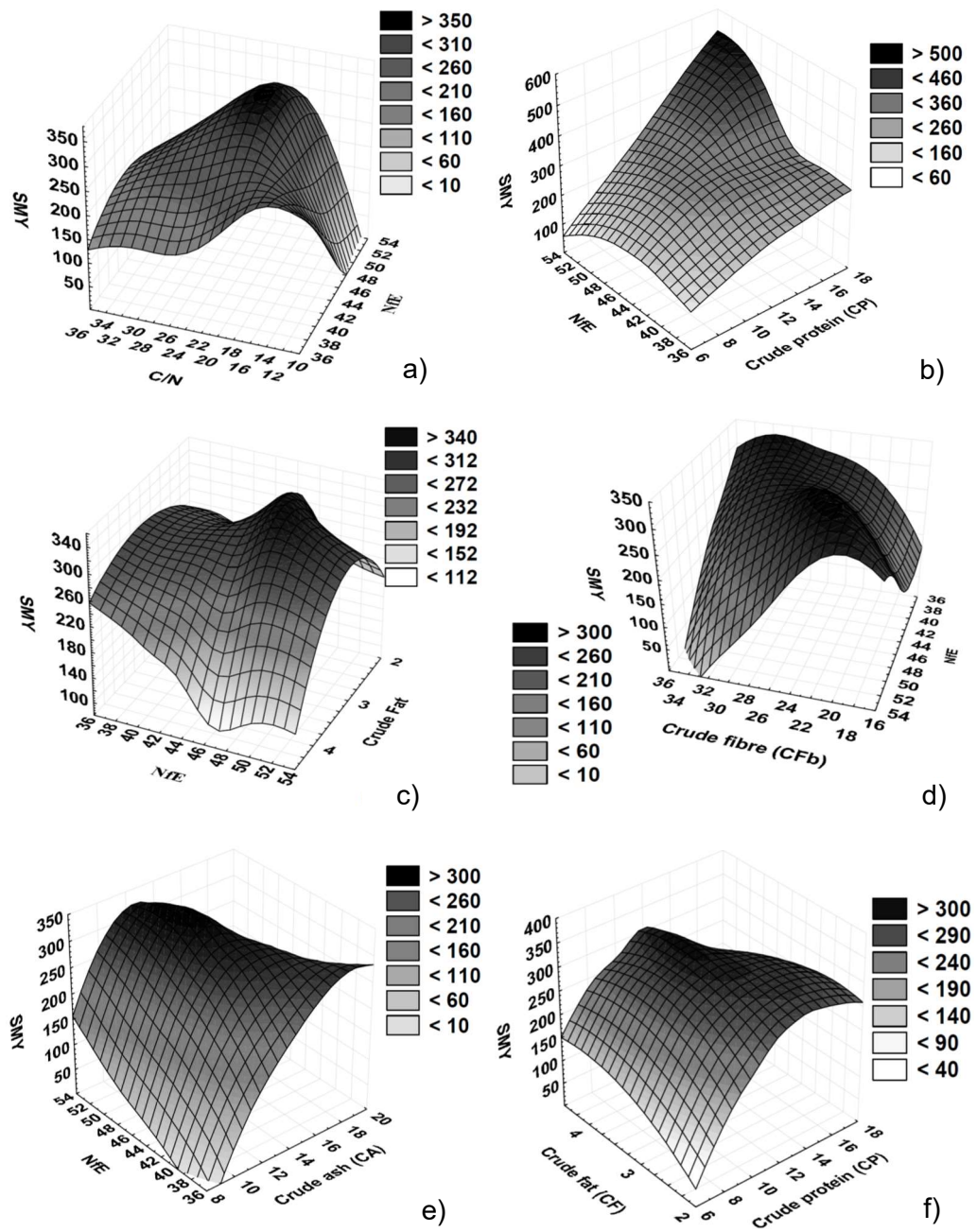
According to the level of the direct (G) and adjusted (G') correlation graph, it was determined that the productive indicators of biogas yield SMY and  $R_m$  in the case of anaerobic fermentation of oilseed radish silage in the system of dependencies belong to the average deterministic ones in the applied complex of the correlation galaxy for 19 traits (according to Sokal & Rohlf, 2012; Lockwood, 2016)) with the level of determination according to the adjusted correlation graph (G') at the level of 19.62% and 23.62%.

At the same time, the highest levels of determination were established for such indicators as  $k_1$ ,  $t_{50}$  and  $\lambda$  with the corresponding value of the general correlation graph (G) at the level of 11.0–12.1 with the level of determination by the adjusted correlation graph (G') at the level of  $d_{yx}$  32.72–40.70%. In the system of factor indicators, the highest level of determination by the size of the correlation graphs was established for the crude fiber content (CFb) (G at the level of 12.6, and G' in the expression  $d_{yx}$  49.00%).

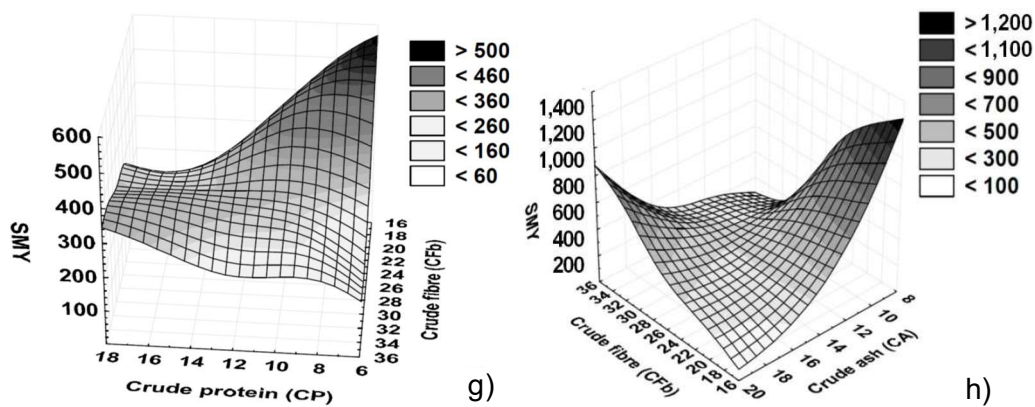
It should be noted that the acidity (pH) of the oilseed radish silage samples has a tendency to influence the biomethane productivity of substrates. Based on the fact that silages from cruciferous crops have a predominantly pH in the range of 3.5–5.3 and for radish 3.8–5.3 (Herrmann et al., 2016), optimization of silage acidity to the level of 4.6–5.0 positively affects the levels of biomethane production in terms of SMY and  $R_m$  and, given the established dependencies, allows for their conjugate growth with a decade-long decrease in acidity (respectively, an increase in the nominal pH) by at least 9.0–11.0%.

These conclusions are consistent with the findings of Jayaraj et al. (2014), Cerón-Vivas et al. (2019), and Zhang et al. (2022).

The presence of a complex variation in the size of correlation graphs allowed us to conclude that the dependencies between the factors taken for the analysis of the kinetics and productivity of biomethane production from silage-fermented oilseed radish mass are more complex. Based on this, a system of graphical regression correlation of a number of indicators to the resulting SMY (specific methane yield,  $L_N \text{ kg}^{-1}_{ODM} \text{ d}^{-1}$ ) was applied using the method of weighted least squares distances (Fig. 4). The presented regression surfaces demonstrated the complex nature of the formation of the main resulting indicator SMY (specific methane yield) from the binary combination of a number of biochemical parameters of oilseed radish silage in the systematic comparison of the general combinatorics of options. Thus, in the system of dependencies SMY–C/N–NfE (Fig. 4, a), the maximum level of SMY (330–350  $L_N \text{ kg}^{-1}_{ODM}$ ) is predicted to be formed at the content of nitrogen-free extractives (NfE) in the silage mass of oilseed radish at the level of 50–52% $_{DM}$  at a C/N ratio of 20–22.



**Figure 4.** Response surfaces of dependencies (method of weighted least squares distances) of the 'specific methane yield' (SMY) indicator depending on the basic chemical composition of oilseed radish silages (inoculum subtracted) (in the summary system of variants-repeat-years). Regression comparisons: a: SMY-C/N-NfE; b: SMY-NfE-CP; c: SMY-CF-NfE; d: SMY-CFb-NfE; e: SMY-CA-NfE; f: SMY-CF-CP.



Continued Figure 4. Response surfaces of dependencies (method of weighted least squares distances) of the 'specific methane yield' (SMY): g: SMY–CFb–CP; h: SMY–CFb–CA.

For the system of SMY–NfE–CP dependencies (Fig. 4, b), the same level of SMY can be predictably achieved at the following NfE values of 50–52%<sub>ODM</sub> at a crude protein content (CP) of 15–16%<sub>ODM</sub>.

For the system of SMY–CF–NfE dependencies (Fig. 4, c), the level of SMY 312–340 L<sub>N</sub> kg<sup>-1</sup><sub>ODM</sub> can be predictably achieved at the following values of the comparison factors NfE 48–50%<sub>ODM</sub>, crude fat (CF) 3.1–3.2%<sub>ODM</sub>.

For the SMY–CFb–NfE dependence system (Fig. 4, d), the level of SMY > 300 L<sub>N</sub> kg<sup>-1</sup><sub>ODM</sub> can be predictably achieved with the following values of the matching factors NfE 50–52%<sub>ODM</sub> and crude fibre (CFb) 20–23%<sub>ODM</sub>.

For the system of SMY–CA–NfE dependencies (Fig. 4, e), the level of SMY > 300 L<sub>N</sub> kg<sup>-1</sup><sub>ODM</sub> can be predictably achieved at the following values of the matching factors CA 14–16%<sub>ODM</sub> and NfE 48–50%<sub>ODM</sub>.

For the SMY–CF–CP system (Fig. 4, f), the level of the maximum resulting SMY index > 300 L<sub>N</sub> kg<sup>-1</sup><sub>ODM</sub> is achievable at CF values of 3.2–3.35%<sub>ODM</sub> and CP values of 14–16%<sub>ODM</sub>.

For the SMY–CFb–CP system (Fig. 4, g), the maximum resulting SMY > 360 L<sub>N</sub> kg<sup>-1</sup><sub>ODM</sub> is achieved at CFb 18–22%<sub>ODM</sub> and CP 8–12%<sub>ODM</sub>.

For the SMY–CFb–CA system (Fig. 4, h), the level of maximum resulting SMY up to 500 L<sub>N</sub> kg<sup>-1</sup><sub>ODM</sub> is achieved at CFb 16–18%<sub>ODM</sub> and crude ash (CA) 10–12%<sub>ODM</sub> or (binary peak reaction surface) in the variant: CFb 26–28%<sub>ODM</sub> and CA 18–20%<sub>ODM</sub>.

The constructed reaction surfaces are fully consistent with the results of the evaluation of a significant number of bioenergy crops for biogas productivity (Herrmann et al., 2016; Oleszek & Matyka, 2020; Aravani et al., 2021; Lallement et al., 2023) and take into account the special characteristics of oilseed radish under different technological solutions, taking into account the timing of sowing and the timing of leaf mass selection for anaerobic fermentation.

## CONCLUSIONS

Oilseed radish as a multidisciplinary bioenergy crop has demonstrated high biogas and biomethane potential with the possibility of long-term use and biomass harvesting in a wide range of phenological phases. From the point of view of the potential share of methane yield in combination with biogas productivity for the phenological phase of budding (BBCH 50–53) and flowering (BBCH 64–67) with the subsequent process of silage fermentation, the variant of the first sowing term with the level of biochemical methane potential ( $BMP_{Gomp}$ ), respectively, 304.94 and 344.13  $L_N kg^{-1}_{ODM}$ , specific methane yield (SMY) 296.91 and 319.66  $L_N kg^{-1}_{ODM}$  with a maximum specific methane production rate ( $R_m$ ) 27.88 and 33.74  $L_N kg^{-1}_{ODM} d^{-1}$ .

For the phenological phase of the green pod (BBCH 73–75), the variant of the fourth sowing term (summer term as a possible option for intermediate cultivation of oilseed radish in crop rotation) with the corresponding parameters was determined as expedient:  $BMP_{Gomp}$  287.26  $L_N kg^{-1}_{ODM}$ , SMY 283.18  $L_N kg^{-1}_{ODM}$  at  $R_m$  29.46  $L_N kg^{-1}_{ODM} d^{-1}$ .

The optimal biochemical idio-type of oilseed radish plants was determined in the system of pairwise correlation and multiple regression comparison from the point of view of realization of biomethane potential of the crop when harvesting its biomass in the interphase period of budding–green pod (BBCH 50–75): CP 14–18% $_{DM}$ , CFb 16–20% $_{DM}$ , CA 14–18% $_{DM}$ , CF 3.1–3.2% $_{DM}$ , NfE 48–50% $_{DM}$  at a C/N ratio of 20–22 with the acidity of the subsequent silage-fermented mass at the level of pH 4.6–5.0.

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