Towards Increasing the Utilization of Anaerobic Digestate from Biogas Production in Agrotechnologies

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Abstract. The paper presents the results of a comprehensive study that focused on the composition and properties of digestate obtained through mesophilic anaerobic co-fermentation of broadleaf cattail suspensions with yeast waste inoculum. Additionally, bioindication studies were conducted to evaluate the effect of the digestate on the germination of ryegrass and barley under lab-scale conditions. The initial total solids in suspensions before digestion varied from 5%wt. to 10%wt., and the mass fraction of the inoculum ranged from 0.05 to 0.2. Through thermogravimetric analysis, it was observed that digestate samples with higher initial inoculum content exhibited lower thermal stability. One of the limiting factors for the use of digestate was its high water content, ranging from 95.6% to 97.9%. To address the high water content, centrifugation of the digestate samples was performed for 2 minutes at 5000 rpm. This process led to significant dewatering, particularly for samples with a higher inoculum content. The maximum possible reduction in water content of the digestate was achieved at 31.65%. The bioindication study involved evaluating the germination of ryegrass and barley in soil samples with different digestate content. The results indicated that the highest germination rates were achieved with a digestate content of 20%wt. For ryegrass, the germination rate was 93.33%, which was 1.67% higher than the soil control sample and 0.33% higher than the sterile control. Similarly, for barley, the germination rate was 91.33%, surpassing the soil control by 4.00% and the sterile control by 0.67%. The findings of this study confirm the potential of utilizing digestate in agricultural technologies as an additional source of plant nutrients. The comprehensive analysis of the digestate's composition, properties, and its positive impact on germination rates further supports its viability as a valuable resource in agricultural practices.

Keywords: anaerobic digestate; *Lolium perenne; Hordeum vulgare;* digestate utilization; fermentation inoculum; bioindication studies; thermogravimetric analysis.

1. Introduction

Anaerobic digestion of organic waste with biogas production is a broad and promising direction for the development of renewable energy resources as an important component of sustainable development strategies (Appels et al., 2011; Vambol et al., 2023; Voytovych et al., 2020).

The technical and economic success of biogas projects largely depends on the efficiency of anaerobic digestate utilization (Al Seadi et al., 2013; Lamolinara et al., 2022). In many cases, the high humidity of the digestate, low content of organic matter and the presence of specific contaminants make the task of digestate utilization very problematic (Nag et al., 2020; Pivato et al., 2016; Rozylo et al., 2017). It is important that the composition of the digestate significantly depends on the type of organic raw material and is quite variable over time for nominally the same type of raw material (Zirkler et al., 2014). Difficulties with digestate utilization in such cases cause problems with implementing anaerobic technology as a whole, so the processing and utilization of digestate are often compared with the bottleneck of anaerobic digestion of organic waste (Fuchs & Drosg, 2013; Sobhi et al., 2021; Xia & Murphy, 2016).

The problem of using digestion, on the other hand, has significant positive potential. A comprehensive solution to the problem of digestate utilization contributes to the effective closure of nutrient cycles, providing positive environmental impacts (Czekała et al., 2020; Makadi et al., 2008; Tymchuk et al., 2020,Vankovych et al., 2021, Tymchuk et al., 2021). In recent decades, there has been an active search for rational ways to dispose of digestate of various origins and compositions (Hanoshenkoet al., 2022). The effectiveness of digestates as substrates for growing algae and microalgae has been established. A wide range of microalgae species can efficiently absorb the nutrients from anaerobic digestate, producing energy- and nutrient-valuable biomass for further bioprocessing (Xia & Murphy, 2016). Thus, the cultivation of microalgae *Scenedesmus* sp. and *Chlorella* sp. in the anaerobic digestion of piggery effluent demonstrated functional changes in bacterial communities that improve nitrogen removal and, accordingly, the efficiency of biological wastewater treatment (Ayre et al., 2021). Wastewater treatment plant digestate can be an effective substrate for the growth of mixed microalgal culture dominated by *Scenedesmus* sp., promoting the increase in biomass total suspended solids as high as 2.6 g/L with a growth rate up to 0.9 day^{-1} (Uggetti et al., 2014).

At the same time, the main direction in terms of capacities and potential of anaerobic digestate utilization was and remains its agricultural use as a highly nutrient valuable biological fertilizer (Lukehurst et al., 2010; Jurgutis et al., 2021; Lee, et al., 2021), which can be an effective alternative to mineral fertilisation (Gissén et al., 2014; Głowacka et al., 2020; Koszel & Lorencowicz, 2015).

In the process of methanogenesis, raw materials are transformed (changes in ammonia content, pH, carbon-nitrogen ratio, etc.), which is relevant and increases the proportion of macro- and micronutrients available to plants (Möller & Müller, 2012).

The use of solid digestate in olive groves has shown the ability to improve soil fertility and increase the number of available forms of C and N, organic matter, and soil microflora activity (Badagliacca et al., 2022; Coelho et al., 2019). The use of digestate in a mixture with other types of organic waste (ash after the combustion of plant products or compost) confirms that the biological and chemical components in digestate allow their use as an additive to improve fertility and microbiological activity (Garcia-Sanchez et al., 2015; Jimenez et al., 2020). Digestates have a good effect on the physicochemical properties of acidified soils, increasing the content of protein, phosphorus and magnesium in the cultivated biomass (Głowacka et al., 2020). Digestate can be used in various forms, both solid and liquid (Jurgutis et al., 2021). Digestate also affects soil fauna. Studies demonstrate its positive indirect short-term and long-term effects on earthworm density and biomass (Koblenz et al., 2015).

The use of digestate as fertilizer, especially in areas adjacent to biogas plants, also improves the economic impact of biogas production and follows the principle of nutrient recovery (Czekała et al., 2020).

Higher aquatic plants are an effective natural means of biological treatment of different types of wastewater (Jozwiakowski et al., 2020). Integrated use of additional biomass requires the creation of a kind of open biological conveyors based on different types of wetlands with anaerobic fermentation of the obtained green plant mass (Banaszuk et al., 2020; Malovanyy et al., 2021). As a result, the task of searching for the optimal way to utilize the anaerobic digestate of aquatic plants is of special importance.

The aim of the article is to study the composition and properties of digestate obtained after mesophilic anaerobic co-fermentation of broadleaf cattail suspensions with yeast waste inoculum, as well as bioindication studies on the digestate effect on germination of ryegrass and barley in the lab-scale conditions.

2. Materials and methods of research

2.1. Materials

The object of this study was a digestate obtained by mesophilic anaerobic fermentation of broadleaf cattail (*Typha latifolia*) suspensions, enhanced by the addition of active anaerobic biomass from an industrial digester of yeast-produced waste used as a fermentation inoculum.

A sample of broadleaf cattail was collected on the wetlands of Lake Yaniv (Lviv region, Ukraine), and plant biomass was grounded to a homogeneous suspension with a particle size of up to 2 mm. As a result of standard laboratory measurements performed in accordance with the requirements of (Standard Methods, 2017), the content of total solids (TS) in the suspension before its fermentation was 13.2%, and the content of volatile solids (VS) was 70.5% of the TS.

To intensify the fermentation process and increase the degree of biodegradation of lignocellulosic compounds of broadleaf cattail, an active anaerobic medium was used. It was selected from the industrial digester plant of a yeast company (Lviv, Ukraine) producing baker's yeast, mainly *Saccharomyces cerevis*. The TS content in the fermentation inoculum at the start of the process was equal to 6.95% and the VS content was 66.7% of TS.

Four different mixtures of broadleaf cattail suspension with yeast fermentation inoculum were used for mesophilic anaerobic digestion. The ratios of the components were calculated so that the total initial TS content in the mixtures at the beginning of the anaerobic fermentation process was 5 wt.% and 10 wt.% including relative TS content of fermentation inoculum of 5% and 20% of total dry matter of the mixture (Table 1). The total initial weight of all mixtures was equal to 500 g.

Table 1. Main parameters of mixtures of broadleaf cattail suspensions with yeast fermentation inoculum before mesophilic anaerobic digestion.

Sample	**TS (wt.%)**	X_{in}	**TS_{in} (wt.%)**	**VS_{tot} ($g \cdot L^{-1}$)**
No.1	5	0.05	0.25	35.16
No.2	10	0.05	0.5	70.31
No.3	5	0.2	1.0	34.87
No.4	10	0.2	2.0	69.74

2.2. Laboratory installation for anaerobic fermentation

Anaerobic fermentation of mixtures based on broadleaf cattail was performed on a specially designed lab-scale installation consisting of two units - a thermostated fermentation unit and a control unit for collecting and controlling the quantity and quality of biogas. The total duration of anaerobic fermentation of mixtures was 24 days, until practical depletion of the methanogenesis process in all four types of mixtures was observed. Research has determined that the optimal conditions for biogas synthesis are achieved when a yeast-containing fermentation seed is included in the composition of raw materials. Therefore, one of the tasks of the research was to evaluate the influence of yeast-containing fermentation seed on the consumer quality of digestate. Adherence to anaerobic conditions inside the flask reactors was controlled for the absence of oxygen in the biogas samples.

2.3. Thermogravimetric analysis of the digestate

To assess the impact of yeast-containing fermentation inoculum on the degree of biodegradation of broadleaf cattail biomass in the process of anaerobic digestion, a series of thermogravimetric studies of the obtained digestate samples was performed. Thermogravimetric studies of the digestate of the mixtures, as well as the biomass of the fermentation inoculum itself, were performed on a derivative Q-1500 D system 'Paulik - Paulik – Erdei' with the registration of the analytical signal of weight loss and thermal effects using a computer. Samples were analyzed in a dynamic mode with a heating rate of 5°C/min in the air medium. The weight of the samples was 100 mg, and aluminium oxide Al_2O_3 was the reference substance.

2.4. Preparation of the digestate for use as a component of the substrate

One of the main tasks for the possibility of practical use of digestate as a component of soil substrate is its partial dehydration. In different samples of obtained digestate the dry matter content was as low as 2–5%.

To correct the moisture content in digestate, in order to prevent waterlogging of the soil, part of the gravitational moisture was separated mechanically using anOPn-8 centrifuge. The dehydration in a centrifuge has been performed for 2 minutes at a rotational speed of 5000 min^{-1} without the use of any flocculants to exclude the possible influence of flocculants on the further results of bioindication studies.

The primary condition for the possibility of using the digestate as an additive to soil substrate is to find the content of hazardous compounds, in particular heavy metals, which may be a limiting factor for using the digestate as an additive to growth substrates (Kostenko et al., 2017; Malovanyy et al., 2019). The content of macro-and microelements in the obtained digestate samples of broadleaf cattail was determined on an EXPERT 3L X-ray fluorescence analyzer using standard methods.

2.5. Testing the germination of ryegrass and barley in laboratory conditions

At this stage, the influence of the content of digestate on the germination of ryegrass and barley was studied. 100 seeds of ryegrass (*Lolium perenne*) and 100 seeds of barley (*Hordeum vulgare*) were selected for bioindication and placed in Petri dishes on different substrates according to the following scheme:

1) CS - control on sterile medium (distilled water on filter paper), m = 25 g;

2) C - control on soil (dark gray podzolic soil), m = 25 g;

3) D 10% - digestate 10% (mixture of dark gray podzolic soil and digestate, 90%:10%), m = 25 g;

4) D 20% - digestate 20% (mixture of dark gray podzolic soil and digestate, 80%:20%), m = 25 g.

All test samples were stored under the same conditions at t = 25°C in a dry air thermostat (Fig. 1) for 7 days, the humidity of the substrates was about 70%. Based on the results of the studies, the intensity of germination of the cultures on the respective substrates was determined.

Thus, with these studies, the effect of digestate on the germination of crops is determined excluding all external environmental factors, such as lighting, temperature and humidity.

Figure 1. Examples of digestate in a dry air thermostat

The overall views of the studied samples with ryegrass (*Lolium perenne*) and with barley (*Hordeum vulgare*) are presented in Figure 2. All soil mixtures contained four replicates to ensure the reliability of the study results and minimize errors.

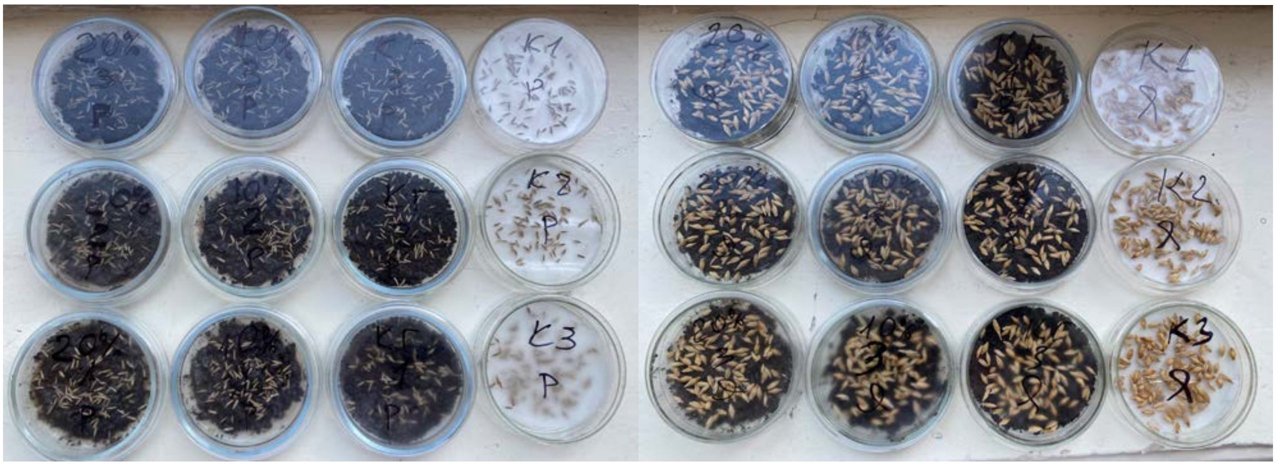

Figure 2. Soil mixtures with the addition of digestate on the day of sowing ryegrass (*Lolium perenne*) and barley (*Hordeum vulgare*)

The statistical treatment of germination results was conducted separately for each plant species, and the data were not transformed prior to the statistical analysis. In order to assess the reliability of the results, tests were conducted to examine the homogeneity and normality of the germination data. Considering the limited sample sizes of seed germination, statistical evaluations were performed using a t-test comparing soil control with the best germination treatment. This test allows for a comparison of mean differences between groups and helps determine if the observed differences are statistically significant.

3. Results and Discussion

3.1. Thermal analysis of the digestate obtained after methanogenesis

To determine the quality of methane fermentation and the degree of decomposition of the organic component, complex thermal analysis of the digestate samples was performed. The results of thermal analysis are presented in the form of thermograms (Figs 3-7).

Thermogravimetric curves (TG) show the mass loss of the sample during heating. Differential thermogravimetric analysis (DTG) curves show the rate of the sample weight loss. These curves are the result of differentiating TG curves. The differential thermal analysis (DTA) curves show the thermal effects of the corresponding sample transformations.

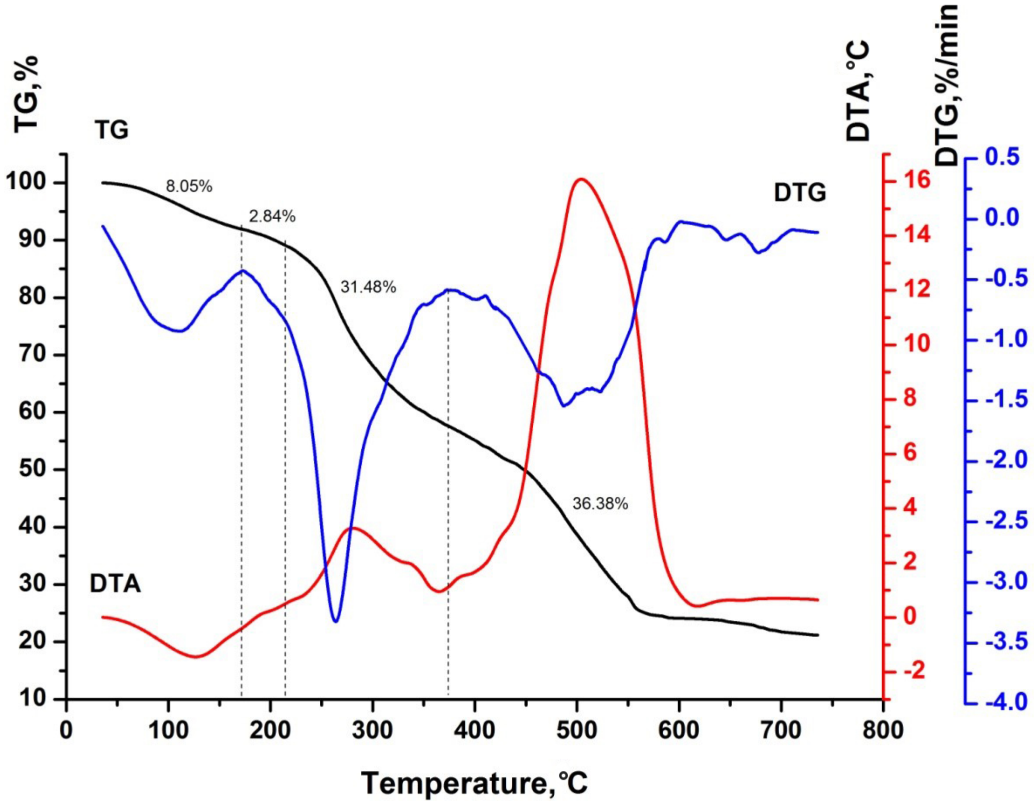

Figure 3. Thermogram of the sample of fermentation inoculum

In Figure3, a thermogram of a sample of seed biomass is shown. In the temperature range of 20-171°C, there is a release of volatile components contained in the biomass sample. This process is accompanied by a weight loss of the sample (8.05%) and the appearance of an endothermic effect on the DTA curve. The slight loss of mass of the enzyme sample (2.84%) in the temperature range of 171-215°C, accompanied by a characteristic kink in the DTG curve, corresponds to the first destructive processes in the sample.

Profound destructive processes in the sample of fermentation inoculum begin at temperatures above 215°C. In the temperature range of 215-374°C on the DTA curve, there is a clear exothermic effect corresponding to thermooxidative destruction of the sample. This process is accompanied by a significant loss of the sample of the fermentation nucleus (31.48%) and a deep extremum on the DTG curve. In the temperature range of 374-750°C, combustion residues are burned. This process corresponds to a weight loss of the sample of 36.38% and the appearance of a rapid exothermic effect on the DTA curve.

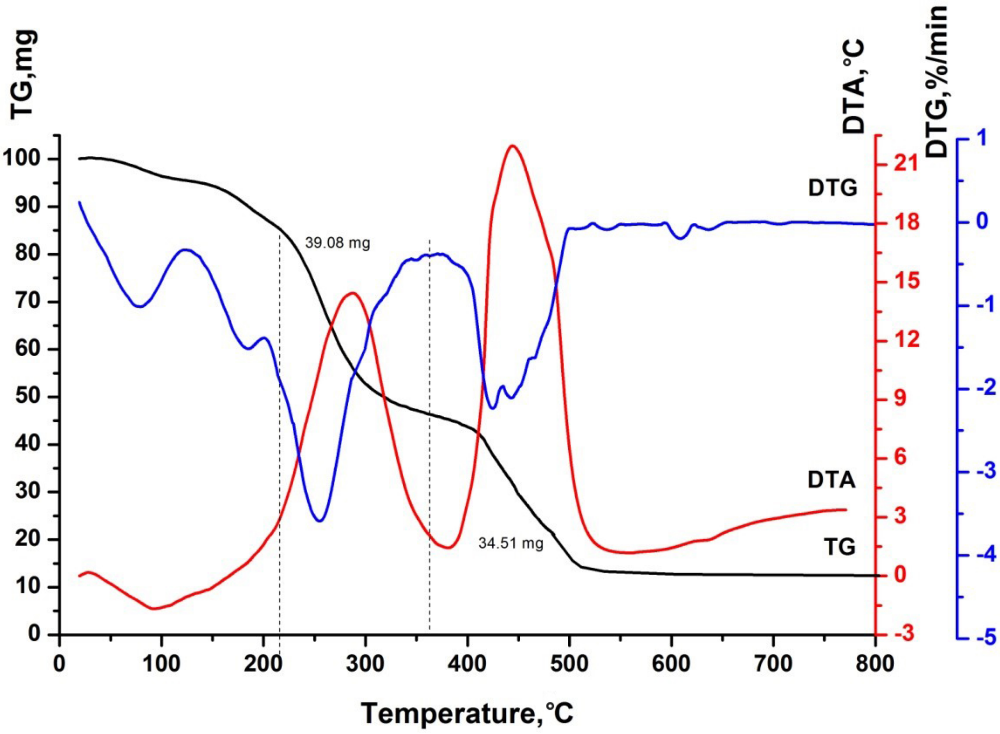

Figure 4. Thermogram of the digestate sample No.1 (не відповідає табл. 2!)

Figures 4-7 show the thermograms of the digestate samples No.1, No.2, No.3, No.4. Thermograms show, that the thermolysis of samples 1 - 4 proceeded in four stages.

At the first stage of thermolysis, in the temperature range of 20-134°C, hygroscopic water is released. This process is accompanied by a loss of the sample mass and the appearance of endothermic effects on the DTA curves.

At the second stage of thermolysis, in the temperature range of 125-201°C, the dehydration of the cellulose residues present in the digestate samples occurs (Yalechko et al., 2014). In the same temperature range, there is a partial destruction of the fermentation nucleus, which is part of the samples.

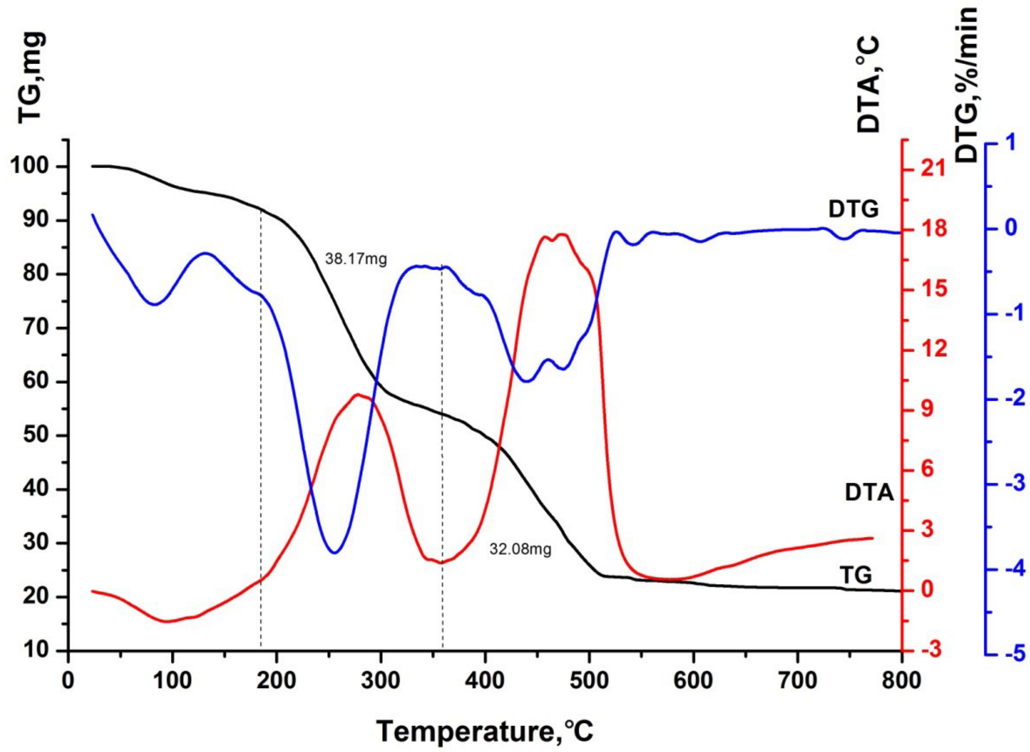

Figure 5. Thermogram of the digestate sample No.2

At the third stage of thermolysis, in the temperature range of 185-369°C, thermal-oxidative destruction of the sample components and combustion of the destruction products occur. This process is accompanied by a sharp loss of weight of the samples and the appearance of rapid exothermic effects on the DTA curves.

At the fourth stage of thermolysis, in the temperature range of 355-700°C, the pyrolytic residue of the samples burns, which is accompanied by the appearance of rapid exothermic effects on the DTA curves.

It should be noted that samples containing more fermentation inoculum have lower thermal stability. The onset of destructive and thermooxidative processes in these samples at the third stage of thermolysis shifts to the range of lower temperatures. Thus, thermal decomposition of sample No.2 (TS = 0.05, X_{in} = 0.2) begins at a temperature of 185°C, sample No.4 (TS = 0.1, X_{in} = 0.2) - at a temperature of 189 °C. The decomposition temperatures of samples No.1 (TS = 0.05, X_{in} = 0.05) and No.3 (TS = 0.1, X_{in} = 0.05) correspond to temperatures of 200°C and 201°C, respectively. It should be noted that the sample of the fermentation substrate is characterized by higher thermal stability compared to the samples of the fermentation substrate (Fig. 3). The onset of intensive destructive processes of the enzyme is observed only at temperatures above 215°C.

The samples with higher content of fermentation inoculum are able to thermo-oxidize more at the third stage of thermolysis and form lower content of charred residues as a result of combustion. Samples No.2 and No.4 are characterized by a shift of the maxima of the exothermic effects of the third stage of thermolysis to the range of lower temperatures.

In the case of the fermentation residue sample No.3, which is characterized by the low content of fermentation inoculum, the weight loss due to the thermal oxidative destruction and combustion of the organic component at the third stage of thermolysis is 38.90 mg. At the fourth stage of thermolysis, 34.4 mg of digestate sample No.3 is lost due to the combustion of the charred residue. Since the weight loss of the sample at the third and fourth stages of thermolysis is 73.3 mg, the content of organic components that can be thermally oxidized and burned is 53.06% at the third stage of thermolysis (Table 2).

Table 2. The results of the thermal analysis of digestate samples.

Sample	Stage	Temperature range, °C	Weight losses, mg	Organic matter losses, %
Digestate No.1 TS = 5% wt., X_{in} = 0.05	I	20 - 129	4.64	-
	II	120 - 200	7.64	-
	III	200 - 369	39.08	53.11
	IV	369 - 700	34.51	46.89
Digestate No.2 TS = 5% wt., X_{in} = 0.2	I	20 - 134	4.92	-
	II	134 -185	3.11	-
	III	185 - 355	38.17	54.33
	IV	355 - 700	32.08	45.67
Digestate No.3 TS = 10% wt., X_{in} = 0.05	I	20 - 125	4.05	-
	II	125 - 201	10.73	-
	III	201 - 369	38.90	53.06
	IV	369 - 700	34.40	46.94
Digestate No.4 TS = 10% wt., X_{in} = 0.2	I	20 - 128	6.78	-
	II	128 - 189	5.06	-
	III	189 - 366	35.56	51.91
	IV	366 - 700	32.94	48.09

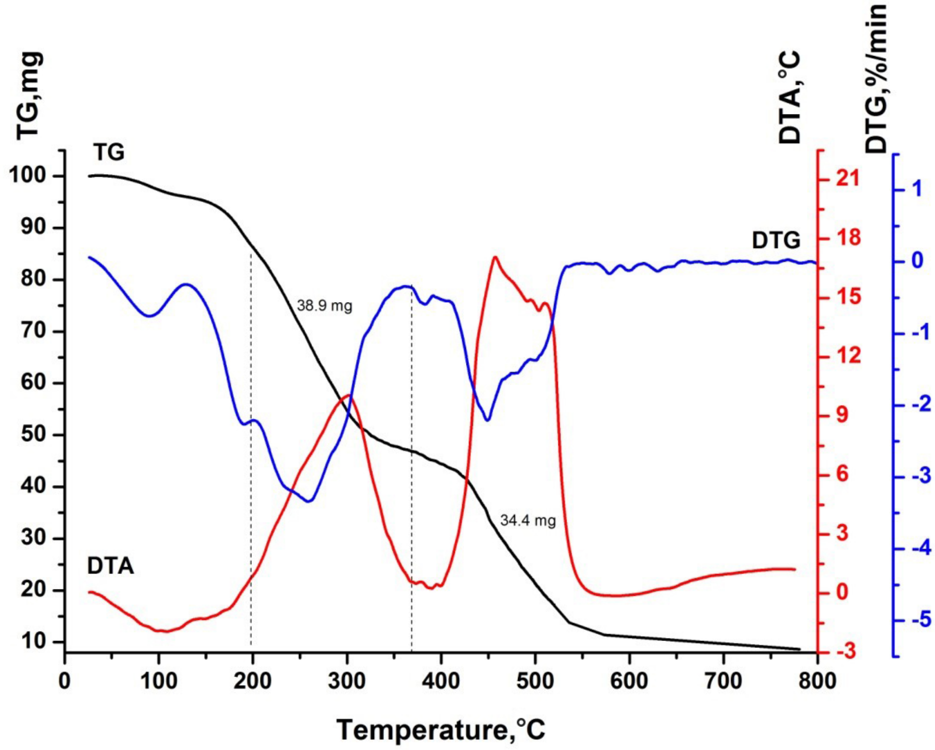

Figure 6. Thermogram of the digestate sample No.3

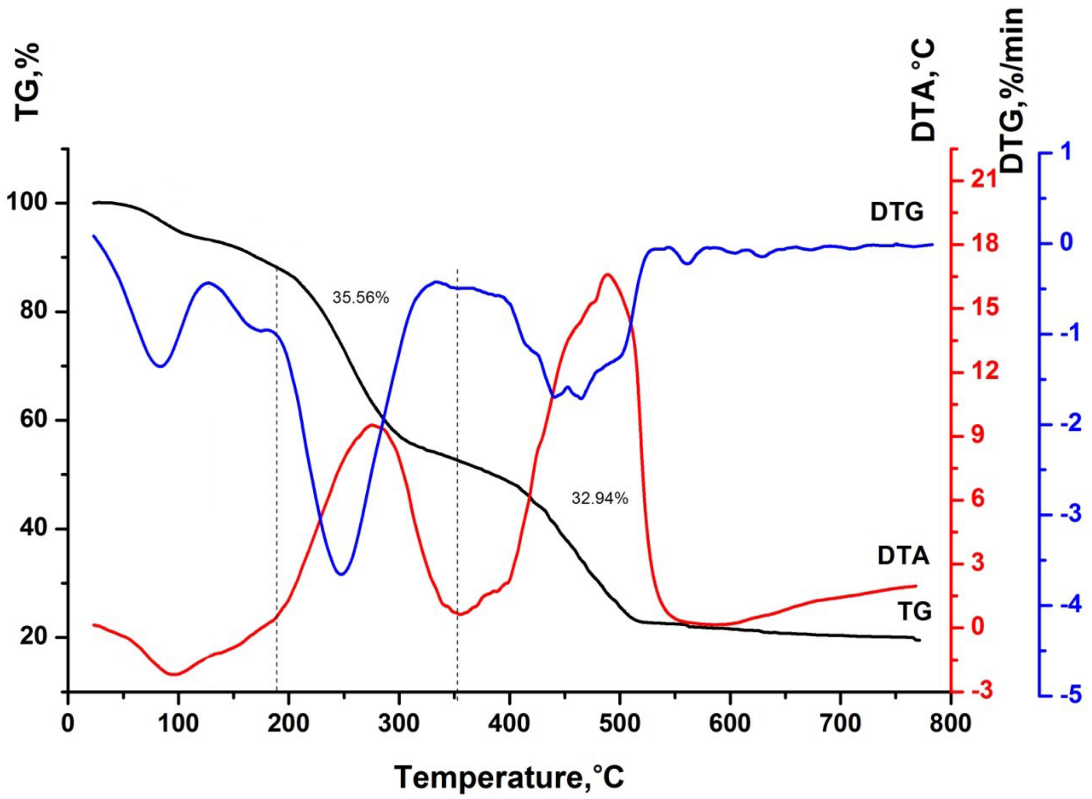

Figure 7. Thermogram of the digestate sample No.4

In the sample of fermentation residue No.2, which has the high inoculum content, the weight loss at the third stage of thermolysis is 38.17 mg. This corresponds to 54.33% of the organic matter losses at the third and 45.67% at fourth stage of thermolysis. (Table 2).

3.2. Preliminary preparation of the digestate

One of the most important tasks in the use of digestate as a component of the substrate is the release of excess moisture, because study has shown that all obtained digestates contain a significant amount of water in the range of 95.6-97.9% (Figs 8, 9).

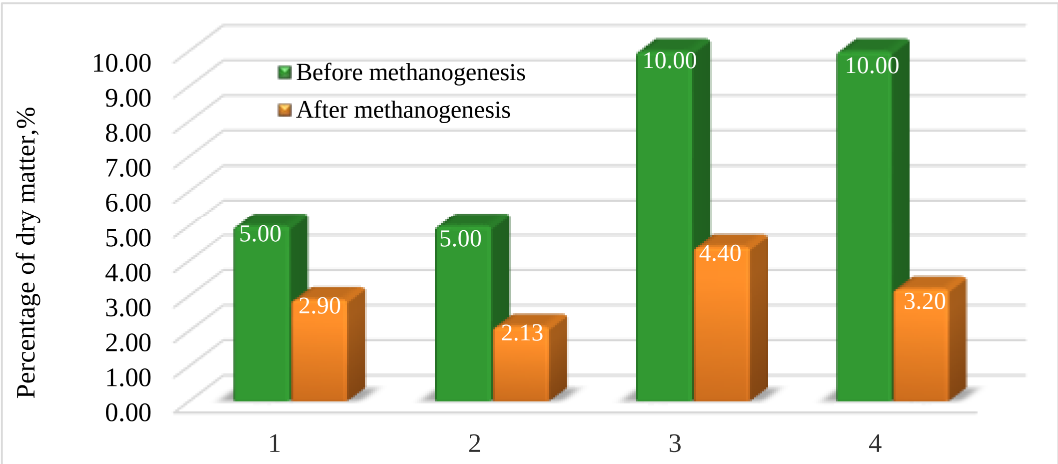

Figure 8. Dry matters in digestate before and after methanogenesis: 1 − TS = 5% wt.; X_{in} = 0.05; 2 − TS = 5% wt.; X_{in} = 0.2; 3 − TS =10% wt.; X_{in} = 0.05; 4 − TS =10% wt.; X_{in} = 0.2

Thus, each digestate sample differed in the amount of dry matter, which is due to the different ratio of biomass and inoculum and, accordingly, the process of methanogenesis. Thus, in the samples No.2 and No.4, with more inoculum, decomposition was more efficient, and more biogas was released. Therefore, the amount of dry matter during methanogenesis varied in different ways. Thus, in flask No.1, it decreased by 42.04% or 1.73 times, in flask No.2 - by 57.39% or 2.35 times, in flask No.3 - by 56.01% or 2.27 times, and the greatest decomposition occurred in flask No.4, where the amount of dry matter decreased by 68.01% or 3.13 times.

To add more digestate and prevent waterlogging, it is necessary to remove some of the excess moisture. We did this mechanically with an OPn-8 centrifuge (2 min at 5000 rpm) to eliminate the possible influence of flocculant research. The average results of the excess moisture are shown in Figure 9.

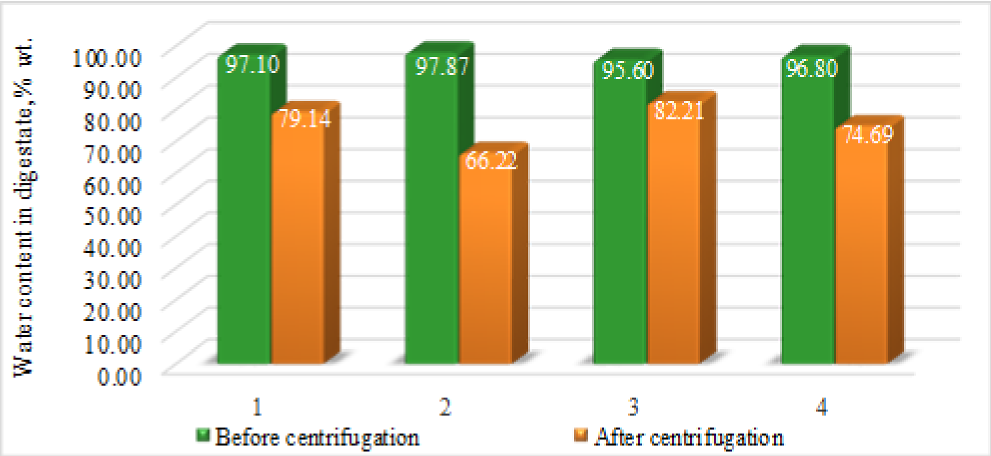

Figure 9. Water content in digestate before and after centrifugation: 1 – TS = 5% wt.; X_{in} = 0.05; 2 – TS = 5% wt.; X_{in} = 0.2; 3 – TS =10% wt.; X_{in} = 0.05; 4 – TS =10% wt.; X_{in} = 0.2

The release of excess moisture contributed to decrease the moisture content of the digestate before the bioindication studies by 17.96% for the first sample, 31.65% for the second, 13.39% for the third, and 22.11% for the fourth one. In this case, the following dependence is also observed: samples with a higher content of inoculum released moisture much better than those with a lower content. Thus, the second sample released 1.76 times more moisture than the first, and the fourth sample - 1.65 times more than the third.

3.3. Influence of digestate on crop germination

At this stage, the possibility of the influence of digestate on the germination of the studied crops was determined.

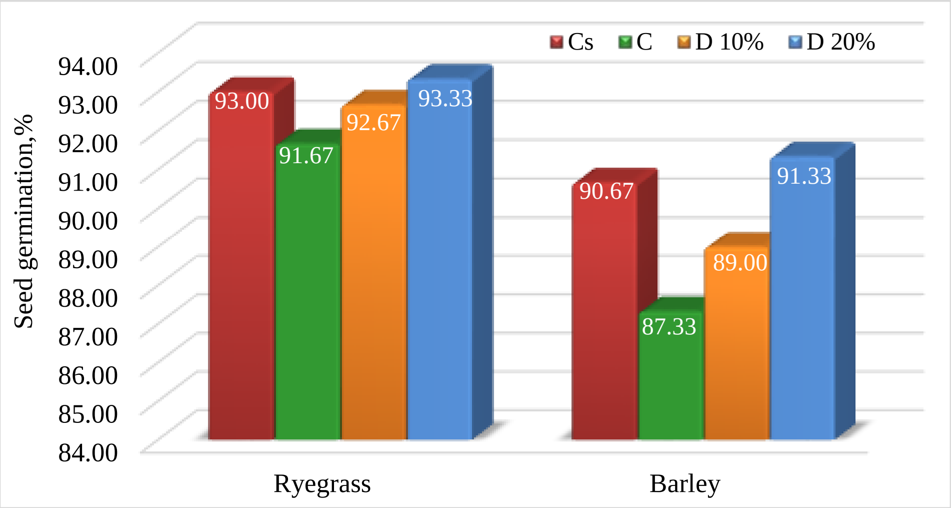

Figure 10. The influence of digestate on crop germination

The average results of the study are shown in Figure 10. Germination results were quite good in all studied variants and were equal to 87.3-93.3%. Ryegrass generally showed a better percentage of plant germination (91.7-93.3%), but barley shows a better positive effect of digestate on crop germination. Thus, the best germination of the two crops was observed in samples with digestate content of 20%, for ryegrass it was equal to 93.33% (1.67% more than the soil control, and due to the T-test this difference is statistically significant with a probability of 85.0%), in barley – 91.33% or 4.00% more than the soil control, corresponding to statistical significance of 78.1%. The probabilities that the variances of individual series are equal, according to two-sample F-tests, are in the range from 0.5 to 1.0. Figures 11 and 12 show the overview of samples on the 7th day of the study.

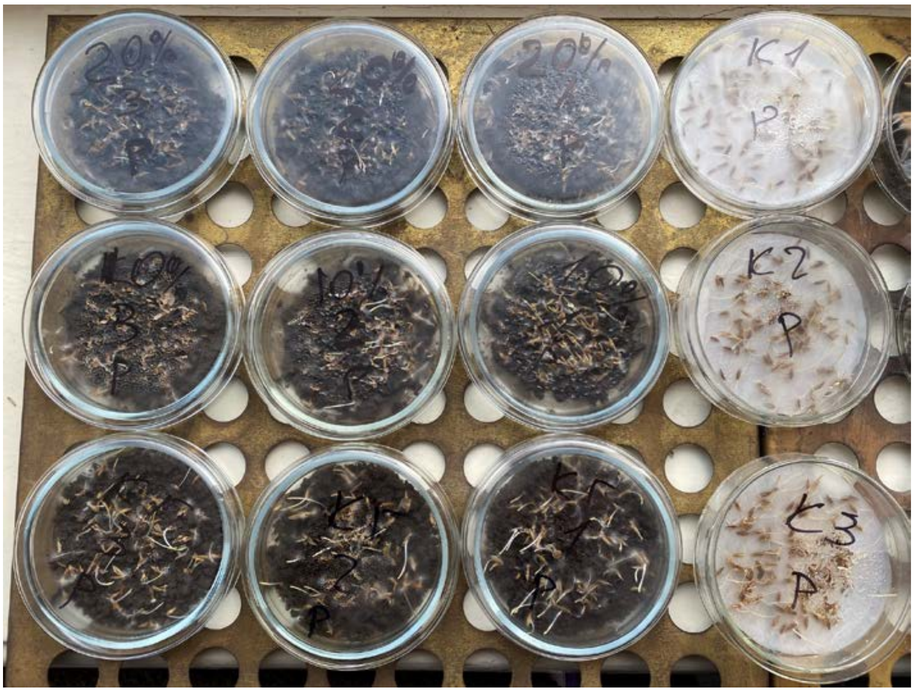

Figure 11. Soil samples with ryegrass (*Lolium perenne*) on the 7th day of the bioindication study

Figure 12. Soil samples with barley (*Hordeum vulgare*) on the 7th day of the bioindication study

Summarizing the data of the bioindication study, it can be concluded that soil samples with adding the digestate showed quite good results, so in the future it is advisable to continue research on plants to determine the effect of digestate on plant growth and development.

4. Conclusions

The analysis of the digestate after the mesophylic anaerobic fermentation using the thermal method showed that the content of inoculum has a significant effect on the decomposition of the organic component of broadleaf cattail biomass. Samples of digestate with higher initial inoculum content have lower thermal stability. The onset of destructive and thermooxidative processes in these samples, which can be observed at the third stage of thermolysis, shifts to the range of lower temperatures. The increased ability of samples No.2 and No.4 to thermooxidative degradation is due to the increased content of less thermally stable components, which are products of biological decomposition of the cattail biomass during its anaerobic fermentation.

The limiting factor for the use of digestate can only be its significant water content (95.6-97.9%), which requires prior dewatering. The degree of digestate dewatering by mechanical methods depends on the amount of undecomposed organic matter after methanogenesis. The less organic matter remains in the digestate, the easier it is dewatered. The release of excess moisture helped to reduce the water content of the digestate. A much better dewatering is observed in samples with a higher content of inoculum, and the maximum possible reduction in the water content of the digestate was achieved at 31.65%.

Bioindication study on the use of digestate in soil mixtures shown that crop plants (*Lolium perenne and Hordeum vulgare*) thrive in all studied samples compared to the soil control and the sterile control. This confirms the perspective of using digestate in agricultural technologies as a source of plant nutrients.

Acknowledgments

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