Testing the Impact of the Waste Product from Biogas Plants on Plant Germination and Initial Root Growth

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Abstract

During biogas production, anaerobic digestion of plant material rich in nutrients results in the so-called whole digestate. The application of nutrient-rich material present in digestate could have fertilising effects, especially in intensively used agricultural soils, and in crop yields that can affect the nutrient cycle. The aim of this article is to inform about possibilities of using mixture of digestate and haylage (use the fertilizing effect of both matters), and at the same time contribute to the improvement of agrochemical properties of soil. This study evaluates the effect of applying the mixture of digestate and haylage on germination and early stages of plant development. This article deals with primary test mixtures of digestate and haylage at ratios 10:1, 5:1 and 3:1 and compares the results with whole digestate applications. Simplified statistically calculated quantities showed that all examined mixtures better fertilizing effect in comparison with the control growing media. Based on the chemical analysis of the growing medias, a growing media with mixtures of digestate and haylage characterizing as growing medias with a high content of nutrients and a low amount of hazardous metal was investigated. The examined growing media thus met the limits for organic and commercial fertilizers. Fertilizing effects of growing media with mixture of digestate an haylage can also be noted on increasing the proportion of macronutrients in the soil, reducing fertilization only throughout whole digestate.

Keywords: *digestate, germination, haylage, soil, Petri dishes, biogas station, pH*

Introduction

In the future, humanity will face an increase in the human population leading to more inputs needed to produce food, including arable land, while inorganic minerals and fossil fuels have been on the decline. On the other hand, biogas technology offers a competitive process to manage biodegradable waste streams and to produce renewable energy in a sustainable way. Furthermore, the nutrients present in the waste materials are preserved in the anaerobic digestate, which can be further refined into value-added products, such as organic fertilizers and soil improvers.

Anaerobic digestion is an effective method of biomass processing in biogas plants, in which the organic matter decomposes in the absence of oxygen to give two valuable products, i.e. biogas and digestate. Biogas is a very useful source of renewable energy, while digestate is considered a valuable bio-fertilizer (Yu at al., 2010; Scaliglia at al., 2017). However, Govasmark et al. (2011) and Heviánková et al. (2013) proved the possible occurrence of pathogenic bacteria and heavy metals in digestate. This is why it is important that digestate is safe if used to substitute mineral fertilizers (Vázquez-Rowe et al. 2015)

If digestate is compared with conventional organic fertilizers, i.e. livestock fertilizers, the digestate has a relatively high total nitrogen content from 0.2 to 1% by weight, higher pH (7-8), lower carbon and dry matter content ranging from 2-13%. The content of easily degradable organic substances depends on the technical solution of Biogas plant. According to Vítěz et al. (2013), however, the longer the residence time of the BPS substrate, the less readily degradable substances will be present in the resulting digestate.

Many authors have agreed that digestate can be used in agricultural practice as a fertilizer. For example, according to Dimambro (2015), its introduction into agricultural practice can reduce the amount of inorganic fertilizers. According to Stoknes et al. (2016), digestate can serve as the main growing media component for the production of vegetables and mushrooms, and thus significantly improve the commercial profitability of tomatoes, cucumbers and lettuce.

Digestate is often considered as an organic fertilizer, but it contains stable organic matter, so it is rather mineral fertilizer (Heviánková et al., 2014). The use of digestate as fertilizer is limited primarily by hygiene requirements, the presence of hazardous elements and salinity. In a number of fermentation residues, higher concentrations of Cu and Zn were found, which did not comply with legislative requirements (Alburquerque et al, 2012). The export of digestate as fertilizer to agricultural land is governed by the Council Directive 91/676/EEC.

Fertilization is one of the main factors generating yield. Used properly, it helps maintain or increase soil fertility and productivity in an environmentally friendly manner. When used incorrectly, especially over a long period of time, it results in adverse changes in soil properties and other agroecosystem components, reduces plant productivity and degrades yield quality (Edmeades 2003, Gamzikov et al. 2007).

A combination of mineral fertilizer and farmyard manure enables to supply plants with nutrients for more than one vegetative growth season. Such fertilization system secures good plant nutrition with mineral nutrients for late growth and development stavage of plants (Bagdoniene, 1997).

The main weakness of digestate, however, is its pH, which is usually ranges from 8.2 to 8.6, in a wide range of digestates

Tab. 1. Average germination of samples – Petri dish test
Tab. 1. Średnie kiełkowanie próbek – test na szalce Petriego

AGR (%)	10:1	5:1	3:1	Digestate	Control
One dose	100	100	97,33	100	100
Double doses	92	94.67	96	96	97.33
Tree times doses	97.33	94.67	90.67	93.33	94.67
Four times doses	93.33	93.33	89.33	90.67	96
Five times doses	96	98.67	89.33	90.67	96
Six times doses	93	94.67	90.67	97.33	96

Tab. 2. Scattering coefficient for average germination – Petri dish test
Tab. 2. Współczynnik rozproszenia dla średniego kiełkowania – test na szalce Petriego

CVG	10:1	5:1	3:1	Digestate	Control
One dose	0	0	4.75	0	0
Double doses	7.53	2.44	4.17	7.22	2.37
Tree times doses	2.37	6.45	10.19	6.55	2.44
Four times doses	8.92	2.47	5.17	2.55	4.17
Five times doses	0	2.34	5.17	6.74	4.17
Six times doses	2.47	2.44	17.83	4.75	4.17

(Heslop and McCabe, 2012; N. Voća et al., 2005; Dimambro, 2012). The optimum pH range for most soil grown crops is between 5.5 and 7.0, being the range where plant nutrients are most available (Jensen, 2010). The pH value is most often adjusted with lime, according to a study by Jaskulska et al. (2014), however, it has been shown that this can lead to a deterioration of the agrochemical properties of the soil in the long term.

Therefore, in our study, we tried to adjust the pH using haylage, which is also organic matter and improves agrochemical conditions in agricultural land.co je cílem článku?

Materials and methods

Plant material, experimental design and growing conditions

For testing of germination we used Watercress (Lepidium sativum). Watercress is an annual herb growing to a height of 60 cm. Stems at the top of the branches, the leaves are pinnate, the leaves growing at the ground are more pronounced stalked. The flowers are arranged in clusters on the tops of the stems, are white or reddish and only about 2 mm in size. This plant was selected for testing based on the recommendations of EN 16086-2: 2012-01.

Characteristics of the growing media and nutrient solutions

We tried to bring our experiment as close as possible to real conditions, but at the same time maintain the methodology given by the EN 16086-2 standard. We used white top peat with a particle size of less than 10 mm, quartz sand in the range of 0.05-0.2 mm and kaolin Sigmaas substrate. The material would be mixed in a weight ratio of 74:20:5. After mixing, the soil was allowed to settle and then its pH was adjusted to 5.5-6.5, density 90 $kg.m^{-3}$ ± 20%, and EC 0.1 $dS.m^{-1}$. the tested digestate came from the family farm Stonava, Czech Republic. The optimal pH range for most crops grown in soil is between 5.5 and 7.0, which is the range in which plant nutrients are most available (Jensen, 2010). Digestate had ~95.67% water content, and the chemical parameters of the digestate were: TOC (39%), nitrogen (N 7.76%), phosphorus (P_2O_5 10.5%),potassium (K_2O 30.2%), and pH 7,76. The haylage was prepared by grinding it into smaller pieces corresponding to a size of 10 mm. At the same time it contained haylage ~3223% water content, and the chemical parameters of the haylage were: TOC (41.7%), nitrogen (N 1.58%), phosphorus (P_2O_5 222%),potassium (K_2O 24,2.%), and pH 7.76.

Phytotoxicity test, microbiological analyses and agronomic traits

To assess the influence of mixtures of digestate and haylage, a phytotoxicity test was performed according to DIN EN 16086-2:2012-01, i.e. by incubating twenty seeds of Lepidium sativum (cress), a high sensitive reference species used in phytotoxicity bioassays according to Wang et al. (2001), at 20°C in Petri dishes, replicated tree times.

We prepared growing media in Petri dishes by adding digestate and haylage under conditions of 10:1 (3.15 g of digestate and 0.35 g of haylage), 5:1 (2.92 g of digestate and 0.58 g of haylage) and 3:1 (2.62 g digestate and 0.88 g of haylage) and whole digestate (3.5 g of digestate), three Petri dishes were used as controls. The sealed foil-sealed Petri dishes are incubated for 72 hours at an angle of 70° to 80° to the horizontal, with the seeds sown at the top and the growing media at the bottom, in the dark at a constant value of 25±5 °C.

Seventy two hours after germination, the germination index percentage (GI%) was calculated according to the formula GI% = 100 × (G1/G2) × (R1/R2), where G1 and G2 are germinated seeds in the sample and control, and R1 and R2 are mean root lengths for the sample and for the control, respectively.

The concentration of mixures matters (digestate and haylage) was then increased by adding a given proportion of digestate and hay again to the same Petri dishes, and new watercress seeds were reseeded after 4 hours. The fifth replicates of these increasing concentration were performed in this way.

Flame AAS analysis and GC analysis

These analyses were performed on flame atomic absorption spectrometry (AAS) using Mehlich-3 extraction (Mehlich,

Tab. 3. Root length index – Petri dish test
Tab. 3. Wskaźnik długości korzenia – test na szalce Petriego

RI (%)	10:1	5:1	3:1	Digestate	Control
One dose	104.27	119.03	110.30	111.97	100
Double doses	118.23	125.48	120.92	128.76	100
Tree times doses	142.17	126.37	120.29	126.74	100
Four times doses	139.18	129.54	117.53	133.66	100
Five times doses	137.63	159.94	128.35	131.49	100
Six times doses	144.15	134.04	131.26	131.45	100

Tab. 4. Munoo-Liis vitality index – Petri dish test
Tab. 4. Wskaźnik witalności Munoo-Liisa – test na szalce Petriego

MLV (%)	10:1	5:1	3:1	Digestate	Control
One dose	104.27	119.03	107.32	111.97	100
Double doses	112.03	122.03	119.62	127.53	100
Tree times doses	**146.16**	126.68	115.30	124.97	100
Four times doses	136.29	126.13	109.77	126.36	100
Five times doses	137.63	**164.51**	119.98	124.66	100
Six times doses	140.37	132.30	**128.14**	**133.07**	100

1984; Wolf and Beegle, 1994). The suitability of the extraction solution Mehlich 3 for determination of fertiliser requirement was tested using the correlations between the contents of the corresponding plant nutrients in the parallel soil-plant samples. According to Sen Tran & Simards (1993), the extraction solution according to the Mehlich 3 method consists of : 0.2 N CH_3COOH, 0.25 N NH_4NO_3, 0.013 N HNO_3, 0.015 N NH_4F and 0.001 M EDTA – combines microelements into complex compounds and avoids precipitation of Ca. Phosphorus, potassium, magnesium, copper and manganese were determined from the plant material by dry ashing of office analytical method: K – 71/250 EEC; Mg, Mn – 78/633 EEC.

For determination of nutrients in soil (phosphorus, ammonia nitrogen - $N\text{-}NH^{4+}$, nitrite nitrogen - NO^{3}) by GC analysis was carried out with a gas chromatograph equipped with a flame photometric detector (P-filter) with using Mehlich-3 extraction (Mehlich, 1984; Wolf and Beegle, 1994). Macronutrients in soil (Ca, Mg, K, Na) were determined using Gilligam solution (Gillman, 1976).

All chemical analyses were made at a Czech accredited laboratory.

Statistical analysis

Experimental data were analysed using software Statistica for factorial analysis of variance (ANOVA). Multivariate data analysis was performed using principal component analysis (PCA) to assess the existing relationships between the variables examined and the parameters recorded.

Results and discussion

Today, soilless cultivation systems use large amounts of non-renewable materials (Ronga et al., 2016); Therefore, alternative growing medias are needed to improve the sustainability of production. Digestate (mainly solid digestate) appears to be suitable as a growing media (Stoknes et al., 2016), although, as some reports on compost suggest, high pH promotes their mixing with other matrices capable of reducing this value (Bugbee, 1996; Ronga et al., 2016).

The results of the measured root lengths and the calculated percentage of germination from the number of germinated seeds, from tests on Petri dishes, were further used in the calculations of statistical quantities determined by the standard EN 16086-2: 2011. Interestingly, no effects of phytotoxicity on Watercress were observed in this study (Table 1): all mixtures, including digestate, gave germination index values above 50%, which is commonly recognized as a threshold value for many crops (Zucconi et al., 1981).

In particular, all doses of mixtures and digestate gave a very similar germination value as the control samples (their difference compared to the germination of the control sample ranged from 5-7%), the most significant improvement in germination was achieved with a triple dose of digestate and haylage in a ratio of 10: 1. Similar results were obtained by Gell et al. (2011) and Sánchez et al. (2008), who evaluated digestive phytotoxicity on lettuce, radish, wheat and watercress.

Responses in terms of germination of seeds of Lepidium sativum can be used to evaluate toxic effects on plants, thus allowing the determination of the quality of waste through a simple assay (Zucconi et al., 1985). The toxic effects on plants are a combination of multiple factors (Zucconi et al., 1981).

Table 2 contains data on the coefficient of variation for the root length. It is valid that the larger the coefficient, the more the data differ from the average (the difference is again determined on the basis of the squares and its difference between the arithmetic average and the measured value). Here, too, it can be seen that the coefficients are relatively low, so in most tests we worked with a homogeneous set, which showed a relatively low variability of measured values.

In the case of determining the root length index according to EN 16086-2: 2012-01, the control sample is taken as the default value, which is assigned a value of 100%, for other mixtures it is then calculated and determined whether their lengths are greater (or shorter) than the root in the control sample. In general, we could say that the rule is that the longer the root, the better the fertilizing effect of the mixture. In our

Tab. 5. Germination indices of watercress phytotoxicity test
Tab. 5. Wskaźniki kiełkowania testu fitotoksyczności rukwi wodnej

	10:1	5:1	3:1	Digestate	Control
lv (mm)	5.02	4.59	3.81	4.58	4.59
kv (%)	79.2	79.2	72	73.8	72
IK (%)	**120.39**	**110.00**	**83.05**	**102.25**	**100.00**

Tab. 6. Average elemental composition of the soil after the experiment
Tab. 6. Przeciętny skład pierwiastkowy gleby po doświadczeniu

	units	10:1	5:1	3:1	Digestate	Control
pH		6.7	6.6	6.6	7.1	6.8
Calcium (Ca)	mg/kg of dry matter	300	284	268	336	256
Magnesium (Mg)	mg/kg of dry matter	38	36	36	33	31
Potassium (K)	mg/kg of dry matter	37	41	49	31	18
Sodium (Na)	mg/kg of dry matter	18	20	18	19	16
Exchange acidity (Al+H)	mmolchekv/ kg	<1	<1	<1	<1	<1
Ammonia nitrogen	mg/kg	1440	1410	1520	1280	1560
Total nitrogen	% of dry matter	0.15	0.17	0.18	0.14	0.16
Total organic carbon	% of dry matter	3.51	3.35	3.6	2.77	2.64

monitored samples, the root length index was above 100% for all examined samples (see Table 3).

In addition to the average root lengths, the Munoo-Liis vitality index (Table 4) also takes germination into account. Here, too, all the samples examined were above 100%. It again confirms that all the mixtures examined can be used as fertilizer. All mixtures showed increased germination compared to the control sample. In the case of digestate, we reached the same values as in the study of Maunuksel et al. (2012), which, in addition to pot tests with Chinese cabbage (Brassica pekinensis) and barrels, also performed germinating ten cress seeds (Lepidium sativum). No similar studies were found to compare other mixtures.

According to Table 5 of results of the germination index of tested samples from the watercress phytotoxicity test, samples 3: 1, digestate and haylage can be interpreted as well-mature "compost" and samples 10:1, 5:1 and digestate as compost with stimulating ability. All tested samples came out above 80%, which means that the fertilizing effect decreases and the effect of humus is stronger, i.e. that nutrients are more bound. Nitrogen and phosphate release is slower and nutrients do not leach into groundwater. All examined samples are therefore satisfactory from the point of view of the determined phytotoxicity on watercress germination.

The best ratio of germination indices was 10:1 with the resulting germination index of 120.39%. Another ratio is divided by a ratio of 5:1 with a separate with a germination index of 110%. Another digestion index, which was above 100%, was a digestate sample.

These results confirmed a study performed by Dimambr (2015), which showed that appropriately diluted digestate (in terms of NH_4-N concentrations, pH and electrical conductivity) can generally achieve similar or higher yields compared to standard growing methods.

The most important indicators in the table are probably pH and ammoniacal nitrogen. When the pH rises above 6.5 some of the nutrients, micro-nutrients begin to precipitate out of the solution and can no longer be absorbed by the crop (Rush, 1987).

Hence for some horticultural crops reducing the pH below 7 is recommended, such as for hydroponic tomato (Neal and Wilkie, 2014), cucumber (Liedl, 2006) and lettuce production (Liedl, 2004b). At the same time a number of studies have highlighted the importance of considering the quantity of mineral N in digestates as this is the portion of N which is readily available to the crop (WRAP, 2012; Risberg, 2015). For horticultural purposes, a number of authors have highlighted the importance of matching the NH4-N concentration of the digestate to the crop requirements (Neal and Wilkie, 2014; Liedl, 2004a).

From this point of view, the values in the case of digestate appear to be the least suitable. As in the study according to Calamai (2020), all mixtures showed a significant increase in the content of secondary macronutrients (Ca, Na, K, Mg). The degree of alkaline saturation is an important indicator of agricultural soil quality. The value of this characteristic below 50% indicates a degraded soil (fertilisation, crop rotation), at the level of 50–75%, also agrotechnical errors, only values above 85% indicate a good physicochemical condition of the soil. As emphasised by Brodowski et al. (2005) and Cheng et al. (2006), this parameter plays a crucial role in the retention of water and nutrients for plants. Cation exchange capacity is important for maintaining adequate quantities of plant-available Ca, Mg, and K in soils. The applied fertilisation also influenced the share of cations in the sorption complex, generally decreasing the share of H+ ions and increasing slightly the share of Ca^{2+} ions and more clearly increasing the share of Mg^{2+} and K^{+} cations. The optimal Ca to Mg ratio should be 7:1 (Łabetowicz, 1999). According to Sanik et al. (1952), the Ca:Mg ratio has an impact on the solubility of cations in the soil solution. In our study, the ratio in the case of mixtures was in the range of 7.8-7.4:1, in contrast, in the case of digestate, the range was 10.1:1. The test results for the digestate are the same as for the research by Glowack et al. (2020).

Conclusion

In all cases of the examined samples of the determined test with watercress on Petri dishes, the growth of the root length was stimulated in comparison with the control growing media. From all statistically calculated quantities it can be deduced that all tested growing medias are in a positive position compared to the control growing media. Growing medias therefore have a positive effect on plant development. Based on chemical analyses of growing medias performed in the accredited laboratory Morava, the mixed growing medias can be characterized as growing medias with high nutrient content and heavy metal content meeting the limits for organic and livestock fertilizers according to EU regulation 2019/1009., On setting requirements for fertilizers, as amended. From the results of the watercress phytotoxicity test according to Plíva et al. (2006), it can again be deduced that the researched mixture matters are growing medias with fertilizing effects and can be used as fertilizer in all aspects. Due to the low homogeneity of the investigated growing medias, the results were not objectively comparable, and therefore it is not possible to say unambiguously which mixture of the tested ratios was the most suitable for root growth and germination. However, all mixtures achieved a more significant improvement in the agrochemical properties of the soil compared to whole digestate fertilization. For a clear conclusion, it would be necessary to continue testing in a longer time horizon and in real conditions, such as in the case of a long-term experiment in Jaskulska et al. (2014) or Glowack (2020), who examined the impact of different agricultural practices on soil quality and yields.

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Badanie wpływu produktów odpadowych z biogazowni na kiełkowanie roślin i początkowy wzrost korzeni

Podczas produkcji biogazu fermentacja beztlenowa bogatego w składniki pokarmowe materiału roślinnego skutkuje powstaniem tzw. pofermentu. Zastosowanie materiału bogatego w składniki odżywcze obecnego w pofermencie może mieć działanie nawozowe, zwłaszcza dla intensywnie użytkowanych gleb rolniczych oraz dla plonów, może wpływać na cykl składników odżywczych.
Celem artykułu jest przedstawienie możliwości wykorzystania mieszanki pofermentu i sianokiszonki (wykorzystanie efektu nawozowego obu substancji), a jednocześnie przyczynienie się do poprawy właściwości agrochemicznych gleby.
W pracy oceniono wpływ zastosowania mieszanki pofermentu i sianokiszonki na kiełkowanie i wczesne etapy rozwoju roślin. Artykuł dotyczy podstawowych mieszanek testowych pofermentu i sianokiszonki w proporcjach 10:1, 5:1 i 3:1 i porównuje wyniki z zastosowaniem całego pofermentu. Uproszczone statystycznie obliczone ilości wykazały, że wszystkie badane mieszanki mają lepsze działanie nawozowe w porównaniu z kontrolnymi podłożami uprawowymi. Na podstawie analizy chemicznej podłoży uprawowych zbadano podłoża uprawowe z mieszaniną pofermentu i sianokiszonki, charakteryzujące się jako podłoża uprawowe o wysokiej zawartości składników odżywczych i niskiej zawartości metali niebezpiecznych. Badane podłoża uprawowe spełniały tym samym limity dla nawozów organicznych i komercyjnych. Nawozowe efekty podłoży uprawowych mieszanką pofermentu i sianokiszonki można również zauważyć na zwiększenie udziału makroskładników w glebie, ograniczając nawożenie tylko w samym pofermencie.

Słowa kluczowe: *poferment, kiełkowanie, sianokiszonka, gleba, szalki Petriego, stacja biogazowa, odczyn pH*