

The Application of Z-Ion Substrate to Support Energy Crop Growth (*Dactylis Glomerata L.*) on Degraded Soil

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ABSTRACT

Investigations concerned the effect of raising the dose of new Z-ion zeolite substrate on cocksfoot (*Dactylis glomerata L.*) growth. During the pot experiment, plants were grown on degraded soil, arable soil and mixtures of degraded soil with increasing Z-ion substrate additions (1%, 2%, 5%, 10% v/v). When the experiment was terminated, the mean values of the vegetative parameters of test species were calculated. The carbon to nitrogen ratio for cocksfoot stem biomass was determined. The enzyme diversity of the degraded soil enriched with substrate additions after cocksfoot growth (Shannon's diversity index) was also evaluated. The application of Z-ion additions positively influenced the cocksfoot growth – the additions in the range of 1–10% v/v to degraded soil significantly increased wet and dry stem biomass, dry root biomass and total dry biomass of plants. It turned out that the Z-ion substrate addition not exceeding 1% v/v can be considered as one which – after introducing into a specific degraded soil – would give similar biomass yield of cocksfoot to that obtained on the selected arable soil. At 1% substrate dose, the carbon/nitrogen ratio in the plant material (27.17) was within the range of values ensuring the proper methane fermentation course. The preliminary studies have shown that a significant increase in enzyme diversity can be observed when there is a certain degree of root development caused by a sufficiently high addition of Z-ion substrate to the degraded soil – under experimental conditions it was 5% v/v Z-ion dose.

Keywords: energy crop, cocksfoot, Z-ion substrate, degraded soil

INTRODUCTION

In the face of the fossil fuels depletion, the increasingly common use of renewable energy sources in the form of wind, sun, water and biomass is an urgent need. From an ecological point of view, biomass is the organic matter produced by plants during photosynthesis and used by heterotrophic organisms as a source of carbon and energy. According to Directive 2009/28/EC [2009], biomass is the biodegradable fraction of products, waste and residues of biological origin from agriculture (including vegetal and animal substances), forestry and related industries including fisheries and aquaculture, as well as the biodegradable fraction of industrial and

municipal waste. Currently, biomass occupies the largest share in energy production among the renewable energy sources in Poland [Igliński et al. 2009], either through its direct combustion or conversion into biofuels in thermochemical (e.g. gasification), chemical (e.g. transesterification) or biological (e.g. methane fermentation, alcohol fermentation) processes [Klimiuk et al. 2012]. As shown in the definition of biomass mentioned above (Directive 2009/28/EC), it can come from various sources, including the cultivation of energy crops/species such as: maize, rape, fodder and sugar beets, willow or perennial grasses [Seppälä et al. 2009, Hattori and Morita 2010, Butkute et al. 2014, Del Grosso et al. 2014]. Energy crops can be cultivated on typical

agricultural lands, but in view of the indicated problem of competition for land for food and feed production [Herrmann et al. 2016, Fritsche et al. 2010] it is proposed to establish plantations of less demanding energy species on marginal or degraded soils [Nabel et al. 2014, Mocek-Płóćiniak 2014]. Cultivation of selected energy plants on degraded areas should bring significant ecological benefits. In fact, the plant roots present in the degraded soil are a source of organic matter (exudates, tissue residues), which provides nutrients for the developing populations of different microorganisms, influencing their species richness, biomass and enzymatic activity [Wallenius et al. 2011, Fageria and Moreira 2011, Natywa et al. 2014]; organic matter is also the starting material for the forming process of typical humic compounds that determine the sorption properties of soils as well as affect their structural, thermal and edaphic conditions [Zawadzki 2009]. Furthermore, the cultivation of perennial energy species, especially grasses, can counteract the erosion of degraded land, sequester C with the mitigation of greenhouse gas emissions, create wildlife habitats or increase the landscape and biological diversity [Butkute et al. 2014].

Cultivating energy crops on degraded soils requires the application of agrotechnical management practices, including mineral fertilization. This fertilization can be realized with the use of new zeolite substrates first prepared by workers of the Institute of Physical and Organic Chemistry of the Belarusian Academy of Sciences in Minsk–IFOCH NASB [Kosandrovich et al. 2019]. These substrates (with the “Z-ion” trade mark) are produced from clinoptilolite (loaded with potassium and ammonium ions) and ground phosphate rocks. The nutrient ions concentration in the solution equilibrated with the given substrate variant is regulated by equivalent ion exchange of K^+ and NH_4^+ ions with the plant metabolites and restricted solubility of the minerals containing calcium and magnesium phosphates. Nitrogen in Z-ion substrate is in the ammonium form, which facilitates the assimilation of this element by plants, because NH_4^+ ions are directly involved in the synthesis of amino acids; therefore, there is no energy loss for the reduction of nitrates to ammonium ions. At the same time, unlike conventional nitrogen fertilizers, the Z-ion substrate does not acidify soil when used as an additive, because the zeolite matrix, playing the role of an anion, is not

osmotically active. Another advantage of using zeolite substrates as soil additives is the ability to increase the retention of nutrient cations. After the supply of nutrient ions from the substrate is depleted, the soil can be fertilized with conventional fertilizers – the cations derived from them are retained in the zeolite structure and therefore less washed away by water, which improves the economic aspect of using typical mineral fertilizers and reduces groundwater contamination [Kosandrovich et al. 2019].

Taking into account the need to support the development of energy crops on degraded soils, the main objective of the study was to determine the influence of the increasing additions of a new zeolite substrate on the growth of cocksfoot (*Dactylis glomerata* L.) – the species recommended in biological soil restoration and the species whose biomass is used as a feedstock in biogas production systems. An additional objective was preliminary evaluation of the enzymatic activity of degraded soil enriched with Z-ion additions after the test species growth.

MATERIAL AND METHODS

In the research, degraded soil, arable soil and Z-ion substrate were used as components of prepared growing media. The Z-ion substrate (a nutrient carrier) was produced according to the IFOCH NASB procedure at the experimental plant of “Project WISMUT” Ltd. (Russia). It was a mixture of ammonium and potassium forms of clinoptilolite with weakly soluble calcium and magnesium phosphates. The substrate contained the following nutrients amounts (mmol/kg): N – 324, P – 100, K – 110, Ca – 113, Mg – 80, Na – 101, S – 1.4.

The degraded soil was taken from the area of the sand mine in Rokitno (Lublin province, Poland). The pH value of 1 M KCl–soil extract was 4.25. The soil contained low (S), very low (P, K, Mg) or insufficient (N, Ca) amounts of nutrient elements regarding plant needs (Table 1).

The arable soil was harvested from the farm located in Czesławice (Lublin province, Poland). The pH value of its 1 M KCl extract was 5.4. The soil was characterized by very high phosphorus content, high potassium and magnesium content, as well as low sulfur content (Table 1). The contents of nitrogen and calcium were higher than in degraded soil but they were

Table 1. Contents of plant available nutrients in soils

Soil	N-NH ₄ [mg/dm ³]	N-NO ₃ [mg/dm ³]	P ₂ O ₅ [mg/100g]	K ₂ O [mg/100g]	Mg [mg/100g]	Ca [mg/dm ³]	S-SO ₄ [mg/100g]
Degraded soil	2.60	13.64	3.25	1.55	1.75	196	0.44
Arable soil	4.39	21.71	39	20.15	8.45	538	0.57

Explanations: Plant available contents of macronutrients were determined according to methods described in Ostrowska et al. [1991], Polish Standard, PN-R-04023 [1996], Polish Standard PN-R-04022 [1996], Polish Standard PN-R-04020 [1994].

not fully satisfactory as far as plant demands are concerned; hence, the arable soil could be defined as that of medium quality.

The studies were performed using cocksfoot (*Dactylis glomerata* L.) as the test species. In total, 48 pots of media (360 cm³ volume each) were prepared and split into six series: the two control series (degraded soil and arable soil) and four degraded soil series with increasing Z-ion substrate additions – 1%, 2%, 5%, 10% (v/v) – see Table 2. While preparing media series, CaCO₃ was added to the degraded soil to raise its pH to the pH level of the arable soil (Table 2).

The pot test began on 4th April 2019. Forty cocksfoot seeds were sown in each pot. After seed germination, the plants number in each pot was set at 25. The experiment was performed in a phytotron under the following conditions: 13/11 photoperiod, 25±1/16±1°C day/night temperature. The experiment was finished after 6 weeks from the time of seed sowing. The plant stems were cut down (in all pots) and roots were separated (from five pots of each media series). The wet and dry (105°C) biomass of stems and dry (105°C) root biomass were measured. The total dry biomass of plants was measured as a sum of dry stem and root biomass. The stem biomass was analyzed for total organic carbon and total nitrogen content. The C content was determined using RC 62 LECO apparatus, while the N content was analyzed with Kjeltac™ 8200 Foss Tecator system. The results of carbon and nitrogen analysis were used to calculate the C:N

ratios in the stem biomass of cocksfoot growing in the media series.

The preliminary study of enzymatic activity of degraded soil enriched with Z-ion additions was carried out using the API ZYM semi-quantitative miniaturized system (Bio Merieux). The API ZYM assay enables testing the activity of 19 enzymes involved in the breakdown of peptides, phosphomonoesters, lipids, mucopolysaccharides and polysaccharides (e.g. chitin, cellulose, starch, galactans) – Table 3 (Bending et al. 2002). The API ZYM assay was started at day of the pot experiment termination. Soil (after roots separation) was taken from 3 pots of the media series (degraded soil and degraded soil emended with Z-ion additions) and thoroughly mixed. The soil extracts (three replicates for each media series) were prepared by shaking soil with sterile 0.85% NaCl solution (1:1 w:v) for 20 minutes using Multi Rotator PTR-35, then the soil suspensions were allowed to settle for 10 minutes and centrifuged at 2000 g for 10 minutes. Aliquots of the obtained supernatants (65 µl) were pipetted into the microcupules of the API ZYM strips. The strips were covered and incubated at 22°C for 16 hours. After incubation, 30 µl of two reagents (ZYM A and ZYM B) were introduced into microcupules to develop chromogenic substrates. The color reactions were read by four observers who assigned integer values from 0 to 5 to each reaction according to the color chart prepared by the manufacturer of the API ZYM kit. Relative activities of enzymes expressed as numerical

Table 2. Media series used in the pot test

Media series	Pot number	Soil [cm ³ per pot]	Substrate addition [cm ³ per pot]	CaCO ₃ addition [mg per pot]
Degraded soil (DS)	8	300	-	229.7
Arable soil (AS)	8	300	-	-
Degraded soil+1% Z-ion (DS+1%)	8	297	3	227.4
Degraded soil+2% Z-ion (DS+2%)	8	294	6	225.1
Degraded soil+5% Z-ion (DS+5%)	8	285	15	218.2
Degraded soil+10% Z-ion (DS+10%)	8	270	30	206.7

Table 3. Extracellular enzymes of the API ZYM assay

Phosphatases	Alkaline phosphatase
	Acid phosphatase
	Phosphohydrolase
Esterases	Esterase (C4)
	Lipase (C14)
	Lipase-esterase (C8)
Amino-peptidases	Cystine arylamidase
	Leucine arylamidase
	Valine arylamidase
Proteases	Trypsin
	α -Chymotrypsin
Glycosyl hydrolases	α -Galactosidase
	β -Galactosidase
	β -Glucuronidase
	α -Glucosidase
	β -Glucosidase
	N-acetyl- β -glucosaminidase
	α -Mannosidase
α -Fucosidase	
Enzyme activity: 0 – 0 nanomoles substrate hydrolysed; 1 - 5 nanomoles substrate hydrolysed; 2 - 10 nanomoles substrate hydrolysed; 3 – 20 nanomoles substrate hydrolysed; 4 - 30 nanomoles substrate hydrolysed: 5 - 40 or more nanomoles substrate hydrolysed	

values were recalculated into nanomoles substrate hydrolyzed according to the instruction provided by the manufacturer (Table 3). The data on the enzymatic activity in the test media series were used to calculate Shannon's diversity index using the following formula [Gove et al. 1994]:

$$H' = -\sum_1^i pi \log_2 pi \quad (1)$$

where: i is i -th enzyme,

pi is the ratio of the activity of a particular enzyme to the sum of all enzyme activities.

The calculated Shannon's index can express the enzyme diversity detected in the studied soil or soil microbial functional diversity under the assumption that the origin of most soil enzymes is microbial [Boluda et al. 2014].

The data obtained on plant yield (wet and dry stem biomass, dry root biomass, total dry plant biomass) and enzyme activity (Shannon's diversity indices) were subjected to statistical analysis. After verifying the assumption of data normality (using Shapiro-Wilk's test) and variance homogeneity (using Lavene's test), the significance of differences between mean values for dry stem

biomass, total dry plant biomass and Shannon's diversity indices was tested by ANOVA followed by Tukey's post hoc test at $p \leq 0.05$ [Wolek 2007]. In the case of wet stem biomass and dry root biomass (variance heterogeneity), the data were subjected to F Welch's test and the means were separated using T3 Dunnett's post hoc test at the 0.05 significance level [Shingala and Rajyaguru 2015].

RESULTS AND DISCUSSION

The results on the plant yield are presented in Figures 1–4. Regarding the biomass of test species growing on degraded soil and on degraded soil supplemented with increasing additions of Z-ion substrate, it can be said that the substrate favorably affected the cocksfoot growth. The substrate doses (1%, 2%, 5%, 10% v/v) added to the degraded soil caused a statistically significant increase in the values of vegetative parameters of the test species. Wet and dry stem biomass, dry root biomass and total dry plant biomass in series DS+1%, DS+2%, DS+5% and DS+10% was greater than that obtained in the control series DS by 317–688%, 314–488%, 83–176% and 184–304%, respectively. With rising substrate share in degraded soil, wet and dry stem biomass of cocksfoot

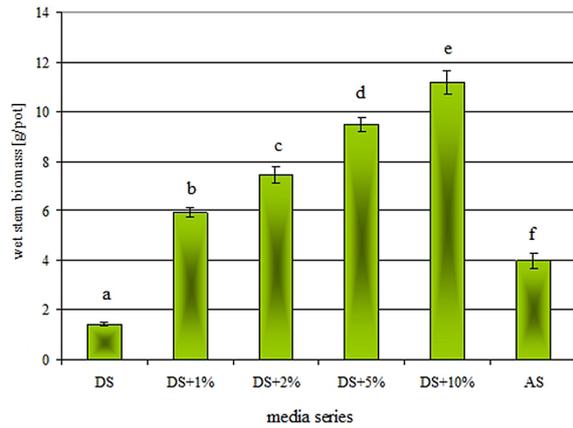


Fig. 1. Wet stem biomass of cocksfoot; explanations: DS – degraded soil, DS+1% – degraded soil with 1% Z-ion addition, DS+2% – degraded soil with 2% Z-ion addition, DS+5% – degraded soil with 5% Z-ion addition, DS+10% – degraded soil with 10% Z-ion addition, AS – arable soil, I – standard deviation, the mean values with different letter differ significantly at $p \leq 0.05$

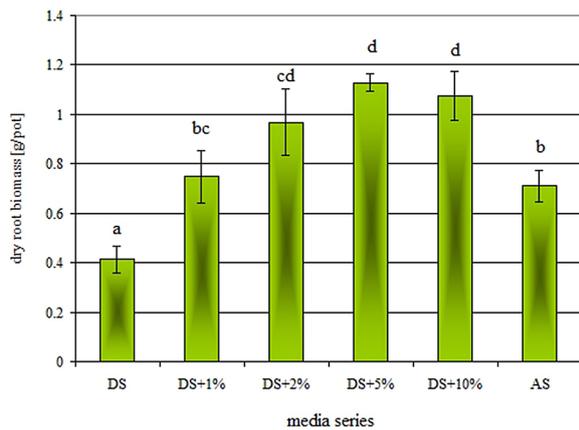


Fig. 3. Dry root biomass of cocksfoot; explanations are identical to **Fig. 1**

increased significantly to a level of 10% substrate addition (Fig. 1 and 2). This trend was not observed for dry root biomass, because the values of this vegetative parameter in series DS+2%, DS+5% and DS+10% did not differ significantly (Fig. 3). This phenomenon is consistent with the regularity described in the literature, according to which, as the doses of some nutrients, e.g. nitrogen (present as NH_4^+ in the Z-ion substrate) are increased, the above-ground biomass of plants increases as well, while the root mass may not, and sometimes it even decreases [Chen et al. 2017, Chen et al. 2020].

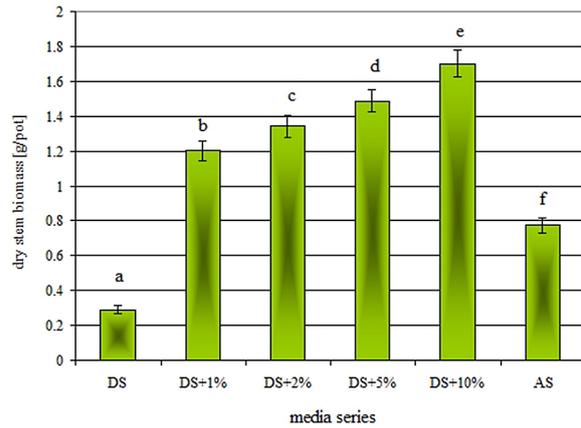


Fig. 2. Dry stem biomass of cocksfoot; explanations are identical to **Fig. 1**

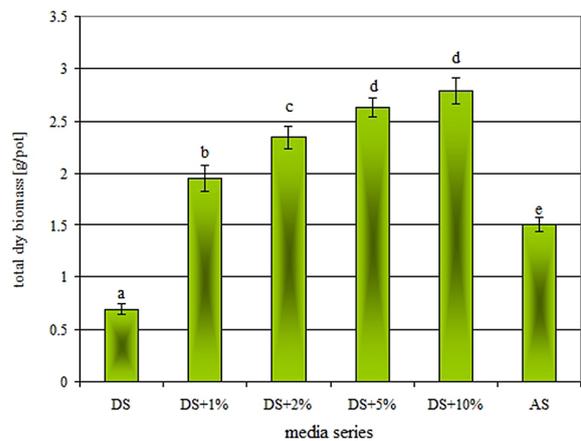


Fig. 4. Total dry biomass of cocksfoot; explanations are identical to **Fig. 1**

The found increases in dry root and stem biomass of cocksfoot, caused by 2% dose of Z-ion substrate, were lower than those reported by Chomczyńska [2013] or Wasąg et al. [2000], who used in their studies 2% (v/v) doses of different ion exchange substrates prepared with the use of ion exchange resins. The dissimilarities in the efficiency of Z-ion substrate and other ion exchange substrates (e.g. Biona®-312, Biona®-111, Mp) could be caused by varying nutrient contents in particular substrates or different nutrient status of amended sand and soil used as growing media in the studies.

Cocksfoot, belonging to perennial grasses, is recommended as a feedstock for methane fermentation for different reasons, including energy and management practice aspects [Tilvikiene et al. 2020]. Thus, it is interesting to compare the values of vegetative parameters of test species growing on arable soil and on degraded soil enriched

in Z-ion additions. It is seen that wet and dry stem biomass, dry root biomass and total dry biomass of plants in series DS+1%, DS+2%, DS+5% and DS+10% exceeded those of plants growing on arable soil by 49–181%, 56–121%, 6–51% and 31–86%, respectively (Fig. 1–4). Regarding the application of the above-ground biomass of cocksfoot for biogas production, it should be noticed that already 1% substrate addition to degraded soil allows obtaining significantly higher dry stem yield than that of test species growing on arable soil (Fig. 2). Simultaneously, the same substrate addition to degraded soil caused intensification root growth so that dry root biomass in series DS+1% was practically the same as in the series AS (Fig. 3). Therefore, the Z-ion addition not higher than 1% v/v can be considered as one which – after adding to degraded soil – would cause obtaining plant yield similar to that on the arable soil of medium quality. This finding needs to be verified for cocksfoot cultivation under field conditions in longer period of time than adopted for the conducted pot experiment.

Since the carbon/nitrogen ratio in feedstock is a crucial parameter for the proper course of methanogenesis in biogas production systems, attention has been paid to the C:N value in cocksfoot stems. Literature implies the carbon/nitrogen ratio ranges of 20–30 [Montusiewicz et al. 2008, Kainthola et al. 2019] or 25–30 [Bohutskyi et al. 2018]. The values shown in Table 4 indicate that the highest C:N proportion was observed in the biomass of cocksfoot growing on degraded soil and the lowest one in plants growing on degraded soil supplemented with 10% Z-ion addition. The application of increasing zeolite substrate doses resulted in a decrease in the C:N ratio in stems of the test species which should be explained by higher availability of nitrogen introduced together with Z-ion substrate into degraded soil. The carbon/nitrogen ratio in the stem biomass of the cocksfoot cultivated on degraded soil

supplemented with 1% Z-ion dose was in the 20–30 range, proposed as the optimal for biogas production.

Due to the fact that the biological/enzymatic activity of degraded soils is generally low [Mocek-Płóćniak 2014], an preliminary attempt was made to determine this activity in the tested soil enriched with Z-ion additions and after the vegetation of cocksfoot, assuming that the development of plant roots may contribute to improving the conditions for the development of microorganisms that are an important source of soil enzymes [Fageria and Moreira 2011, Boluda et al. 2014]. In all considered media series, the activity of alkaline phosphatase, acid phosphatase, phosphohydrolase, esterase, lipase-esterase, leucine arylamidase was detected. Moreover, the activity of valine arylamidase (in series: DS, DS+1%, DS+5%, DS+10%), lipase (in series: DS+5%, DS+10%) and α -glucosidase (in series DS+5%,) was observed. The enzyme diversity (expressed as values of Shannon's index) in degraded soil supplemented with 1% and 2% Z-ion addition did not differ significantly from that in degraded soil alone (Fig. 5). Otherwise, the values of Shannon's index calculated for series DS+5%, DS+10% were significantly higher than those found for series DS, DS+1%, DS+2% (Fig. 5). Thus, it should be stated that under the conditions of the described experiment, a significant increase in microbial functional diversity can be observed only at a certain degree of root development caused by a sufficiently high addition of zeolite substrate to the degraded soil (5% v/v). In light of the information about the effect of mineral nitrogen fertilization on microbial activity in soils [Natywa et al. 2014], it cannot be ruled out that introducing ammonium together with 5% substrate dose also stimulated bacterial growth. This observation should be confirmed in field studies, because soil microorganisms play a key role in the functioning of ecosystems, being responsible

Table 4. The carbon/nitrogen ratio in stems of cocksfoot

Media series	C content [% d.m.]	N content [% d.m.]	C:N
Degraded soil	43.98±0.21	1.33±0.02	33,07
Degraded soil+1% Z-ion	44.01±0.27	1.62±0.01	27.17
Degraded soil+2% Z-ion	43.80±0.79	2.36±0.01	18.56
Degraded soil+5% Z-ion	42.53±0.24	3.41±0.02	12.47
Degraded soil+10% Z-ion	41.14±0.29	4.32±0.05	9.52
Arable soil	42.90±0.10	1.62±0.04	26,48

Explanations: d.m. – dry matter (in 105°C), ± – standard deviations.

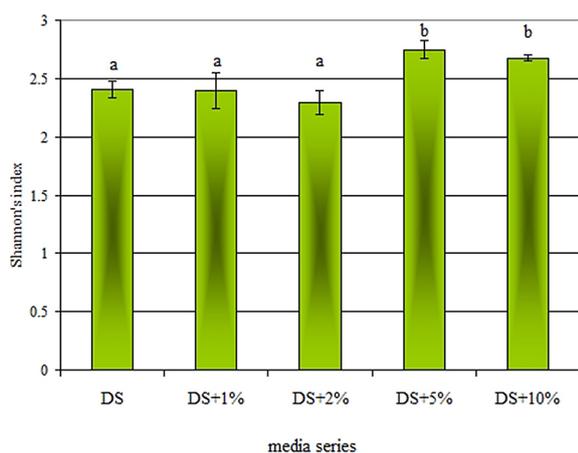


Fig. 5. The microbial functional diversity (values of Shannon's index) in degraded soil and in degraded soil supplemented with Z-ion additions; explanations are identical to **Fig. 1**

for the processes of organic matter decomposition as well as degradation and detoxification of many environmental pollutants.

CONCLUSIONS

Z-ion substrate can be recommended for fertilization of degraded soils – its additions in the range of 1–10% v/v to degraded soil (poor in nutrients) influenced the plant growth positively, significantly increasing wet and dry stem biomass, dry root biomass and total dry biomass of cocksfoot. The addition of Z-ion substrate not exceeding 1% v/v can be considered as one which – after introducing into a specific degraded soil – would give similar biomass yield of cocksfoot to that obtained on the selected arable soil of medium quality. The application of Z-ion additions as carriers of nutrients to the degraded soil caused an increase in the nitrogen content and a decrease in the C:N ratio in the stem biomass of the test species, which is important in connection with the possible use of cocksfoot biomass as a substrate in the biogas production process. As a result of using 1% addition of Z-ion substrate, the C:N ratio in the plant material was within the range of values enabling the right course of the methane fermentation process. The preliminary studies revealed that a significant increase in microbial functional diversity can be observed when there is a certain degree of root development caused by a sufficiently high addition of zeolite substrate to the degraded soil – under experimental conditions it was 5% v/v Z-ion dose.

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