THE QUALITY OF PRESERVED BIOMASS OF SOME *POACEAE* SPECIES UNDER THE CONDITIONS OF REPUBLIC OF MOLDOVA

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Abstract

Energy is an essential ingredient of socio-environmental development and economic growth in the modern economy. Anaerobic digestion is an important way of making use of biomass resources and production of renewable energy, environmentally friendly and rapidly expanding in the latest years. Energy crops can be a suitable feedstock and if preserved they can be supplied to biogas plants continuously throughout the year. The aim of the current study was to evaluate the quality and biochemical methane production potential of preserved biomass (silage and haylage) prepared from *Poaceae* plants, 3-year-old perennial species *Miscanthus giganteus* and tall fescue *Festuca arundinacea*, winter cereal crops: rye *Secale cereale* and triticale *Triticum secale.* The silage and haylage prepared from these species mowed in June, by organoleptic characteristics (smell, colour and consistency) and biochemical indices (pH, content and correlation of organic acids, chemical composition of the dry matter), largely, met the standards. The chemical composition of the *Miscanthus giganteus* silage did not differ essentially compared to *Triticum secale* haylage. The biochemical methane production potential of *Miscanthus giganteus* silage made as a result of the first mowing in June reached 355 L/kg, *Festuca arundinacea* silage - 340 L/kg, *Secale cereale* haylage -333 L/kg, *Triticum secale* haylage - 358 L/kg organic matter, respectively.

Key words: biochemical methane production potential, *Festuca arundinacea*, *Miscanthus giganteus*, *Secale cereale, Triticum secale,* preserved biomass

Energy is an essential ingredient of socio-environmental development and economic growth in the modern economy. Rapid deployment of renewable energy and energy efficiency, and technological diversification of energy sources, would result in significant energy security and economic benefits. At the same time, there are growing opportunities and demands for the use of biomass to provide additional renewables, energy for heat, power and fuel, pharmaceuticals and green chemical feedstocks. Anaerobic digestion is an important way of making use of biomass resources, achieving a number of benefits through biogas technology, in the production of energy, the protection of the environment and improvement of the ecology. For sustainable development of biogas production is important to ensure a stable and continuous supply of feedstock with suitable quality and quantities. Storage conditions play a vital role in maintaining biomass quality for various bioenergy opportunities. The well-known techniques of biomass preservation include ensiling and drying.

Maize, *Zea mays* is the most popular *Poaceae* species used for livestock feeding and as energy crop for biogas production, but its cultivation, harvest and mineral fertilization require high financial and fossil fuel inputs. The adverse climatic conditions, water deficiency in soil, associated with high temperatures and strong evapotranspiration from the last decades, had serious consequences on the maize production.

Given the climatic conditions and the limited water resources in the Republic of Moldova, it is necessary to reintroduce traditionally cultivated *Poaceae* species in crop rotation and to mobilize new and non-traditional species, characterized by high tolerance and stable productivity, for growing on marginally productive farms, restored degraded lands and denuded community lands. Latterly much attention has been focused on some winter cereals and perennial grasses with multiple-use. Rye, *Secale cereale* is generally the most winter hardy of the cool-season annual grasses, is also the most productive on low fertility, well-drained sandy soils, matures earlier in the spring than most wheat varieties but generally produces more forage in the fall than wheat. Triticale, *Triticum secale* is a cross between wheat and rye. Triticale has an extensive fibrous

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root system, can scavenge nitrogen very effectively, and utilizes early spring moisture to grow rapidly, tolerates drought and pests better than wheat, often produces more forage than many varieties of wheat or rye.

The tall fescue, *Festuca arundinacea* cool season perennial grass species, C_3 photosynthetic pathway, native to Europe, common in the spontaneous flora of the Republic of Moldova, have a productivity of 50-65 t/ha of fresh mass or 15-17 tons/ha of hay, implemented in different regions of the Earth, not only as a source of fodder, but also as feedstock for bioenergy production.

Promising perennial grasses, C_4 photosynthetic pathway, natural hybrid *Miscanthus giganteus*, discovered in Japan in 1935, has very high photosynthetic capacity, exceptionally vigorous growth and remarkable adaptability to different environments, make suitable for cultivation and distribution under a range of European and North American climatic conditions. *Miscanthus giganteus* is a high-yielding lignocellulosic crop.

The aim of the current study was to evaluate the quality and biochemical methane production potential of preserved biomass (silage and haylage) prepared from *Poaceae* plants: *Miscanthus giganteus, Festuca arundinacea*, *Secale cereale* and *Triticum secale.*

MATERIAL AND METHOD

The *Poaceae* species, 3-year-old perennial species *Miscanthus giganteus* and tall fescue *Festuca arundinacea*, winter cereal crops: rye *Secale cereale* and triticale *Triticum secale*, served as subjects of the research, maize *Zea mays* - control.

The green mass was harvested manually. The samples were collected in middle of June *Miscanthus giganteus* in stem elongation period, *Festuca arundinacea*, *Secale cereale* and *Triticum secale* in milk ripe. The control *Zea mays* was harvest in kernel milk-wax stage in middle of August. The *Miscanthus giganteus*, *Festuca arundinacea* and *Zea mays* silage was prepared from harvested green mass, but *Secale cereale* and *Triticum secale* haylage was prepared from wilted green mass (32-hour wilting time after cuting), shredded and compressed in well-sealed glass containers. After 30 days, the containers were opened, the organoleptic characteristics were analyzed and the biochemical composition of the silage and haylage were evaluated in accordance with standard laboratory procedures for forage quality analysis. Dry matter or total solid (TS) content was detected by drying samples up to constant weight at 105 °C. Crude protein – by Kjeldahl method; crude fat – by Soxhlet method, crude cellulose – by Van Soest method; ash – in muffle furnace at 550°C. Nitrogen-free extractive substance (NFE) was mathematically appreciated, as difference between organic matter values and analytically assessed organic compounds. Organic dry matter or volatile solids (VS), was calculated through differentiation, the crude ash being subtracted from dry matter. The content of neutral detergent fibre, acid detergent fibre and acid detergent lignin was evaluated using the near infrared spectroscopy (NIRS) technique PERTEN DA 7200 of the Research-Development Institute for Grassland Brasov, Romania. The biochemical biogas potential (Yb) and methane potential (Ym) were calculated according to the equations of Dandikas V. et al 2014, based on the chemical compounds – acid detergent lignin (ADL) and hemicellulose (HC) values:

biogas potential $Yb=727+0.25\ HC-3.93\ ADL$

methane potential $Ym=371+0.13HC-2.00\ ADL$

The carbon content of the substrates was obtained from data on volatile solids, using an empirical equation reported by (Badger C.M. *et. al*, 1979).

RESULTS AND DISCUSSIONS

It is known that the ratio of stems to leaves influences the chemical composition of biomass intended for livestock feeding or for use as a means of obtaining various industrial products. The results regarding some bio-morphological characteristics of the studied *Poaceae* species and the structure of the harvested biomass are presented in *table 1*. The studied species differed in the growth and development rate, which affected the accumulation of aerial biomass and its structure. At the time of harvesting, mid-June, *Miscanthus giganteus* plants were in the intense stem elongation stage, the other species – in the generative period - milk-ripe stage. Plant height varied from 137 cm (*Festuca arundinacea*) to 195 cm (*Secale cereale*), stem thickness 9-12 mm in *Miscanthus giganteus* and 1-3 mm in the other species. The biomass of *Miscanthus giganteus* had the largest proportion of leaves (51.8 %), followed by *Triticum secale* (37.7 %), but *Secale cereale* contained the largest proportion of stems (61.2 %). The species *Festuca arundinacea* and *Triticum secale* had the highest content of dry matter in the freshly harvested biomass: 41.1 % and 37.1 %, respectively. During the research carried out in USA, it was found that, in the milk stage of grain fill, the rye fresh mass contained 7.1 % leaves, 70.0 % stems and 22.9 % spikes (Beck P., Jennings J., 2014). In Canada, the natural fodder of triticale contained 24.0 % leaves, 35.0 % stems and 40.8 % spikes (Khorasani G. R. *et al,* 1997).

The preserved biomass of the investigated *Poaceae* species had different smell, colour and dry matter content. *Miscanthus giganteus* silage had pleasant smell, specific to pickled vegetables, but tall fescue silage – had a pungent, unspecific odour, somehow similar to the smell of fresh pinewood, but it disappeared later. The rye and triticale haylage had pleasant smell, specific to baked rye bread. During the organoleptic assessment, it was found that the colour of *Festuca arundinacea* silage was dark green leaves and yellow stems; the *Miscanthus giganteus* silage and *Triticum secale* haylage were homogeneous green-olive, but the *Secale cereale* haylage was homogeneous yellow-green.

Table 1

Some biological peculiarities and the structure of the harvested biomass of the investigated *Poaceae* species

Plant species	Plant height, cm	Stem, g		Leaf,g		Spike, g	
		green mass	dry matter	green mass	dry matter	green mass	dry matter
Miscanthus giganteus	157	60.18	10.16	42.83	10.94	0	0
Festuca arundinacea	137	6.95	2.28	5.78	1.82	1.38	0.58
Secale cereale	195	7.62	2.38	1.11	0.44	3.12	1.07
Triticum secale	148	6.31	2.19	1.92	0.75	4.48	1.78

Table 2

The fermentation quality of the investigated preserved *Poaceae* biomass

Indices	*Miscanthus giganteus* silage	*Secale cereale* haylage	*Triticum secale* haylage	*Festuca arundinacea* silage	*Zea mays* silage
pH index	4.15	5.37	4.78	5.04	3.61
content of organic acids, g/kg	26.7	45.7	57.0	34.4	32.8
free acetic acid, g/kg	4.4	0	1.7	0.1	1.3
free butyric acid, g/kg	0	0	0	2.9	0
free lactic acid, g/kg	6.8	3.9	5.1	1.4	13.7
fixed acetic acid, g/kg	5.7	2.9	5.8	2.6	2.0
fixed butyric acid, g/kg	0	0	0	8.7	0
fixed lactic acid, g/kg	9.8	38.9	44.4	18.7	15.8
total acetic acid, g/kg	10.1	2.9	7.5	2.7	3.3
total butyric acid, g/kg	0	0	0	11.6	0
total lactic acid, g/kg	16.6	42.8	49.5	20.1	29.5
acetic acid, % of organic acids	37.83	6.35	13.16	7.85	10.06
butyric acid, % of organic acids	0	0	0	33.72	0
lactic acid, % of organic acids	62.17	93.65	86.42	58.43	89.94

Table 3

Dry matter content and biochemical composition of the investigated preserved *Poaceae* biomass

Indices	*Miscanthus giganteus* silage	*Secale cereale* haylage	*Triticum secale* haylage	*Festuca arundinacea* silage	*Zea mays* silage
Dry matter,g/kg	199.4	596.2	508.5	309.4	314.0
Raw protein, g/kg	84.0	76.2	92.4	55.4	53.4
Raw fats, g/kg	28.6	23.7	28.9	23.9	28.6
Raw cellulose, g/kg	449.9	398.2	375.6	508.4	225.2
Nitrogen free extract, g/kg	340.8	455.1	449.3	273.1	601.9
Minerals, g/kg	96.7	46.9	53.7	102.9	49.9

Table 4

The cell wall content, digestibility of dry and organic matter of the investigated preserved *Poaceae* biomass

Indices	*Miscanthus giganteus* silage	*Secale cereale* haylage	*Triticum secale* haylage	*Festuca arundinacea* silage	*Zea mays* silage
Acid detergent fibre, g/kg DM	452	393	354	489	303
Neutral detergent fibre, g/kg DM	760	652	601	817	514
Acid detergent lignin, g/kg DM	28	36	24	37	46
Total soluble sugars, g/kg DM	32	177	167	8	276
Dry matter digestibility,%	56.4	53.8	62.4	41.9	72.3
Organic matter digestibility,%	48.5	47.4	55.6	33.0	68.3
Cellulose, g/kg DM	424	357	333	452	257
Hemicellulose , g/kg DM	308	259	244	328	211
Carbon/ Nitrogen	37	43	36	56	62
Bio biogas potential, L/kg VS	694	651	700	664	599
Biomethane potential, L/kg VS	355	333	358	340	306

The fermentation quality of preserved *Poaceae* biomass, are shown in *table 2*. As a result of the performed analysis, it was determined that the pH index of the preserved biomass varied from 4.15 to 5.37. The pH index of the silage prepared from *Miscanthus giganteus* was low and met the standard SM 108 for the 1st class quality.

It is known that during the process of ensiling, epiphytic bacteria produce organic acids. During the process of ensiling, epiphytic bacteria produce organic acids. Analyzing the data regarding the overall content of organic acids, we concluded that the concentration of organic acids was higher in the haylage made from *Secale cereale* and *Triticum secale* (45.7-57.0 g/kg), lower – in *Miscanthus giganteus* silage (26.7 g/kg), compared with maize silage (34.4 g/kg). The lactic acid level in *Festuca arundinacea* and *Miscanthus giganteus* silage were lower (16.6- 20.1 g/kg), but in *Secale cereale* and *Triticum secale* haylage – higher (42.8-49.5 g/kg). Lactic and acetic acids were present in all samples of silage and haylage, being predominantly in fixed state, which is desirable because organic acids in the fixed state contribute more to the preservation of nutrients in the silage. *Triticum secale* haylage and *Miscanthus giganteus* silage were characterised by high concentration of acetic acid (7.5-10.1 g/kg). Butyric acid was present, in very high amounts, in *Festuca arundinacea* silage (33.72 % of organic acids) which caused the pH level to rise and the fermentation quality to worsen in these silages. The share of the lactic acid from the total amount of organic acids accumulated in the preserved winter cereal crops was obviously higher and varied from 86.4 % in *Triticum secale* haylage to up to 93.7 % in *Secale cereale* haylage.

Analyzing the data on the biochemical composition of the dry matter of the preserved *Poaceae* biomass, shown in *table 3*, we found that a higher crude protein content, in comparison with the control, was in *Miscanthus giganteus* silage (8.4 %), and in the haylage from *Secale cereale* and *Triticum secale* (7.6-9.2 %). The fats from fodder provide energy to the body and contribute to the quality of animal products. Among the studied species, *Secale cereale* and *Festuca arundinacea* provided a low fat content in the preserved biomass, but *Triticum secale* and *Miscanthus giganteus* contained about the same amount as the control – maize. The preserved *Poaceae* biomass contained high amount of crude cellulose (37.6-50.6 %) and low amount of nitrogen-free extract (30.9-45.5 %). There was a moderate content of raw cellulose and nitrogen-free extract in the haylage from *Secale cereale* and *Triticum secale*. The silages made from *Miscanthus giganteus* and *Festuca arundinacea* were richer in minerals (9.7-10.3 %) than maize silage (5.0 %).

The availability of feedstock depends on fibre composition, structure, degradability and digestibility. The main source of fibre in feeds is plant cell wall, consisting of cellulose, hemicellulose and a non-saccharidic component – lignin. To measure the quality, wet chemical analyses are needed, analyses that are laborious and time consuming. Near infrared reflectance spectroscopy (NIRS) has been used in agriculture research for years, as a robust method, low cost and doing non-destructive measurements with limited sample preparation, providing quantitative and qualitative information (Vidican R. M. *et al,* 2000). The cell wall content, digestibility of dry and organic matter of the preserved biomass of the studied *Poaceae* species, determined by the NIRS method, are presented in *table 4*. The results show that the neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents in preserved *Poaceae* biomass varied from 601 g/kg to 817 g/kg and 354 g/kg to 489 g/kg, which are larger amounts than in maize silage. The studied preserved biomass of *Poaceae* is characterised by lower amount of acid detergent lignin (24-38 g/kg) in comparison with the control (46 g/kg). *Festuca arundinacea* and *Miscanthus giganteus* silage contained the highest amounts of NDF, ADF, cellulose, hemicellulose, and very low amounts of soluble sugars. The cell wall content in *Triticum secale* haylage was reduced, but the concentration of soluble sugars – higher. The hemicellulose content was approximately at the same level in the prepared haylage (244-259g/kg DM). In the preserved *Poaceae* biomass, dry matter digestibility varied from 41.9 % (*Festuca arundinacea*) to 62.4 % (*Triticum secale*) in comparison with 72.3 % in *Zea mays* silage. Organic matter digestibility had the same tendency.

Some authors mentioned various findings about the quality of preserved *Poaceae* biomass. Lukianciuc V.N., 2005, remarked that, under the climatic conditions of Rostov region, Russia, *Triticum secale* haylage with pH index 4.88 contained 444.7 g/kg dry matter, 47.4 g/kg raw protein, 12.3 g/kg fats, 141 g/kg cellulose, 211 g/kg nitrogen-free extract, 31.3 g/kg ash and 24.4 g/kg organic acids including 13.7 g/kg lactic acid, 9.8 g/kg acetic acid and 0.9 g/kg butyric acid. According to Askel E.J. *et al,* 2017, in Guarapuava, Brazil, the chemical composition of the haylage made from winter cereals as follows: from *Secale cereale* – 9.08 % protein, 73.93 % NDF, 45.95 % ADF and 5.19 % ash; from *Triticum cereale* –

10.55 % protein, 66.08 % NDF, 39.91 % ADF and 6.64 % ash; from *Hordeum vulgare* – 8.26 % protein, 71.50 % NDF, 39.56 % ADF and 4.21 % ash; from *Triticum aestivum* – 9.28 % protein, 71.98 % NDF, 42.32 % ADF and 4.30 % ash. Geren H., 2014, reported that silage made from *Secale cereale* and *Triticum secale* harvested in the milk stage, under the Mediterranean climatic conditions of Turkey, contained 9.4-10.9 % crude protein, 60.6-59.3 % NDF, 41.9-34.6 % ADF, pH index 4.44-4.20. In South Korea, Lee S.S. *et al*, 2018, noted that the silage from *Secale cereale* harvested in the dough stage contained approximately 42.1-44.1 % dry matter, 4.52-4.64 % protein, 1.87-2.45 % fats, 74.4-77.2 % NDF, 45.1-48.2 % ADF and 4.15 - 4.50 % ash; fermentation characteristics pH index 4.51-6.03, concentration of organic acids 36.2 - 98.7 g/kg, lactate 11.6-44.2 g/kg, acetate 22.3-71.6 g/kg; *in vitro* dry matter digestibility 42.8-44.4 %. Jancik F. *et al*, 2011, remarked that, under the climatic conditions of Tábor region, Czech Republic, the silage made from *Festuca arundinacea*, harvested in May-June, contained 158-198 g/kg raw protein, 26.2-29.0 g/kg fats, 71.4-100 g/kg ash, 479-545 g/kg NDF, 273-344 g/kg ADF, 21.1-32.1 g/kg ADL, parameters of the fermentation process of grass silages - pH index 4.65, organic acids: 24.7 g/kg lactic, 6.66 g/kg acetic, 1.41 g/kg butyric.

Whittaker C. *et al* (2016), examined the ability of *Miscanthus giganteus*, harvested in September, to be stored as silage for later use in anaerobic digestion and mentioned that untreated *Miscanthus giganteus* silage was of poor quality in comparison with *Zea mays*, as indicated by pH 5.2, higher ethanol content (6-7 %), but reduced starch, lactic and acetic acid content. Borso F. *et al*, 2018, remarked that the amount of crude protein was the highest in *Miscanthus giganteus* silage prepared in August (40.1 g/kg) and the lowest – after winter harvest (20.6 g/kg). Baldini M. *et al*, 2016, reported that dried biomass of *Miscanthus giganteus* silage prepared after the 1-st mowing contained 4.27 % raw protein, 1.07 % fat, 43.0 % cellulose, 28.0 % hemicellulose and 7.33 % ADL, but after the second mowing – 2.77 % raw protein, 1.04 % fat, 41.9 % cellulose, 28.8 % hemicellulose and 7.97 % ADL. According to Herrmann C. *et al*, 2016, the biochemical composition of *Secale cereale* and *Triticum secale* silage was 8.9-9.0 % protein, 2.0-2.2 % fats, 50.4-54.9 % nitrogen free extract, 52.3-55.6 % NDF, 31.3-36.6 % ADF, 4.5-5.5 % ADL, 3.5-4.6 % lactic acid, 1.5-1.6 % acetic acid and 0.0-0.3 % butyric acid, pH 4.0-4.2, but *Miscanthus giganteus* silage (prepared in July-August) – 4.8 % protein, 1.4 % fats, 40.9 % nitrogen free extract, 76.2 % NDF, 55.4 % ADF, 13.3 % ADL, 0.3 % lactic acid, 1.2 % acetic acid and 1.6 % butyric acid, pH index 4.8.

The nitrogen concentration and the type of structural carbohydrate can also be very important in determining the plant utility for the production of food, feed or energy. Due to the increasing concern upon the effect of greenhouse gases and crude oil price, biogas has become of major interest as an alternative energy source. At the beginning of the anaerobic digestion, bacteria need nitrogen to start the process, but in later stages, high nitrogen could act as an inhibitor. Most research results suggest that biogas production is mostly influenced by the carbon to nitrogen (C : N) ratio. The optimal C/N ratio is expected to be in the range 15-25, when the anaerobic digestion process is carried out in a single stage, and for the situation when the process develops in two steps, the optimal C/N ratio will range: for step I: 10-45; for step II: 20-30 (Dobre P. *et al*, 2014). In the preserved *Poaceae* substrates, the carbon to nitrogen ratio was higher. *Miscanthus giganteus* silage and *Triticum secale* haylage have the same C/N ratio, lower in comparison with *Zea mays* substrates due to the higher nitrogen concentration.

The differences in the concentrations of carbohydrates affected the potential of biogas and methane production of substrate. Lignification of cell walls during plant development was identified as the major factor limiting nutrient digestibility, degradation of feedstock for anaerobic digestion and concomitantly biomethane productivity (Klimiuk E. *et al*, 2010; Dandikas V. *et al*, 2014). The biochemical methane production potential of investigated preserved biomass varied from 333 L/kg VS (*Secale cereale* haylage) to 358 L/kg VS (*Triticum secale* haylage). The methane potential of the *Miscanthus giganteus* silage did not differ essentially compared to *Triticum secale* haylage.

Various studies reported about of the methane potential of *Poaceae* substrates. According to Heiermann M. *et al* (2009), the highest biogas yields were obtained from rye harvested in the milk stage (618-743 l/kg ODM) and triticale in anthesis stage (733-819 l/kg ODM), methane contents 59-61 %. Herrmann C. *et al*, 2016, reported that the production potential of biochemical methane of silages from *Miscanthus giganteus* was 176.6-242 L/kg, *Triticum secale* – 275.4-365.7 L/kg, *Secale cereale* 262.5-327.9 L/kg but *Zea mays* 294.5-376.2 L/kg. Based on the batch experiments, Dandikas V. *et al*, 2014, mentioned that the average methane yield of the studied grass silage varied from 177 to 371 L/kg VS, but maize silage – from 327 to 401 L/kg VS. Whittaker C. *et al*, 2016, remarked that methane yield averaged 186 L/kg VS, from untreated *Miscanthus giganteus* silage prepared in October, in contrast, Klimiuk E. *et al*, 2010, observed lower yields, 100 L/kg VS in *Miscanthus giganteus* silages prepared in autumn. Borso F. *et al*, 2018 reported that, under Mediterranean climate, *Miscanthus* harvested in August had 171.4 L/kg VS methane yield, but harvested in winter – 120.5 L/kg VS.

CONCLUSIONS

The obtained results showed that the leaves/stems ratio, the dry matter content, the fermentation quality, the concentrations of digestible protein, fats, raw cellulose and nitrogen-free extract, ash, neutral detergent fibre fraction, acid detergent fibre and acid detergent lignin in preserved *Poaceae* biomass and its digestibility differed significantly depending on the species, which have influenced the methane yield.

The silage obtained from *Miscanthus giganteus* harvested in June, by organoleptic characteristics and biochemical indices (pH, content and correlation of organic acids, chemical composition of the dry matter), largely, met the criteria for the 1^{st} class quality SM 108 standard.

The production potential of biochemical methane of *Miscanthus giganteus* silage made after the first mowing, in June, reached 355 L/kg, *Festuca arundinacea* silage – 340 L/kg, *Secale cereale* haylage – 333 L/kg, *Triticum secale* haylage – 358 L/kg organic matter, respectively.

Preliminary scientific researches allow mentioning that *Miscanthus giganteus*, harvested in June, can be used to produce silage, which, in turn, can be used as feedstock for biogas production.

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