THE QUALITY OF SILAGE FROM *FESTUCA ARUNDINACEA* AND *MISCANTHUS GIGANTEUS* AS FEEDSTOCK FOR BIOMETHANE PRODUCTION IN REPUBLIC OF MOLDOVA

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Abstract: Biomethane production is environmentally friendly and rapidly expanding in the latest years. Energy crops can be a suitable feedstock and if ensiled it can be supplied to biogas plants continuously throughout the year. The aim of the current work was to evaluate quality and biochemical methane production potential of silage prepared from *Poaceae* plant species: *Festuca arundinacea* and *Miscanthus giganteus* grown in experimental land of the National Botanical Garden (Institute), Chişinäu. The samples were collected from the 3-year-old *Miscanthus giganteus* (June 16, August 17, October 2) and *Festuca arundinacea* (June 16). The biochemical methane production potential of *Miscanthus giganteus* silage prepared from first mowing in June reached 355 L/kg, but second mowing in October – 318 L/kg, single mowing regime in August – 290 L/kg; *Festuca arundinacea* silage – 340 L/kg, respectively.

Keywords: biochemical methane production potential, Festuca arundinacea, Miscanthus giganteus, silage.

Introduction

A continuous increase in energy demand, in the light of running low its conventional carriers, forces the mankind to produce energy from renewable sources. At the present time, renewable energy accounts for 19.3% of the global final energy consumption. Biomass plays a key role in the considerations how to secure enough amount of energy for the next generations, while biomass is a source of energy which is largely available; it provides 63.7% of the global renewable energy supply [REN21, 2017]. Sustainable bio energy represents a huge potential for making a significant contribution to rural and economic development, enhancing energy purposes allows the consumption of air CO₂ during photosynthesis, while its release back into the atmosphere is closed in a relatively short time. The ideal energy crop has to have good capacity for energy transformation from solar to harvestable biomass with maximum efficiency, minimal input requirements and favourable environmental influence [ROMAN & al. 2016].

The technology of biomass conversion through anaerobic digestion is a quite promising option, as biomethane production represents the source of energy with great potential, environmentally friendly and rapidly expanding in the latest years [AMON & al. 2007; KLIMIUK & al. 2010; VINTILĂ & al. 2012; DANDIKAS & al. 2014]. The digestate serves as an excellent fertilizer and soil improver of high quality, replacing mineral fertilizer [BORSO & al. 2018]. The use of biogas for the needs of the transport sector has increased significantly in the USA and has continued to increase its share in the fuel mix in European Union [REN21, 2017]. The profitability of many biogas investments depends on the substrate costs and certificate price. In Europe, maize is the most commonly used energy crop as biogas

feedstock [AMON & al. 2007], its cultivation, harvest and mineral fertilization require high financial and fossil fuel inputs. However, the cultivation in fertile agricultural land with high input crop-management techniques made maize, as energy crop, responsible of elevated environmental impact, increment in food price volatility and in associated risks for food security. Latterly, much attention has been focused on identifying suitable non-food biomass species. Perennial grasses are promising candidates as bioenergy crops. The mobilization and selection of new perennial species, as well as the elaboration of specific agro-technical measures cultivation of plants as bioenergy crops are an important priority to meet the need of biomass production [VALENTINE & al. 2012; ROMAN & al. 2016].

Currently, grasses from the genus *Miscanthus*, which includes about 16-25 species with C₄ photosynthetic pathway, native to the south-eastern Asia, from China, Japan to Polynesia and few species originating from Africa, are considered to be key renewable raw materials for industrial processing and transformation into energy, which can play an important role in the biorefining industry and energy production. The natural hybrid *Miscanthus* \times *giganteus*, discovered in Japan in 1935, the most commonly planted miscanthus type, has very high photosynthetic capacity and growth rate at low temperature. The exceptionally vigorous growth and remarkable adaptability of *Miscanthus* \times *giganteus* to different environments make this novel crop suitable for cultivation and distribution under a range of European and North American climatic conditions *Miscanthus* is a high-yielding lignocellulosic crop providing up to 40 t/ha/year of dry matter [KIESEL & LEWANDOWSKI, 2016; FARRAR & al. 2018].

The tall fescue, *Festuca arundinacea* Schreb. (syn. *Schedonorus arundinaceus* and *Lolium arundinaceum*), is a cool season perennial grass species, C₃ photosynthetic pathway, native to Europe, common in the spontaneous flora of the Republic of Moldova. Tall fescue has been cultivated since the beginning of the 20th century. It has been investigated in many scientific centres and implemented as crop in different regions of the Earth, not only as a source of fodder and a phytoremediation plant, but also as feedstock for bioenergy production. The selected forms and new cultivars have a productivity of 50-65 tons/ha of fresh mass or 15-17 tons/ha of hay [JANČÍK & al. 2011; MARUŞCA & al. 2011; BAHCIVANJI & al. 2012].

Ensiling is one of the most effective methods of storage and conservation of harvested green mass, playing an essential role in livestock feeding, but in recent decades, it has also been used as substrate in biogas production. Energy crops can be a suitable feedstock for anaerobic digestion and if ensiled it can be supplied to biogas plants continuously throughout the year [TRULEA & al. 2013; FRANCO & al. 2016; WHITTAKER & al. 2016; BORSO & al. 2018].

The aim of the current study was to evaluate the quality and the biochemical methane production potential of the silage prepared from *Festuca arundinacea* and *Miscanthus giganteus*, grown under the conditions of the Republic of Moldova.

Material and methods

Perennial plants of the *Poaceae* family, the local ecotype of *Festuca arundinacea* and cv. *Titan* of *Miscanthus giganteus*, which was cultivated in the experimental plot of the National Botanical Garden (Institute) Chişinău, latitude 46°58'25.7"N and longitude N28°52'57.8"E, served as subjects of this study.

The green mass of 3-year-old perennial grasses was harvested manually. The samples of Miscanthus giganteus were collected under different harvest regimes and on different dates: single mowing regime (June 16, August 17) and double mowing regime -1^{st} mowing (June 16) and 2nd mowing (October 2), but *Festuca arundinacea* – under single mowing regime in the full flowering (June 16). The green mass was 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 was determined in accordance with the Moldavian standard SM 108. Dry matter or total solid content was detected by drying samples up to constant weight at 105 °C. Organic dry matter or volatile solids, 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 Braşov, România. The biochemical biogas potential (Yb) and methane potential (Ym) were calculated according to the equations of DANDIKAS & al. (2014), based on the chemical compounds - acid detergent lignin and hemicelluloses values:

biogas potential Yb=727+0.25 HC-3.93 ADL methane potential Ym=371+0.13HC-2.00ADL

Results and discussions

It is known that the growth and development rates of plants influence biomass accumulation, dry matter content and biochemical composition. In our previous papers [TELEUȚĂ & ȚÎŢEI, 2013; ŢÎŢEI, 2015, 2016], we mentioned that studied grasses species were characterized by a different growth and development rates. Thus, in the first year of vegetation, *Miscanthus giganteus* was distinguished by faster growth, developing shoots, which reached 152-183 cm, while *Festuca arundinacea* did not develop shoots. In the following years, the regrowing season for *Festuca arundinacea* started in the first half of March, when the average soil temperature was above 3-5 °C and for *Miscanthus giganteus* – in April, when the temperature was above 10-12 °C. Thus, by late April, *Festuca arundinacea* plants grew over 70 cm, *Miscanthus giganteus* was 8 cm tall at that time and, by full flowering, the studied plants reached 131 cm and 324 cm, respectively.

Some biological peculiarities of *Festuca arundinacea* and *Miscanthus giganteus* in the third growing season are described in Table 1. It was determined that *Festuca arundinacea* resumed growth in the first days of March, but *Miscanthus giganteus* – in April. The studied perennial grasses were characterized by faster growth rates. The peak growth of *Festuca arundinacea* occurred during the period of reproductive growth (middle May – June), when shoots were over 135 cm tall. *Miscanthus giganteus* plants developed shoots that reached a height of 157 cm in mid-June, 260 cm in mid-August and in the period when the panicle development started, the first days of October – 385 cm.

Analyzing the results of the study on the influence of the harvest time on the leaf : stem ratio of *Miscanthus giganteus*, we found that stem dry matter increased from 10.16 to 25.53 g, but the leaf mass – from 10.94 to 16.36 g, which caused a decrease in the leaf content in the harvested biomass from 53.50 to 39.05%. The studied perennial grasses were distinguished by different dry matter content in harvested green mass.

We may mention that after mowing in June, the plants of the cultivar *Titan* of *Miscanthus giganteus* were characterized by a moderate rate of revival and, in early October,

the stems reached 193 cm, but *Festuca arundinacea* was characterized by slow growth, the secondary peak of vegetative growth occurs in autumn, this species did not develop shoots.

Horvosting	Plant	Stem, g		Leaf, g		Leaves		
period	height,	green	dry	green	dry	content,		
	cm	mass	matter	mass	matter	%		
Festuca arundinacea								
16 June (1 st mowing)	137	6.95	2.28	7.16	2.40	40.73		
Miscanthus giganteus								
16 June (1 st mowing)	157	60.18	10.16	42.83	10.94	53.50		
17 August (1st mowing)	260	65.95	25.53	43.42	16.36	39.05		
2 October (2nd mowing)	193	30.11	11.76	26.54	10.30	46.69		

Table 1. Some biological peculiarities of *Festuca arundinacea* and *Miscanthus giganteus*

The investigated silage from studied perennial grasses was distinguished by a different dry matter content and organoleptic characteristics. When opening the glass vessels with silage made from tall fescue *Festuca arundinacea*, there was a pungent, unspecific odour, somehow similar to the smell of fresh pine wood, but it disappeared later. The silage made from green mass of *Miscanthus giganteus* harvested under double mowing regime (June and October) had a pleasant smell, specific to pickled vegetable, but the silage made from green mass harvested in August, after opening the glass vessels, intensively eliminated carbon dioxide – a by-product of fermentation, the smell was unpleasant but, about 5 minutes after opening, it changed and became specific, like corn silage.

During the organoleptic assessment, it was found that the colour of the *Festuca* arundinacea silage was dark green leaves and yellow stems; the silage made from *Miscanthus giganteus* green mass harvested under double mowing regime in June and October, it was similar – homogeneous green-olive, but the silage from green mass harvested in August – yellow-green leaves and stems. The consistency of the silage from perennial grass species was retained, in comparison with the initial green mass, without mould and mucus.

It is known that the microflora in the harvested green mass is totally different from that of the future silage. During the process of ensiling, epiphytic bacteria produce organic acids. Optimal ensiling results in rapid lactic acid and acetic acid fermentation, causing a decrease of the pH to 4-4.5 within several days.

The fermentation quality of silage prepared from the studied grass species are shown in Table 2. As a result of the performed analysis, it was determined that the pH index of the prepared silage varied from 4.02 to 5.45. The pH index of the silage prepared from *Miscanthus giganteus* green mass in double mowing regime met the standard SM 108.

Analyzing the data regarding the overall content of organic acids, we conclude that the concentration of organic acids was higher in *Festuca arundinacea* silage and lower in *Miscanthus giganteus* silage prepared from green mass harvested in August. The lactic acid concentration in *Festuca arundinacea* silage – 2.01%, but *Miscanthus giganteus* silage was characterised by lower concentration (1.12-1.66%). The *Miscanthus giganteus* silage prepared from green mass harvested in June was characterized by high acetic acid concentration (10.1 g/kg), but the silage prepared from green mass harvested in August – by lower acetic acid concentration (0.9 g/kg). Butyric acid was not found in the silage prepared from *Miscanthus giganteus* harvested under double mowing regime, but it was present, in very high amounts in *Festuca arundinacea* (11.6 g/kg) and *Miscanthus giganteus* harvested in August (4.6 g/kg). As previously mentioned, butyric acid had a share of 27.63 - 33.72%, which caused the pH level to rise and the fermentation quality to worsen in these silages. A high content of butyric acid indicated large production of CO₂ and ammonia as well, which was observed while opening the glass vessels.

According to WHITTAKER & al. (2016), *Miscanthus giganteus* silage, made from biomass harvested in October, indicated pH higher than 5 and 7% ethanol content, but lower lactic and acetic acid, sugar and starch contents.

	Festuca	Miscanthus	Miscanthus	Miscanthus
Indices	arundinacea	giganteus	giganteus	giganteus
	16 June	16 June	17 August	2 October
pH index	5.04	4.15	5.45	4.02
content of organic acids, g/kg	34.4	26.7	16.7	28.1
free acetic acid, g/kg	0.1	4.4	0.4	2.3
free butyric acid, g/kg	2.9	0	0.2	0
free lactic acid, g/kg	1.4	6.8	1.8	8.8
fixed acetic acid, g/kg	2.6	5.7	0.5	4.6
fixed butyric acid, g/kg	8.7	0	4.4	0
fixed lactic acid, g/kg	18.7	9.8	9.4	12.4
total acetic acid, g/kg	2.7	10.1	0.9	6.9
total butyric acid, g/kg	11.6	0	4.6	0
total lactic acid, g/kg	20.1	16.6	11.2	21.2
acetic acid, % of organic acids	7.85	37.83	1.38	24.55
butyric acid, % of organic acids	33.72	0	27.63	0
lactic acid, % of organic acids	58.43	62.17	66.99	75.44

Table 2. The fermentation quality of Festuca arundinacea and Miscanthus giganteus silages

The quality of feedstock for biogas production depends on how accessible the biomass is to enzymes and microbes. To measure the quality, wet chemical analyses are needed, analyses that are laborious and time consuming. Near infrared reflectance spectroscopy has been used in agricultural research for years, as a robust method, low cost and doing non-destructive measurements with limited sample preparation, providing quantitative and qualitative information [VIDICAN & al. 2000; MAYER, 2015; VANCE & al. 2016].

The Near infrared reflectance spectroscopy study revealed the compositional content of carbohydrates and biochemical methane potential of prepared grass silage, and the results are presented in Table 3. The obtained data showed that the concentrations of carbohydrates and their compositional content in silage differed significantly, depending on the species and harvesting period. The prepared grass silage was characterized by the highest concentrations of structural carbohydrates and low concentrations of soluble sugars and this fact affected the quality of silage fermentation. The total soluble sugars content it is important to create favourable conditions for the development of lactic acid bacteria responsible for a successful ensilage process. In the silage from *Festuca arundinacea* and *Miscanthus giganteus* harvested in August, there was a significant decrease in soluble sugars (8-19 g/kg DM) and an increase in cellulose (452-489 g/kg DM), which also affected the quality of silage fermentation. The hemicellulose content was approximately at the same level in the prepared grass silage (308-328 g/kg DM).

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 & al. 2010; TRIOLO & al. 2011; DANDIKAS & al. 2014]. The concentrations of acid detergent lignin in the investigated silage varied from 28 g/kg to 61 g/kg. In the silage from *Miscanthus giganteus* harvested in August, the acid detergent lignin content increased to 61 g/kg, probably because from late July the lower leaves started to dry and soluble nutrients moved back to the rhizome.

The differences in the concentrations of carbohydrates affected the potential of biogas and methane production of silage substrate. The biochemical gas forming potential of obtained grasses silages varied from 567 to 694 L/kg VS. The biochemical methane production potential based on the chemical compounds – acid detergent lignin and hemicelluloses of *Miscanthus giganteus* silage made after the first mowing in June reached 355 L/kg, but after the second mowing in October – 318 L/kg, under single mowing regime in August – 290 L/kg; *Festuca arundinacea* silage – 340 L/kg, respectively.

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	Festuca	Miscanthus	Miscanthus	Miscanthus				
Indices	arundinacea	giganteus	giganteus	giganteus				
	16 June	16 June	17 August	2 October				
Acid detergent fibre, g/kg DM	489	452	550	485				
Neutral detergent fibre, g/kg DM	817	760	869	804				
Acid detergent lignin, g/kg DM	37	28	61	47				
Total soluble sugars, g/kg DM	8	32	19	24				
Cellulose, g/kg DM	452	424	489	438				
Hemicelluloses, g/kg DM	328	308	319	319				
Bio biogas potential, L/kg VS	664	694	567	622				
Bio methane potential, L/kg VS	340	355	290	318				

 Table 3. The concentrations of carbohydrates in *Festuca arundinacea* and *Miscanthus giganteus* silages and potential of biochemical methane production

Some authors mentioned various findings about the quality of grasses silage and specific methane yield. According to JANCIK & al. (2011), the chemical composition of of silage dry matter prepared in May, in Czech Republic, from Festuca arundinacea was: 17.80% protein, 2.76% fat, 8.59% ash, 51.20% NDF, 31.10% ADF and 2.66% ADL, but from Dactylis glomerata -14.90%, 3.08%, 4.66%, 54.10%, 33.30% and 3.12%, respectively. BALDINI & al. (2016), reported that dried biomass of Miscanthus giganteus silage prepared from 1st mowing consists of 4.27% raw protein, 1.07% fat, 43.0% cellulose, 28.0% hemicellulose and 7.33% acid detergent lignin, but 2nd mowing – 2.77% raw protein, 1.04% fat, 41.9% cellulose, 28.8% hemicellulose and 7.97% acid detergent lignin. AMON & al. (2007), clearly demonstrated the grass grown at the valley site produced 190–392 L/kg VS, the highest specific methane yield was measured for the biomass from the second cut from the "four-cuts variant". HERRMANN & al. (2016), reported that Miscanthus silages contained 30-40% dry matter, 5-7% crude ash and methane yield 200-260 L/kg VS, but meadow fescue (late 1st cut) silages 27-44% dry matter, 5-7% crude ash and methane yield 277-342 L/kg VS. Based on the batch experiments DANDIKAS & al. (2014), published that average methane yield of grassland silage varied from 177 to 371 L/kg VS, but maize silage – from 327 to 401 L/kg VS. WHITTAKER & al. (2016), remarked that methane yield averaging 186 L/kg VS from untreated Miscanthus giganteus silage prepared in October, in contrast, KLIMIUK & al. (2010) observed lower yields, 100 L/kg VS in Miscanthus giganteus silages prepared in autumn. BORSO & al. (2018) reported that in Mediterranean climate Miscanthus harvested in August showed 171.4 L/kg VS methane yield, but harvested in winter period 120.5 L/kg VS.

Conclusions

The obtained results showed that fermentation quality, dry matter content, concentrations of neutral detergent fiber, acid detergent fiber, lignin and cellulose in silage from studied grass species differed significantly depending on the species and harvesting period, which have influenced the methane yield.

The silage obtained from *Miscanthus giganteus* harvested in double mowing regime, by organoleptic characteristics and biochemical indices (pH, content and correlation of organic acids, chemical composition of the dry matter), largely, met the standards.

The biochemical methane production potential of *Miscanthus giganteus* silage made as a result of the first mowing in June reached 355 L/kg, but second mowing in October – 318 L/kg, single mowing regime in August – 290 L/kg; *Festuca arundinacea* silage – 340 L/kg, respectively.

Preliminary scientific researches allow mentioning that the local ecotype of *Festuca* arundinacea and cv. *Titan* of *Miscanthus giganteus* can be used to produce silage and possibility of its use as feedstock for biogas production.

Notes on contributor

Victor $\hat{T}\hat{T}EI$ – is Head of the Plant Resources Laboratory "Alexandru Ciubotaru" National Botanical Garden (Institute), Chişinău, Republic of Moldova, with a PhD in Biology – Plant Physiology and Applied Botany with a special interest in the mobilization plant genetic resources, breed new cultivars and exploit their potential as forage, honey and energy crops, identification promising plant species for valorification marginal and degraded lands.

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