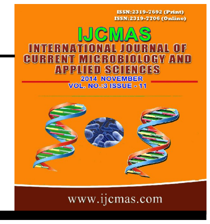


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**Original Research Article**

# Comparative study of microflora of new energy crops for biogas production

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**Keywords**

*Paulownia* sp., *Miscanthus giganteus*, *Arundo formosana*, *Salix viminalis*, Microflora, digester, Dry matter, Epizootiological safety

**A B S T R A C T**

Studies were carried out on the quantities of microorganisms from some major groups in fresh leaves of *Paulownia elunchati*, *Paulownia shangton*, *Paulownia* sp. hybrid, *Paulownia kawakami*, *Arundo formosana* and the elephant grass *Miscanthus giganteus*, which are introduced in Bulgaria as potential energy crops for biogas production, also of willow *Salix viminalis* (branches with leaves), fresh sludge from urban waste water treatment plant (UWWTP) and poultry litter. It was found that in a unit of the fresh materials greatest amount microorganisms contains the poultry litter, in the second position are *P. kawakami* and *Paulownia* sp. hybrid, and in the third - the sludge of UWWTP and the willow. The lowest was the microbial content of *M. giganteus* and *A. formosana*, also of *P. elunchati* and *P. shangton*. In a unit of dry matter, however, the greatest amount of microorganisms contained the sludge of UWWTP, more than the poultry litter and *P. kawakami*. The lowest was the microbial content of *P. elunchati*. In contrast to the poultry litter and sludge, the tested energy crops did not contain the sanitary indicative microorganisms (*E. coli*, *Salmonella enterica* and *Clostridium perfringens*) and their agricultural use could not be risky of epizootiological point of view. Furthermore, the results showed that the comparative assessment of the quantities of microorganisms in the incoming materials in the digester is appropriate to be made not only in a unit of fresh material, but also per unit of dry matter.

## Introduction

The anaerobic digestion for biogas production is an important strategy for replacing fossil fuels, approved by Directive 2009/28/EC of the European Union. Valuable raw materials for the production of methane are the biological wastes from agro-industrial origin. Besides these increasingly use special energy crops, but it is important to be cultivated on secondary lands in order to avoid limiting and appreciation of agricultural production (Barbantia *et al.*, 2014).

Shindarska *et al.* (2013) reported that at alone using of substrates of organic fertilizers or sewage sludge the biogas yield is insufficient, therefore they offer and use of energy crops as feedstock for this purpose. Baykov *et al.* (2013) presented opportunities for successful application of *Paulownia* spp. like new energy crops for our country. By their use is achieved an increase in efficiency of the production of biogas, and improvement of the soil structure after application of the final product. Popova *et al.* (2013) found that during the ensilage of poultry manure mixed with the sheet of *Paulownia elongata*, also with maize or beets, the amounts of microorganisms therein are reduced. All species of genus *Paulownia* are fast-growing trees, thus some of them are used for industrial production of timber, feed, ethanol, paper, etc. Foliage of *Paulownia* spp is particularly suitable for preparation of feed for herbivores (Velboy Ltd., 2010).

The perennial grasses show many useful properties as energy crops, and there is increasing interest in their use in the United States and Europe since the mid-1980s. In Europe about 20 perennial grasses are tested and four of them are selected for more extensive research programs, including the giant reed (*Arundo donax*) and *Miscanthus* spp. that originates from Southeast Asia. For efficient production of bioenergy from these is required to choose the most appropriate species for given environmental and climatic conditions. It has been found that in this respect these are suitable for the Central and Southern Europe (Lewandowski *et al.*, 2003). One of the promising energy crops is giant cane *Arundo donax*, since it is effective in that regard, while less demanding and more economical for the cultivation than maize, for example (Barbantia *et al.*, 2014).

It is a perennial herb, growing in meadows and wetlands. It is supposed that has originated in Asia and later spread to the Mediterranean (Mariani *et al.*, 2010). It has long been associated with humans and for thousands of years is grown in Asia, Southern Europe, North Africa and the Middle East. *Arundo* is a cane genus of the family *Poaceae*. Reaches from 3-6 to 10 m in height, and leaves are 30-60 cm long and 3-6 cm wide (Watson and Dallwitz, 2014).

*Miscanthus* is a genus of high perennial grasses, new bioenergy crops for Europe over the past two decades. Compared with other sources of bioenergy they occupy a middle position in terms of the life cycle between some annual crops (canola, sugar beet, etc.) and woody perennial plants (willow, poplar). It is recommended, however, these new plants to be sterile (e.g. triploid) as a precaution against their conversion in weeds (Scurlock, 1998). Still in 1999 Venturi *et al.* predicted prospectively future of lignocellulosic crops such as miscanthus (*Miscanthus giganteus*) and willow (*Salix viminalis*) in European countries as energy crops.

They pointed out the production of willow stems as the most economical. It is not significantly different in this respect from miscanthus, but for the willow there is some appreciation for drying costs. Ordinary willow or wicker *Salix viminalis* originates from Europe and western Asia. It grows mainly near streams and other moist places. It represents a multi-stem shrub reaching between 3 and 6 m (rarely to 10 m) in height. Along with other willows, its flexible branches are often used in basketry (Meikle, 1984; Rushforth, 1999). In recent years, is experimented the effectiveness of its growing in our country as an energy crop for environmentally clean production of energy from renewable sources (Dimitrova, 2013).

The raw materials of energy crops, however, may be carriers of different microorganisms, including pathogenic for animals which fall easily from the soil and fertilizers, as well as through distribution by rodents and other animals. Therefore, this work aims to investigate the leaves and twigs of these new energy crops for the presence of pathogenic microorganisms and evaluation of their epizootic safety when are used in agriculture and biogas production.

## Materials and Methods

### Microbiological studies

Microbiological studies were conducted according to the Ordinance on the terms and manner of utilization of sludge from wastewater treatment through its use in agriculture (Ordinance. 2004). The titers of *E. coli* and *Clostridium perfringens* were established. Additionally were traced amount of bacteria of the genera *Staphylococcus, Enterococcus, Pseudomonas*, Gram-negative aerobic bacteria, fungi and the total number of microorganisms.

### Nutrient media

Elective and selective nutrient media (Sharlau Chemie S. A., Spain) were used for isolation and quantitative determination of the microorganisms from the studied groups and types. The following media have been chosen: Mueller Hinton agar for counting the total number of microorganisms in the examined material, Eosin Methylene Blue agar for *E. coli* and Gram-negative aerobic bacteria, Cetrimide agar for bacteria of the genus *Pseudomonas*, Chapman Stone agar for those of the genus *Staphylococcus,* Sabouraud agar for fungi, selective medium for *Enterococci*, Salmonella-Shigella agar for *Salmonella enterica* and selective agar for *Clostridium perfringens* (Merck-Bio Lab, Bulgaria).

### Quantification

The quantification of the microorganisms was performed by the conventional method in serial tenfold dilutions of the tested materials in a sterile saline solution. Cultures on the selected media were prepared from these dilutions, three for each medium and dilution. After incubation at 37°C for 24–72 h under aerobic and anaerobic conditions (with Anaerocult® A mini – Merck-Bio Lab, Bulgaria), the mean arithmetical number of the developed colonies was calculated and the colony forming units (CFU) in 1 g of the initial material were determined.

### Microscopic studies

Microscopic observations of microorganisms were carried out under immersion at 1200 x magnification after staining by various classical methods (by Gram, by M□eler for spores and by Klett for capsules) of materials from different cultures on the nutrient media.

### Statistical analysis

All of the experiments were done in triplicate. Statistical analysis of results is made using the classic method of Student-Fisher.

### Energy crops

Fresh leaves of the following plants, which are introduced in Bulgaria as potential energy crops for biogas production, were examined: *Paulownia elunchati*, *Paulownia shangton, Paulownia* sp. hybrid, *Paulownia kawakami*, elephant grass *Miscanthus giganteus, Arundo formosana* and willow *Salix viminalis* (twigs with leaves).

**Organic waste, raw materials for biogas production**

Fresh sludge from urban waste water treatment plant (UWWTP) and .mature poultry litter.

The content of dry and organic substance in the tested materials, and the data for pH values in them are shown in Table 1.

## Results and Discussion

The studies on the microflora of the leaves of the plants do not show the presence of pathogens such as *Salmonella enterica*. Microflora of leaves consists mostly of germs, proven microscopic and culturally. The results of tests to determine the total number of microorganisms, fungi and staphylococci in materials from energy crops, sewage sludge and poultry manure are presented in Table 2 They are shown in a unit (1 g) of the materials, and in 1 g of their dry matter.

As can be seen from the pooled data the total number of microorganisms is highest in the poultry manure litter as far outstrips that of other materials (P <0.01). In the energy crops most microorganisms were established in the research material from *P. kawakami* (almost identical to that in the fresh sludge) and willow *S. viminalis* and *Paulownia* sp. hybrid. In these representatives of the genus *Paulownia* is also found the greatest amount of fungi. The number of fungi in Paulownia sp. hybrid was even superior to that in bird litter (P <0.001), also in *P. kawakami* (P <0.001).

The total number of microorganisms was smallest in the species *A. formosana* and *P. elunchati*. The differences with the other tested materials are significant (P <0.001). In these plants, as well as in elephant grass and willow, the amount of fungi was also at least, probably due to their lowest humidity. When comparing the results calculated on the basis of dry matter, however, was found that the highest content of microorganisms was in sewage sludge. Interestingly, of the studied energy crops were not isolated staphylococci, but in the manure and sludge, these microorganisms were contained in relatively high amounts.

On Table 3 can be seen the summary data from investigations for content of the sanitary indicative species *E. coli*, *Clostridium perfringens*, and bacteria of the genus *Enterococcus* in energy crops, sewage sludge and poultry manure, shown in 1 g of the materials, and in 1 g of their dry matter. From the presented results it can be seen that the materials of the test plants do not contain the sanitary indicative microorganisms such as *E. coli*, and *C. perfringens*. *Enterococci* are present in the seven plant species tested, but not in large amounts. In the willow, however, was established a large amount of *Enterococci*, outnumbering even those in sludge from UWWTP, but the difference was not significant (P >0.05). Their amount was relatively high and in *P. kawakami*, and *Paulownia* sp. hybrid. Most *Enterococci* were contained in the poultry litter, and the differences in their number in the other tested materials were high (P <0.001). These cocci were at least in the elephant grass, *P. shangton* and *P. elunchati*. The sludge and poultry litter contained *E. coli*. When comparing the data for the material was shown, that *E. coli* was in significantly greater amount in the poultry manure (P <0.001). Upon comparison of the results calculated in a unit of dry matter, however, it was found that the amount of *E. coli* was greater in the sludge, although the difference with the manure is not significant (P >0.05). The same applies for the quantities of *C. perfringens* - in the manure it was higher than in the sludge ($P < 0.01$), but in a unit of dry matter the amount of *C. perfringens* was higher in the sludge, as the difference with the manure was not significant ($P > 0.05$).

Table 4 shows the amounts of the isolated bacteria of the group of Gram-negative aerobes and facultative anaerobes, as well as of those of the genus Pseudomonas in the tested materials, which also are given in 1 g of the material and in 1 g of dry matter.

It is noteworthy that *P. elunchati* and the elephant grass did not contain *Pseudomonas* spp. Bacteria of this genus were in a very small amount and in *A. formosana* and the willow. The greatest quantity of *Pseudomonas* spp. was found in *P. kawakami*, little more than that in the sludge ($P > 0.05$).

The smallest number of *Pseudomonas* spp. was isolated from the mature poultry litter ($P < 0.001$ compared with the other tested materials). In the quantities of Gram-negative aerobic and facultative anaerobic species the differences were not large, but these bacteria were most numerous at the willow, as the differences with *Paulownia* sp. hybrid and *P. kawakami* were not large ($P > 0.05$), but with *P. elunchati* and the elephant grass were more significant ($P < 0.05$), as well as with the other plant materials ($P < 0.001$). These results were similar and when comparing the corresponding data for 1 g of dry matter. Among the studied cultures the amount of Gram-negative aerobes was the smallest in *P. shangton* and *A. formosana*, but surprisingly the lowest content of these bacteria was established in sludge and manure. When comparing the levels of these bacteria, calculated on a basis of unit dry matter, however, it is seen that in the sludge they were more than in the poultry litter ($P < 0{,}001$), also than *P. shangton*, *A. formosana* and the elephant grass, although differences are not large ($P > 0.05$).

The studied materials are from new energy crops, entering the modern biogas production in some countries. They are new to our country except the willow *Salix viminalis.* Al Seadi *et al.* (2009) pointed out that the biogas production is an effective measure to reduce energy dependence of Bulgaria, while being sure means of limiting emissions of methane and other greenhouse gases.

The waste of animal and human origin used as raw material for this production contains various pathogenic bacteria, parasites and viruses. In our country poultry occupies leading position (23%) and respectively generates the most waste. The most common pathogens in waste from livestock and households are bacteria of the genera *Salmonella*, *Enterobacter*, *Clostridium*, *Listeria*, viruses and fungi. The co-generation of waste material from slaughterhouses and fish industry, sewage sludge, etc., increases the diversity of pathogens. Biomass with a potentially high content of pathogenic microorganisms, however, should not be subjected to anaerobic digestion. The resulting by anaerobic digestion digestate most often is used as fertilizer on arable land. For the best indicator of effective sanitation of co-fermenting materials is considered the absence of *Salmonella* spp. of test samples of 50g%. The tendency of bacteria to agglomerate is a protective response that slows their inactivation. In this regard and the dry matter content has importance. Some cells of Salmonella spp. live longer in raw materials with dry matter content of more than 7% (Al Seadi *et al.,* 2009).

At high moisture content the conditions for growth of microorganisms are favorable and at optimum temperature the contained on plants bacteria and fungi can develop. This inevitably leads to deterioration of the raw material for biogas production, but also has a negative impact on the proportion of the microflora in the bioreactor and productivity of methane forming microorganisms. Some plants, however, contain antimicrobial ingredients that inhibit the growth of microorganisms fallen on them (Popova and Baykov, 2013), which favors the keeping quality of raw material for the production of biogas. When analyzing the results of these studies it is established a dependence between the content of dry matter in the tested plants and the number of microorganisms thereon.

The four species of the genus *Paulownia* have lower values of dry matter, as it is the lowest in *P. elunchati* and *Paulownia* sp. hybrid compared to the others. More dry matter there is in *A. formosana*, and its highest content is in the willow *S. viminalis* and elephant grass *M. giganteus*. Obviously, the higher humidity in *Paulownia* sp. hybrid is a prerequisite for the development and storage of a large number of microorganisms on its surface. This dependence largely can be seen and by comparison of the quantities of fungi on the various kinds of plants, since they are most in those with highest humidity (respectively with lowest dry matter content) as *Paulownia* sp. and at smallest in those with the lowest humidity - willow and elephant grass.

The same dependence is manifested and in the total number of microorganisms, but to a lesser extent. Probably for that matters and the content of herbal ingredients that inhibit the growth of certain microorganisms (Popova and Baykov, 2013). The absence of bacteria of the genus *Pseudomonas* in the elephant grass and their little quantity in A. formosana and the willow is obviously related to the low water content of these plant materials. Like the fungi, *Pseudomonas* spp also requires more moisture for their development, as while exhibit high resistance to adverse chemical factors. This applies to some extent and to the others Gram-negative aerobic and facultative anaerobic bacteria. The *Enterococci*, however, are not demanding to moisture and environmental conditions, and because of their particularly high resistance to various chemical and physical factors are not influenced significantly by the plant inhibitors or less moisture. Anaerobic bacteria such as *C. perfringens* obviously cannot grow on these plants, for which appears to contribute and the effect of the antimicrobial agents found in the extracts of some of these, which may be broadcasted and on the surface thereof.

Particularly favorable is the result, that unlike the manure and sludge, the energy crops tested do not contain the sanitary indicative microorganisms. Obviously on the leaves of these plants do not exist favorable conditions for growth of pathogenic bacteria. The absence of sanitary indicative microorganisms such as *E. coli*, and *C. perfringens* and the not high quantities of *Enterococci* in the materials of the studied plants probably is connected and with the fact that they were not in contact with soil, fertilizers, contaminated environment or diseased animals or humans, nor with a materials thereof.

This shows that in compliance of the hygiene standards in the collection and storage of materials from these plants, their use for biogas or as fodder for animal feed is safe from the epidemiological point of view and do not present a risk of transmission of pathogenic microorganisms. The high level of *Enterococci* in poultry litter is logical since they are normal inhabitants of the large intestines of animals and birds.

**Table.1** Content of the dry and organic matter and a pH values in the studied energy crops and organic waste for production of biogas

| **Material** | **Indicators** | | |
|---|---|---|---|
| | **Dry matter %** | **Organic matter %** | **pH** |
| ***Paulownia elunchati*** | 25,52*±2,01** | 16,24±0,56 | 5,46±0,15 |
| ***Paulownia kawakami*** | 27,00±1,09 | 16,20±0,98 | 5,52±0,07 |
| ***Paulownia* sp. hybrid** | 30,92±1,23 | 20,52±0,19 | 5,31±0,17 |
| ***Paulownia shangton*** | 26,30±1,34 | 14,69±0,15 | 5,63±0,24 |
| ***Arundo formosana*** | 34,89±1,88 | 19,84±0,97 | 5,10±0,09 |
| ***Miscanthus giganteus*** | 43,10±2,12 | 36,68±1,12 | 5,44±0,12 |
| ***Salix viminalis*** | 43,39±2,09 | 38,33±1,01 | 5,21±0,07 |
| **Sludge from UWWTP** | 4,90±0,09 | 29,12±0,22 | 5,53±0,06 |
| **Poultry litter** | 44,59±1,35 | 26,99±0,35 | 8,47±0,07 |

** Average. ** Standard deviation.*

**Table.2** Total number of microorganisms, fungi and bacteria of the genus *Staphylococcus* in the studied energy crops and organic waste for production of biogas, presented in 1 g of the materials (M) and in 1 g of their dry matter (DM)

| **Material** | **Groups of microorganisms - CFU/g** | | | | | |
|---|---|---|---|---|---|---|
| | **Total number** | | **Fungi** | | ***Staphylococcus* spp.** | |
| | **In M** | **In DM** | **In M** | **In DM** | **In M** | **In DM** |
| ***P. elunchati*** | 7,67.$10^{8}$*±0,2** | 3,0.$10^{9}$±0,1 | 1,23.$10^{6}$±0,2 | 4,82.$10^{6}$ ±0,1 | - | - |
| ***P. kawakami*** | 1,05.$10^{10}$ ±0,2 | 3,88.$10^{10}$±0,7 | 4,2.$10^{6}$ ±1,9 | 1,55.$10^{7}$ ±0,7 | - | - |
| ***P.* hybrid** | 6,5.$10^{9}$ ±2,5 | 2,1.$10^{10}$ ±0,8 | 1,3.$10^{7}$ ±0,0 | 4,2.$10^{7}$ ±0,0 | - | - |
| ***P. shangton*** | 1,2.$10^{9}$ ±0,5 | 4,56.$10^{9}$ ±1,9 | 4,8.$10^{5}$ ±2,9 | 1,82.$10^{6}$ ±1,1 | - | - |
| ***A.formosana*** | 6,0.$10^{8}$ ±2,5 | 1,72.$10^{9}$ ±0,7 | 2,0.$10^{3}$ ±0,5 | 5,74.$10^{3}$ ±1,4 | - | - |
| ***M. giganteus*** | 5,67.$10^{9}$ ±5,0 | 1,32.$10^{10}$ ±1,2 | 1,33.$10^{5}$ ±0,5 | 3,08.$10^{5}$±1,2 | - | - |
| ***S. viminalis*** | 7,0.$10^{9}$ ±4,2 | 1,61.$10^{10}$ ±0,9 | 1,2.$10^{6}$ ±0,3 | 2,76.$10^{6}$ ±0,7 | - | - |
| **Sludge** | 1,02.$10^{10}$ ±0,26 | 2,08.$10^{11}$±0,53 | 3,83.$10^{5}$±1,8 | 7,82.$10^{6}$±3,7 | 4,27.$10^{4}$±4,4 | 8,72.$10^{5}$ ± 8,9 |
| **Poultry litter** | 6,9.$10^{10}$ ±0,7 | 1,54.$10^{11}$ ±0,16 | 4,7.$10^{6}$ ±1,0 | 1,05.$10^{7}$±0,2 | 3,3.$10^{6}$ ±0,4 | 7,39.$10^{7}$ ± 0,9 |

*In M – in the material. In DM - in its dry matter. * Average. ** Standard deviation.*

**Table.3** Quantities of E. coli, Clostridium perfringens and Enterococcus spp. in the studied energy crops and organic waste for biogas production, presented in 1 g of the materials (M) and in 1 g of their dry matter (DM)

| Material | Groups of microorganisms - CFU/g | | | | | |
|---|---|---|---|---|---|---|
| | ***E. coli*** | | ***C. perfringens*** | | ***Enterococcus* spp.** | |
| | **In M** | **In DM** | **In M** | **In DM** | **In M** | **In DM** |
| ***P. elunchati*** | - | - | - | - | $2,2.10^3$*$\pm1,0$** | $8,6.10^3 \pm3,9$ |
| ***P. kawakami*** | - | - | - | - | $2,0.10^4 \pm0,8$ | $7,14.10^4 \pm2,9$ |
| ***P.* hybrid** | - | - | - | - | $2,23.10^4 \pm0,7$ | $7,2.10^4 \pm2,3$ |
| ***P. shangton*** | - | - | - | - | $2,0.10^3 \pm1,5$ | $7,6.10^3 \pm5,7$ |
| ***A. formosana*** | - | - | - | - | $1,03.10^4 \pm0,6$ | $2,95.10^4 \pm1,7$ |
| ***M. giganteus*** | - | - | - | - | $6,0.10^2 \pm2,2$ | $1,39.10^3 \pm0,5$ |
| ***S. viminalis*** | - | - | - | - | $7,9.10^4 \pm3,5$ | $1,82.10^5 \pm0,8$ |
| **Sludge** | $1,1.10^4\pm0,1$ | $2,2.10^5\pm2,0$ | $4,8.10^3\pm1,9$ | $9,8.10^4\pm3,8$ | $6,80.10^4\pm0,3$ | $1,39.10^6\pm0,1$ |
| **Poultry litter** | $5,1.10^4\pm0,3$ | $1,1.10^5\pm0,7$ | $1,0.10^4\pm0,3$ | $2,2.10^4\pm0,7$ | $3,3.10^6\pm0,2$ | $7,34.10^6\pm0,45$ |

*In M – in the material. In DM - in its dry matter. * Average. ** Standard deviation.*

**Table.4** Quantities of Gram-negative aerobes and facultative anaerobes and of bacteria of the genus Pseudomonas in the studied energy crops and organic waste for biogas production, presented in 1 g of the materials (M) and in 1 g of their dry matter (DM)

| Material | Groups of microorganisms - CFU/g | | | |
|---|---|---|---|---|
| | **Gram-negative aerobes** | | ***Pseudomonas* spp.** | |
| | **In M** | **In DM** | **In M** | **In DM** |
| ***P. elunchati*** | $2,07.10^5$*$\pm1,4$** | $8,11.10^5\pm5,5$ | - | - |
| ***P. kawakami*** | $2,46.10^5\pm1,4$ | $9,1.10^5\pm5,2$ | $8,05.10^5\pm3,0$ | $2,98.10^6\pm1,0$ |
| ***P.* hybrid** | $3,31.10^5\pm0,6$ | $1,07.10^6\pm0,2$ | $2,24.10^4\pm0,3$ | $7,23.10^4\pm0,9$ |
| ***P. shangton*** | $4,35.10^4\pm3,2$ | $1,65.10^5\pm1,22$ | $1,37.10^4\pm0,4$ | $5,21.10^4\pm1,52$ |
| ***A. formosana*** | $6,35.10^4\pm0,5$ | $1,82.10^5\pm1,4$ | $7,67.10^3\pm4,0$ | $2,2.10^4\pm1,1$ |
| ***M. giganteus*** | $1,73.10^5\pm0,2$ | $4,01.10^5\pm0,46$ | - | - |
| ***S. viminalis*** | $9,2.10^5\pm3,0$ | $2,12.10^6\pm0,69$ | $9,4.10^3\pm2,0$ | $2,16.10^4\pm0,46$ |
| **Sludge** | $2,47.10^4\pm0,45$ | $5,04.10^5 \pm0,92$ | $1,25.10^5\pm0,72$ | $2,55.10^6 \pm1,47$ |
| **Poultry litter** | $3,1.10^4\pm1,6$ | $6,94.10^4\pm3,58$ | $2,7.10^2\pm0,50$ | $6,05.10^2\pm1,12$ |

*In M – in the material. In DM - in its dry matter. * Average. ** Standard deviation.*

The plants with at least microorganisms *P. elunchati* and *P. shangton* are distinguished by a low content of organic and dry matter. The two other tested species of genus *Paulownia* contain at most organic and dry matter within the genus and showed superior microbial content. The willow and elephant grass have the driest and organic matter, but their low humidity is unfavorable factor for the development of microorganisms.

Differences in the dry matter content in the tested materials are significant and it is established a correlation between this content and the quantities and species composition of microorganisms in them. The incoming material into the digester is prepared with certain final dry matter content, regardless of the ingredients used. These data give us reason to consider that a comparison of the quantities of microorganisms is more accurate and appropriate to be made not only for 1 g of fresh material, but also for 1 g of dry matter. Therefore, we propose the comparative evaluation of the microflora and selection of input materials for the digester to take place after calculation based on the content of dry matter.

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