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Researches Regarding the Indices of Anaerobic Fermentation Process for Alfalfa Biomass

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Abstract: Agricultural residues and animal manure are the two most important substrate types used in anaerobic digestion process for biogas production. The effects of particle size on anaerobic digestion of alfalfa biomass were investigated. Alfalfa biomass was ground and divided into four fractions using a clasifier with overlapped sieves (0-0.71 mm, 0.71-1 mm, 1-1.6 mm, 1.6-5 mm). Mixtures of alfalfa biomass and water in varying proportion (5, 6.7% and 10%) were added in Erlenmeyer flasks and thermostated at 37°C, on an orbital incubator, at 150 rpm for several days. Erlenmeyer flasks were sealed with rubber stopper, equipped with plastic tube for sample collection. The tested substrate was characterized by determining the consumption of substrate, total soluble solids and pH after 48, 120 and 144 hours of incubation. Our results showed that reducing the substrate particle size has a positive effect on the biodegradation process of alfalfa biomass. It was observed that the anaerobic digestion parameters had the optimum values for the alfalfa smallest particles. Finally, this study concludes that particle size and alfalfa – water proportion may significantly affect the speed and stability of anaerobic digestion process.

Key words: Alfalfa biomass · Digestion · Total soluble solids · pH · Substrate consumption

INTRODUCTION

Currently, the anaerobic digestion is one of the practical methods widespreaded in Europe and worldwide for bioenergy production. Bioenergy,especially biogas obtained through the anaerobic digestion process, is considered to be one of the high promising alternatives to fossil fuels due to the environmentally and economically significant advantages [1].

Anaerobic digestion is a complex of processes in which microorganisms decompose the biodegradable material in the absence of oxygen. It is used at large scale for energy recovery from biodegradable waste, such as agricultural waste, waste from food industry and wastewater sludge [2, 3]. Anaerobic digestion of farm residues, especially animal manure, slurry and grassland biomass is regarded to be an efficient and ecologically sustainable approach to renewable energy production [4]. During anaerobic digestion, the organic matter is decomposed in the absence of oxygen by a bacterial population and the result is the production of biogas (aproximatively 50-75% CH_4 and 25-50% CO_2, water vapor and trace amounts of hydrogen sulfide) [5].

The solid fraction resulting from the anaerobic digestion process, called digestate, contains nitrogen and phosphorus and is used on large scale as amendment for agricultural soil [6].

Anaerobic digestion takes place in special digesters, in which the digestion conditions are automatically controlled and the digestion parameters are maintained in appropriate boundaries in order to obtain a high yield of biogas production. Fundamental aspects, such as the reactions that occur in the process of anaerobic digestion, the microbial species involved in the process, the effect of raw materials and the effect of operating parameters, such as the pH, the temperature, the C/N ratio, the proportion of volatile fatty acid, the type of digester were analyzed by various researchers in their scientific papers [7].

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Also, there are bioenergy plants cultivated especially for obtaining biogas through anaerobic digestion, whose potential in the production of bioenergy is very high. Lignocellulosic biomass, such as agricultural residues and energy crops represents an abundant organic resource that can be used as substrate in anaerobic digesters. Biomass with hardly degradable components requires pretreatment to enhance conversion of lignocellulose to biogas. Thus, in order to optimize the biodegradability process, physic or chemical pretreatments need to be applied to the tested substrate, mechanical pretreatment being a relatively simple method used to improve biogas production [8, 9]. The general concept of mechanical pretreatment is to enhance the accessibility of microorganisms to the degradable organic matter by increasing the surface area and reducing the cellulose crystallinity [10]. Effective pretreatment can breakdown the linkage between polysaccharides and lignin to make cellulose and hemicelluloses more accessible and more readily degradable to anaerobic bacteria [11].

In the literature can be found a series of experiments conducted on the co-digestion process of animal manure with different agricultural residues or energy crops. Mayer *et al.* [12] concluded that *Miscanthus* crop is one of the best alternative to replace maize silage in the process of anaerobic digestion, recording a biogas production of $5.5 \pm 1x\ 10^3\ m^3\ ha^{-1}$, compared to $5.3 \pm 1x\ 10^3\ m^3\ ha^{-1}$ obtained in the case of maize silage. Li *et al.* [13] assessed the biogas production obtained from rice straw mixed with cow manure in anaerobic mesophilic co-digestion process. The substrate ratio were of 0:1, 1:2, 1:1, 2:1 and 1:0 (rice straw/cow manure). Based on the results of the study, the highest amount of biogas of 383.5 L/kg VS with methane concentration of about 50 – 55%, was obtained for the mesophilic co-digestion of rice straw and cow manure, at a ratio of 1:1, for the organic loading rate of 6 kg VS/(m^3 d).

Other plants, such as maize stalks, alfalfa, switchgrass, sorghum and sunflower were tested in terms of bioenergy production [12].

The use of plant biomass and agricultural waste as a substrate for biogas production has become of great interest, especially in Europe. Ofori and Becker [14] studied the potential of 15 varieties of winter *Brassica rapa* in the production of biogas and also that of 105 combinations of those, concluding that biomass yield may increase by crosses between varieties and by the selection of parental combinations with high productive capacity.

Lately, there were performed lots of experiments on the production of biogas through anaerobic digestion of biomass consisting of various combinations of manure and other biodegradable waste [15-17].

Biogas production from mixtures of goat manure and three types of crop residues (wheat straws, corn stalks and rice straws), was investigated by Zhang *et al.* [18], in different combinations. The combination of goat manure – corn stalks (30:70) had a good yield in biogas production after 55 days of fermentation, but a good efficiency was also obtained from the combinations of goat manure – rice straws (30:70) or goat manure – corn stalks (70:30).

Co-digestion of silage (*Zea mays* L. and *Miscanthus sacchariflorus*) mixed with 0%, 7.5%, 12.5% and 25% swine manure [19] showed that it can lead to high yields in biogas production, compared with separate digestion of silage. The most stable process and the best fermentation efficiency were obtained for the combinations 7.5% and 12.5% swine manure - silage. The higher production ratio was obtained by 12.5% swine manure.

In the present paper was investigated the anaerobic digestion parameters of three different proportions of alfalfa biomass (5%, 6.7%, respectively 10%), mixed with water, for four average sizes of material particles (0-0.71 mm, 0.71-1 mm, 1-1.6 mm, 1.6-5 mm). In order to assess the suitability of alfalfa biomass feedstock for biogas production, were determined the total suspended solids (TSS), pH, moisture and substrate consumption after 48, 120 and 144 hours of anaerobic fermentation.

MATERIALS AND METHODS

Alfalfa plants used for the experimental tests were harvested at maturity, in 2013, from the mountains region Campulung Muscel, Arges county, Romania. They were stored over winter in a dry silage of a local family. Before performing the tests within our paper, it was determined the moisture content of the plants, which ranged between 11.27-12.04 %.

The initial processing of plants consisted in their chopping with an electrical shredding machinery, model VIKING GE-150 (2010, made in Austria), followed by grinding with a Grindomix GM-200 laboratory mill (2012, Retsch – Germany,) equipped with a metal drum and a rotor with two knives made of stainless steel. Initially, the material was pre-chopped manually, the maximum size of stalks being 60-80 mm and the minimum size between

10-25 mm. Rotor speed was of 1500 rpm and sample time was 1 minute. The amount of material introduced into the drum one time was of about 15 grams. After grinding, was performed the size analysis of the grinded material, using a classifier with oscillatory motion, with three overlapped sieves, model VIPO AP-28 (made in Czechoslovakia, 1976), the time of screening was 3 minutes and the oscillation frequency150 osc/min.The sieves had the size of holes of 0.71 mm, 1 mm and 1.6 mm. For the digestion samples were used three ratios of grinded alfalfa and water, respectively: 1:10; 1:15 and 1:20 (representing 10%, 6.7%, respectively 5% proportion of solid – liquid).

The laboratory – scale reactor consisted of Erlenmeyer flasks sealed with rubber stopper, equipped with plastic tube for sample collection.

Each type of substrate was introduced in the ratios mentioned above, in the same quantity of water, in Erlenmeyer flasks. The flasks were introduced in an orbital incubator for six days, at a temperature of 37°C and they were continuously stirred at an oscillation frequency of 150 osc/min. For six days, at regular intervals (every 24 hours), were determined the total soluble solids (TSS) with a thermobalance, after the centrifugation of initial samples at 5000 rpm followed by filtering through a membrane with pores of 0.45 μm. TSS represents a decisive factor for growth and multiplication of microorganisms. Also, were determined, the pH of liquid samples, using a Hanna pH-meter and the substrate consumption. At the beginning of the experiment, the pH value of the tested samples was about 6.4 units.

RESULTS AND DISCUSSION

The results of experimental tests are presented in Table 1. Based on the data presented, were plotted the diagrams of variation of the analyzed indices, depending on the proportion of biomass in liquid and also on the time of digestion or the particle size of the solid faction in the Erlenmeyer flasks. These variations are presented in Figures 1-3.

The total soluble solids, contain soluble sugars, soluble proteins, mineral salts, pigments and other water-soluble compounds that are used as nutritive substrate for different groups of microorganisms involved in anaerobic digestion and biogas production. The most used feedstock is known as vegetal biomass from farms mixed with animal manure.

As expected, from the analysis of data, it can be observed that, in most cases, the TSS tends to increase in relation with the proportion of biomass in the material samples, regardless the time of digestion (respectively the time of parameters determination). The TSS component of the fermentable substrate is more easily solubilized in water in the case of smaller particles thus favoring the growth and multiplication of microorganisms that produce fermentation. This tendency is more obvious for particles with sizes of 0.71-1 mm and over 1.6 mm, after a digestion time of 144 hours (Fig. 1, left up). For the average size of biomass particles, the values of TSS are higher after 120 hours of digestion, having the tendency to decrease over this time. Under these circumstances, the TSS was increased linealry, with a very high correlation coefficient (Fig. 1, right up), for all the values of measurement time. For digestion samples, for which the biomass particles had sizes under 0.71 mm, the TSS only was increased linearly with the proportion of biomass for the digestion time of 120 hours. For measuring time of 48 hours, respectively 144 hours, although the general trend is also an increasing one, it is not linear but is represented by a polynomial regression curve (Fig. 1, left down). The same tendency of polynomial increase of TSS was recorded after 144 hours of digestion for biomass samples with sizes under 0.71 mm or between 1-1.6 mm (Fig. 1, left up). In case of particles with sizes over 1.6 mm, the linear increasing tendency of TSS with increasing biomass proportion is kept for all measuring intervals and the correlation coefficient has values almost equal to 1.

The pH is a parameter that provides significant information on the stability and evolution of the anaerobic digestion process. The evolution in time of the pH variation for each proportion of alfalfa biomass is shown in Fig. 2.

Regarding the pH of material samples subjected to digestion, there is a decreasing trend of it with the proportion of biomass for all four sizes of material particles, for all measuring intervals. After 144 hours of digestion, the linear decreasing tendency of pH is more obvious for the sample with biomass particles higher than 1.6 mm and less obvious for particles between 1-1.6 mm (Fig. 2, left up). A linear decreasing tendency was estimated, for all samples, by the values of correlation coefficient R^2, which were very high in most analyzed cases.

After 48 hours of digestion, the samples of biomass with more obvious decreasing tendency of pH, with the proportion of biomass were those whose particles of material ranged between 0.71-1 mm, but also those with particles over 1.6 mm (Fig. 2, left down). The same phenomenon occurs after 120 hours of digestion (Fig. 2, right down). By making an analysis of pH variation

Table 1: Characteristics ofthe tested substrate after 48, 120 and 144 h of digestion

		TSS (%)			pH			Substrate consumption (%)		
Particle dimensions, mm	Substrate proportion (%)	48	120	144	48	120	144	48	120	144
>1.6 mm	5	1.3	1.2	0.7	6.87	7.22	7.47	2.40	3.77	5.61
	6.7	1.8	1.8	1.7	6.59	6.97	7.35	2.19	3.51	4.9
	10	2.9	2.8	2.7	5.51	5.80	6.35	2.23	3.40	4.96
1-1.6 mm	5	2.2	1.2	1.1	6.00	6.27	6.82	2.28	3.76	5.06
	6.7	2.6	1.9	1.8	5.43	6.27	6.82	2.33	4.18	5.57
	10	3.0	1.8	1.4	5.24	5.67	6.71	2.03	3.78	5.27
0.71-1 mm	5	2.0	1.4	1.3	6.7	6.76	6.97	2.17	4.19	5.71
	6.7	2.6	2.4	1.9	5.41	5.75	6.41	2.32	4.25	5.79
	10	3.3	3.1	3	4.91	5.39	6.15	2.37	3.77	5.42
<0.71 mm	5	2.7	1.9	1.5	6.57	5.72	6.57	2.54	4.40	5.99
	6.7	3.1	1.8	1.9	6.17	5.66	6.36	2.54	5.44	7.19
	10	4.6	2.4	1.8	5.66	5.79	6.33	2.95	5.01	6.45

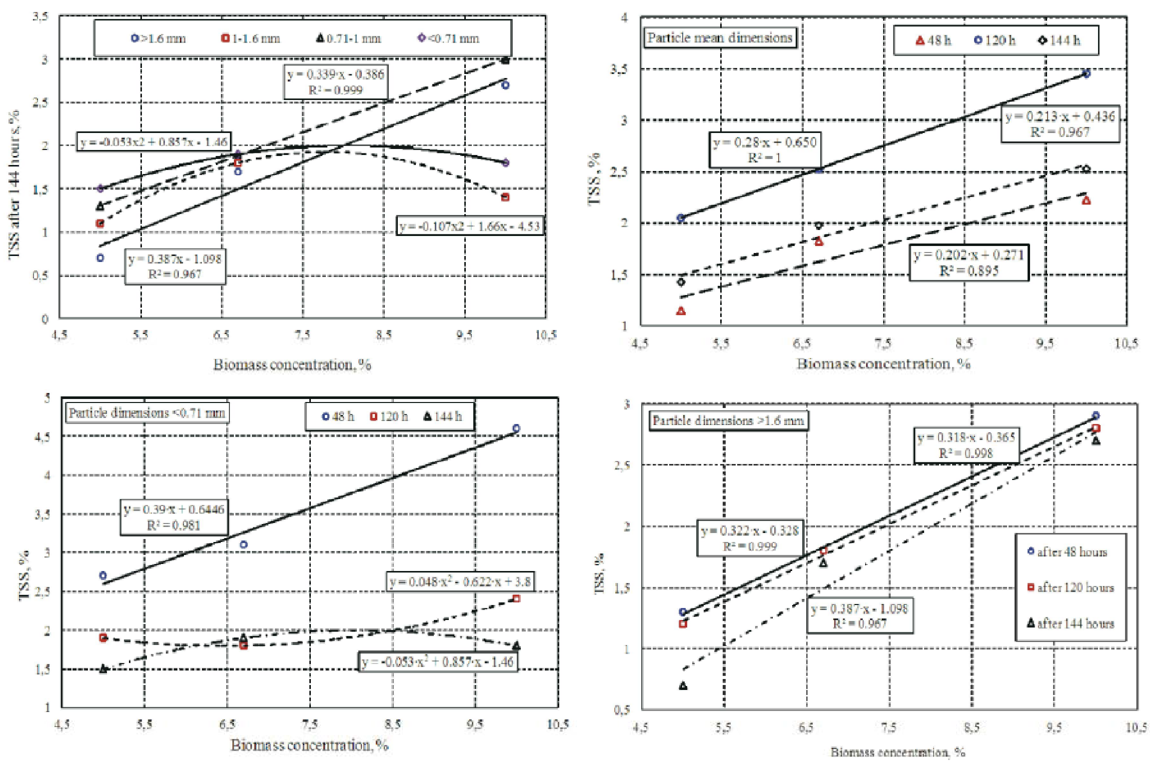

Fig. 1: Variation of the total soluble solids with biomass proportion for four fractions of grinded alfalfa

depending on the average sizes of biomass particles (as average of the values recorded for all four fractions of material with sizes <0.71 mm, 0.71-1 mm, 1-1.6 mm and >1.6 mm), then its decreasing tendency with the proportion of biomass is obvious for all measuring intervals, respectively 48, 120 and 144 hours (Fig.2, right up), with the correlation coefficient R^2 very close to 1. The values of the coefficients of linear equations presented in the figure are closely related both to the amount of biomass and to the sizes of material particles or to the time of determination of digestion parameters.

These results are in accordance with those obtained by Nges and Bjornsson [20], who reported that a pH value above 6.8 units is considered an indicative of anaerobic digestion stable process. Fluctuations of pH in the Erlenmeyer flasks, could be due to the periodic

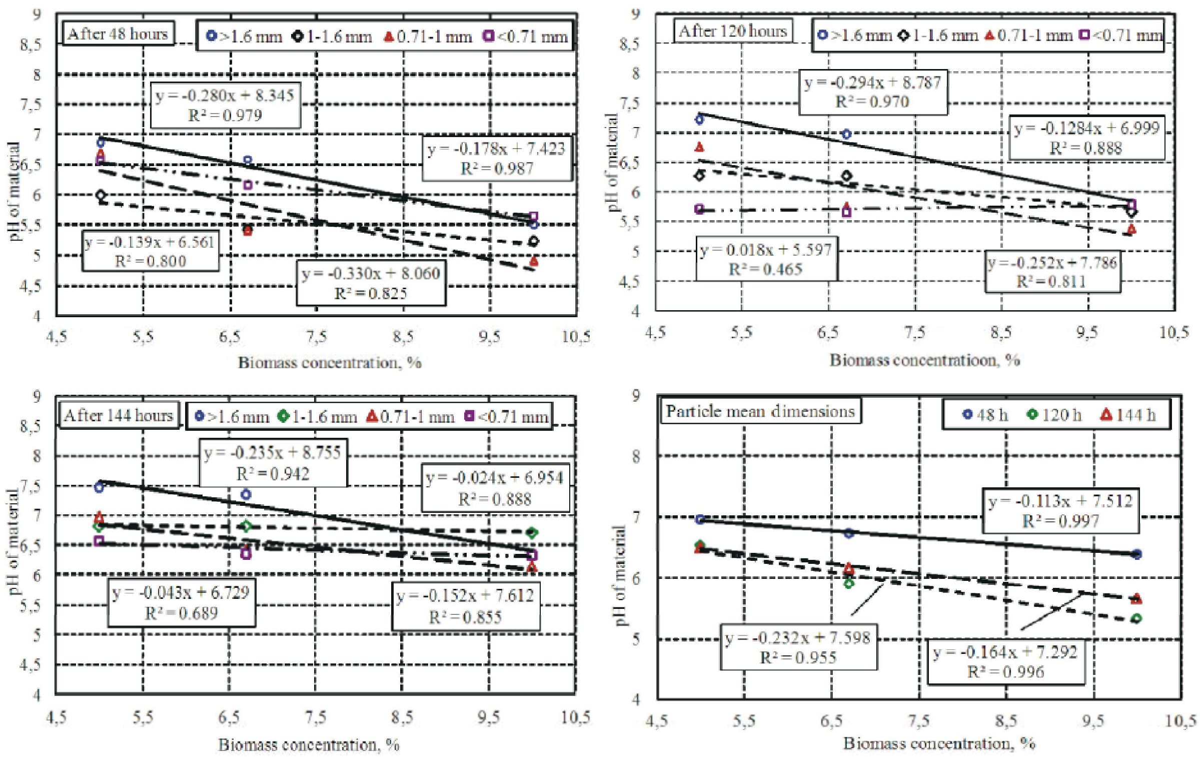

Fig. 2: Variation of pH of material with biomass proportion for the four fractions of grinded alfalfa

accumulation, conversion and consumption of volatile fatty acids in the anaerobic digestion stages. Similar results were also obtained by Anitha *et al.* [21], who tested the anaerobic co-digestion of agricultural wastes and animal manure. They reported that the pH of the reaction mixture fell rapidly from 7.1 to 5.5 units because the more biodegradable organic fraction had been hydrolyzed and converted into volatile fatty acids.

Speed on substrate consumption is the speed of enzymatic reaction in the material during the fermentation of samples. It was determined by weighing, after the above mentioned intervals of measurement and in relation to the initial mass of the samples. Since the substrate is mainly consumed for cell growth and maintenance, the specific ratio of substrate consumption can be expressed in terms of growth yield and ratio of specific consumption of microorganisms, which is also dependent of the proportion of biomass [22].

Analyzing the data in Table 1 and especially the digrams presented in Fig. 3, it was found that the variation of this parameter is more random than specific, related to the proportion of biomass in the analyzed samples. We might say that there is no obvious consumption of substrate for the specified time of digestion, for any fraction of various sizes of material particles. Therefore, the most used regression function in the analysis of substrate consumption trend was polynomial.

It can be concluded that for the sample of material with small particles (under 0.71 mm) there is a increasing tendency of substrate consumption in relation to the proportion of biomass in the sample, although this is only very evident after 120 hours of digestion (Fig. 3, right down). A similar tendency can be seen when taking into account the average sizes of material particles, as the substrate consumption is slightly increasing with the increase of biomass proportion, for all values of the digestion time (Fig. 3, right up).

Some researchers investigated the effect of reducing particle size of agricultural waste on anaerobic digestion process. Sharma *et al.* [23] assessed the effect of five particle sizes of seven agricultural and forest residues (0.088, 0.40, 1.0, 6.0 and 30.0 mm) used as substrate for biogas production through anaerobic digestion at 37°C. They reported that the maximum quantity of biogas with methane concentration between 60 and 73% (v/v) was obtained from raw materials with the smallest particles of 0.088 and 0.40 mm.

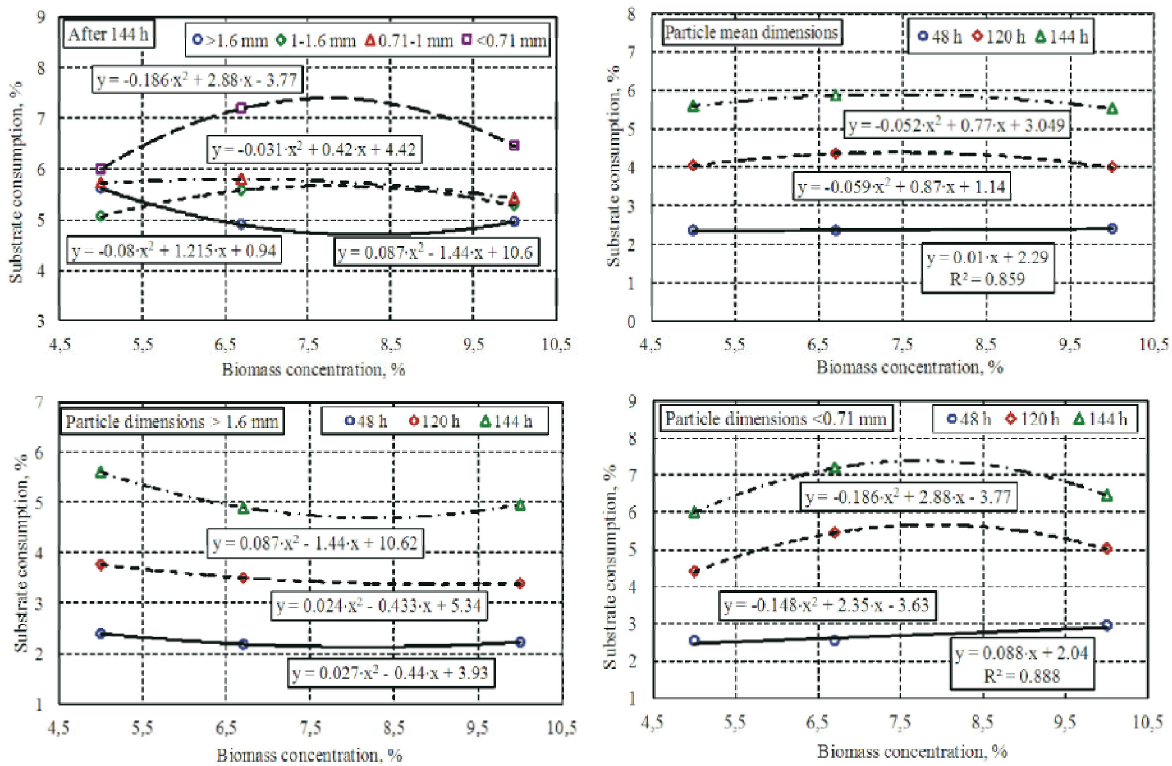

Fig. 3: Variation of substrate consumption with biomass proportion for the four fractions of grinded alfalfa

Dumas *et al.* [24] investigated the biodegradability of ultra-fined ground wheat straw under mesophilic anaerobic conditions. They reported that particle size reduction can be increase the substrate solubility and the kinetics of anaerobic digestion process and can decrease the retention time in the digester. On the other side, the main conclusion was that micronization (particle size between 759–48 µm) do not improve methane yield but has a positive effect on the biodegradation kinetics. Another experiment was conducted by Izumi *et al.* [25]. In this case, the influence of reducing particle size of food wastes on biogas prodution, at mesophilic temperature (37¡ÆC) for 16 days, was evaluated. The results showed that particle size reduction from 0.888 to 0.718 mm improved the methane generation by 28%.

CONCLUSIONS

Alfalfa straw can be found in animal manure and they can influence its degree of decomposition, when it is used in anaerobic digestors for biogas production. The grinding degree of biomass in manure can vary.

For various fractions of alfalfa with particles of different sizes, the indicators of digestion were different, in the conditions in which the material was stirred continuously, in mesophilic anaerobic conditions (37°C).

Experiments have shown that the TSS values have a general increasing tendency with the increase of biomass proportion in the liquid and with the digestion time, for the entire alfalfa mass introduced into the Erlenmeyer flaks. The increase is general linear with the proportion of biomass in the range of 1-2 units for the TSS values (depending on particle size), for decomposition periods from 2 to 6 days.

There was not recorded a sensitive evolution of substrate consumption, for the analyzed digestion periods, therefore, we consider necessary the mixture of alfalfa biomass with animal manure.

The pH of material samples recorded a decrease during decomposition with the increase of biomass proportion, for all fractions of material, the decrease being visible especially after six days of incubation. The range of the decrease rate was 0.2-1.5 units, depending on the proportion of biomass, the decomposition time and the sizes of alfalfa biomass fraction.

These results suggested that optimized reduction of the particle size of the substrate for anaerobic digestion process, could improve the biogas production. Also, the biodegradability of the organic matter in anaerobic digestion process may be increased by reduction in particle size of tested substrate.

The results of our research will be considered in our future research, as we intend to deteremine the parameters of anaerobic fermentation for mixtures of plant biomass and animal manure. Also, they can be useful for other specialists who perform research in this field, but also to those who exploit the anaerobic digestion process.

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