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Influence of Ultrasonic Disintegration on Efficiency of Methane Fermentation of *Sida hermaphrodita* Silage

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ABSTRACT

The technologies related to the anaerobic decomposition of organic substrates are constantly evolving in terms of increasing the efficiency of biogas production. The use of disintegration methods of organic substrates, which would improve the efficiency of production of gaseous metabolites of anaerobic bacteria without the production of by-products that could interfere with the fermentation process, turns out to be an important strategy. The methane potential of commercially available biodegradable raw materials is huge and their effective use gives the prospect of obtaining an important renewable energy carrier in the form of biogas rich in methane. Ultrasonic disintegration may play a special role in the pre-treatment of substrates subjected to methane fermentation. The pre-treatment based on ultrasonic sonication has a positive effect on the availability of anaerobic compounds released from cellular structures for microorganisms. The research was aimed at determining the influence of ultrasonic sonification on the anaerobic distribution of the organic substrate used, which constituted the mallow silage along with cattle manure with hydration of 90%. The research was carried out using the UP400S Ultrasonic Processor. The disintegration process was applied in two technological variants. The efficiency of biogas and methane production was determined depending on the technological variant used and the time of disintegration. The influence of sonication time on the effectiveness of anaerobic transformation was demonstrated. The highest biogas yield and methane production potential was recorded at 120s. The prolongation of the action time of the ultrasonic field did not significantly increase the biogas production. The use of disintegration of liquid manure as the only medium for the propagation of ultrasonic waves was sufficient to increase the production of gaseous metabolites of anaerobic bacteria. Subjecting the substrate additionally containing mallow silage to the process to sonication did not significantly affect the efficiency of the fermentation process. The percentage of methane in the biogas produced was independent of the pre-treatment conditions of the substrate and was in the range of 66–69%.

Keywords: ultrasonic disintegration, methane fermentation, *Sida hermaphrodita* silage, biogas production.

INTRODUCTION

A wide range of waste substrates can be processed into a valuable energy carrier that is methane-rich biogas, with the use of anaerobic fermentation systems. The type of waste submitted to anaerobic degradation is mostly derived from agriculture, e.g. animal waste (liquid manure), industrial waste (waste products from slaughterhouses, blood, fish waste products, etc.), waste and residue matter from plant production (maize silage, wheat straw, barley seed cake, grass, clover leaves, sugarcane bagasse, etc.) as well as household waste [Mata-Alvarez et al. 2000, Alagöz et al. 2018].

The anaerobic fermentation processes are continually improved in terms of the implemented technologies, so as to enhance the acquisition of gaseous metabolites from anaerobic bacteria. One of the factors limiting the rate of anaerobic fermentation is the process of hydrolysis, which prolongs the whole technological process [Alagöz et al. 2018]. In order to improve the efficiency of digestion and shorten the duration of fermentation, various disintegration methods are applied, most often to comminute the sludge itself or the raw material used as a feedstock in fermentation chambers. Physical methods are the most frequent ones in the preliminary disintegration process. The available research reports point to the high efficiency of ultrasound disintegration compared to other approaches to biomass conditioning. The action of ultrasounds in aqueous solutions consists of quick cycles of the compression and decompression of sound waves, which promote the formation of microbubbles within the solution. The rapid release of energy during decompression leads to the destruction of the structure of organic substrate matter subjected to disintegration. At first, this accelerates hydrolysis, which facilitates a more rapid course of the subsequent fermentation stages [Zhang et al. 2008, Grönroos et al. 2005].

The studies conducted thus far have focused on the disintegration of activated sludge, the stage which improves the solubility of organic substances in sludge, contributes to the destruction of cells and flocs, and therefore helps to release the intercellular matter. It also improves the rate and efficiency of methane fermentation processes, which translates into greater production of biogas and methane [Bougrier et al. 2006, Wood et al. 2009]. The inclusion of the ultrasound pretreatment processing of activated sludge prior to the anaerobic fermentation can raise the biogas production by 24% up to 140% [Saha et al. 2011, Zhen et al. 2017].

It has been demonstrated that the essential factors which influence the effects obtained in an ultrasound field are the ones which characterize the emission of ultrasounds, i.e. frequency, power and intensity, but also the exposure time. Many researchers draw the attention to the fact that the ultrasounds generated within a relatively narrow frequency range from 16 to 50 kHz should be used for the disintegration of organic substrates [Eder et al. 2002]. The changes in a medium induced by the active influence of an ultrasound field may vary, being dependent on both the aforementioned characteristics of ultrasounds and the physicochemical properties of the medium, such as viscosity of the liquid, presence of electrolites and polyelectrolites, the macrostructure and character of the suspension, ambient temperature and many other factors. Hence, the ultimate effect results from the interplay of numerous factors which occur simultaneously. Hydration of the substrate is an extremely important parameter. The higher the hydration, the better the transmission of an ultrasound field deep into the structures of organic matter, and therefore the better effects of the action produced by the factor. The exposure of organic substrates to an ultrasound field has a positive impact on the destruction of structures and release of the cellular matter, which becomes more readily available to the microorganisms in the subsequent anaerobic fermentation process. This results in a more effective production of gaseous metabolites and a shorter duration of the whole process. When the cell structures are densely compacted, as in lignocellulose substrates, the high effectiveness of the lysis of cells and release of intracellular substrates to the liquid phase is much more difficult to achieve. It is then necessary to apply either a large dose of energy or long exposure to ultrasounds. Sonification of sludge, like most preliminary substrate conditioning processes, is an energy-consuming process, which raises concerns whether this approach could be implemented in industrial practice. Chu et al. (2001) report that an insufficient dose of ultrasounds does not release insoluble substances directly to the suspension, but allows hydrolytic enzymes to attack organic substances more easily [Chu et al. 2001]. Many researchers have discovered that the ultrasound stimulation promotes the activity of enzymes, growth of cells and permeability of the cell membrane [Xie et al. 2009, Liu et al. 2003, Pitt and Ross 2003].

The purpose of this study has been to determine the effect of a dose of ultrasounds on the disintegration of selected substrates prior to the methane fermentation process.

METODOLOGY

The research on the effect of sonification on particular components in a substrate prior to methane fermentation was conducted under laboratory conditions. The analyzed substrate was a mixture of Virginia fanpetals (*Sida hermaphrodita rusby)* silage and bovine slurry, mixed in a 1:1 ratio. In all the variants, the hydration of the substrate was around 90%. The characteristics of the substrate used for the experiments are specified in table 1.

The study was divided into two parts. Stage I consisted in the sonification of the substrate, which was separated between two technological variants, depending on the material subjected to ultrasounds:

- Variant 1 – mixture of Virginia fanpetals silage + bovine liquid manure V1,
- Variant 2 – bovine liquid manure V2.

Table 1. Characteristics of *Sida hermaphrodita* silage used in the studies

Parameters	Value
TS [%]	27.6±0.78
VS [%T.S.]	91.9±0.97
SS [% T.S.]	6.81±0.076
T_N [g/g]	0.005±0.0001
COD [g O_2/g_{TS}]	1.539±0.043
TC [g/g]	0.417±0.054
TOC [g/g]	0.379±0.007

In variant 2, ultrasound-disintegrated bovine slurry was added in the same ratio to Virginia fanpetals silage and then dosed to fermentation chambers at stage II of the experiment.

During the first stage of the research, a UP400S ultrasound homogenizer was employed for the process of disintegrating the substrate. The specification of the device is given in table 2, and its typical range of applications consists of: homogenisation, deagglomeration, lysis and disintegration of cells, protein extraction and emulsification of liquids. Three substrate disintegration time lengths were tested during the study: 60, 120 and 180 s, with the homogenizer set at its maximum power.

The UP400S (400 W, 24kHz) ultrasound homogenizer is the most powerful laboratory device of this type. The samples submitted to ultrasound processing contained 100 g of substrate each.

The second stage of the research aimed to identify the methane potential of the tested organic substrate depending on the technological variants established in stage 1. An automatic methane potential test system AMPTS II Bioprocess Control was applied. This device is used to measure the flows of biomethane produced during the anaerobic fermentation of biodegradable substrates.

The AMPTS II Bioprocess Control system was composed of three sub-units. The main part consisted of bioreactors immersed in a water bath in which constant temperature was maintained. The capacity of each bioreactor was 500 ml. The reaction chambers were connected to stepper motors, which powered slow rotational blades mixing the contents of the reactors. Mixing was repeated cyclically, every 10 minutes for 30 s at 100 rpm. The second component was a system for measuring the amounts of biogas. It was composed of a water container with measuring cells and motion detectors placed inside. Each reactor chamber had a corresponding measuring cell, which ensured completely automatic measurements of the produced biogas via motion sensors. The last component, a data collection system, enabled the results be seen and controlled throughout the whole experiment.

The research method applied in this study allows the user to determine the activity of anaerobic sludge, biodegradability of substrates and volumes of gaseous products of microorganisms' metabolism. The equipment recorded and analyzed the changes in partial pressure in the measuring chamber caused by the biogas production during anaerobic processes carried out by bacterial microflora.

In each of the experimental variants, the reaction chambers, each of the total capacity of 500 cm^3, were inoculated with anaerobic sludge originating from a digester operating on a semi-technical scale, which was fed with Virginia fanpetals and bovine liquid manure. The characteristics of the inoculant are given in table 3.

Depending on the characteristics of the tested substrates and the technological variants, the sludge was dosed in appropriate amounts of the substrate. The tested load of the fermentation chambers with organic compounds was approximately 5.0 g d.m./dm^3.

At the start of the experiment, in order to ensure anaerobic conditions, the reactors and their contents were deoxygenated by forceful blowing of nitrogen. The volumes of biogas produced in the reactors were measured for 40 days under mesophilic conditions and at a temperature of 38°C.

Table 2. Characteristics of UP400S Ultrasonic Processor

Parameters	
Model	UP400S
Power	400 W
Ultrasound frequency	24 kHz
Automatic frequency adjustment	Yes
Regulated amplitude	100%
Adjustable pulse	100%

Table 3. Characteristics of the anaerobic sediment used in the experiment

Parameter	Value
pH	7.72±0.14
Hydration [%]	94.98±0.21
TS [%]	5.02±0.18
VS [$\%_{T.S.}$]	72.11±2.41
SS [$\%_{T.S.}$]	48.96±1.27

ANALYTICAL PROCEDURES

After both the disintegration and fermentation processes, the analyses of the tested substrate were performed, as required. The contents of dry matter, dry and mineral organic matter were determined with the gravimetric method, which consisted of evaporating a sample, drying the residue at 105°C to constant weight and then weighing it. The COD values were obtained using Hach Lange cuvette tests and a UV/VIS DR 5000 spectrophotometer. The ratio of alkalinity to volatile acids was determined using a TitrLab AT 1000 potentiometric titrator. The content of glucose was monitored with a YSI 2700 Select analyzer. Determination of glucose was achieved through the measurements of D-glucose made with an enzymatic probe. Glucose oxydase is the enzyme immobilized in the membrane. Concentrations of total carbon (TC), inorganic carbon (IC) and total organic carbon (TOC) as well as total nitrogen (TN) were assayed with a Flash 2000 Thermo Scientific elemental analyzer.

The samples for an analysis of the ratio of volatile acids to alkalinity F/T were obtained by centrifugation of the material in a Rotina 380 laboratory centrifuge for 3 min at 9000 rotations per minute.

For assays of COD of sugars, TOC, TC, IC, TN, the samples were additionally vortexed in a Mini Spin Eppendorf laboratory vortex mixer for 90 s at 13000 rpm.

The gas for the qualitative analysis was obtained from respirometers using a needle and a gas-proof syringe. The respirometers were fitted with rubber bungs and tubing, which enabled us to take samples of gas. Each time, 5 cm^3 of biogas was sampled, and the total and percentage compositions of the sampled biogas were determined on a GC Agillent 7890 A gas chromatographer. The chromatograph was coupled with a thermo-conductive detector. The percentage content of the following biogas components was measured: methane CH_4, carbon dioxide CO_2 and oxygen O_2.

STATISTICAL ANALYSIS

The statistical analysis of the results and the calculations pertaining to coefficients of determination R2, were supported by STATISTICA 10.0 PL software. The cultures of microalgae for each series and variant of the experiment were grown in triplicate. All physicochemical analyses were performed in triplicate as well. The verification of the research hypothesis regarding the distribution of each variable was based on the W Shapiro-Wilk test. One-factor analysis of variance (ANOVA) was performed to identify the significance of differences between the variables. The homogeneity of variance in groups was checked with the Levene's test. The RIR Tukey test served to determine the significance of differences between the analyzed variables. The level of significance in the above-mentioned tests was set at $p = 0.05$.

RESULTS AND DISCUSSION

The study on the impact of sonification of substrate prior to the methane fermentation was conducted in order to improve the efficiency of biogas production during the methane fermentation. The first stage of the research consisted in disintegration of substrate (variant 1) and liquid manure (variant 2) before fermentation.

During sonification, the changes in temperature and energy supplied to carry out the process of substrate conditioning were observed (Tab. 4). As the time of exposure of substrate to ultrasounds was prolonged, the amount of supplied energy increased proportionally.

The greatest changes in concentrations of the analyzed parameters were noticed between the research variants. When liquid manure was submitted to disintegration, the concentrations of glucose as well as the ratio of volatile acids to alkalinity F/T were nearly twice as high as in the control sample or variant 1 of substrate conditioning. Statistically significant differences were also observed between the variants with respect to the content of organic content expressed through COD (Table 5). Within the individual technological variants, statistically significant differences were observed with respect to the nitrogen compounds. The exposure of substrate to ultrasounds for 60 s did not cause changes in this parameter relative to the control. Continuing sonification for another 60 s, resulted in an increase of the nitrogen content in both technological variants by about 10%. The subsequent dose of ultrasounds did not have such an effect. In variant 2, where bovine liquid manure was conditioned, the content of organic carbon increased significantly compared to the control and variant 1 samples (Table 5).

In the second stage of the study, disintegrated substrate was fed into fermentation chambers to analyze the biogas production potential. The

Table 4. Parameters of the ultrasonic disintegration process

Type	60 s			120 s			180 s		
	E [Ws]	E[Ws/g]	T [°C]	E [Ws]	E[Ws/g]	T [°C]	E [Ws]	E[Ws/g]	T [°C]
V1	7551.3	75.51	28.5	13585.6	135.86	68.2	18247.6	182.48	87.4
V2	10889.58	108.90	37.6	21794.18	217.94	45.2	30124.4	301.24	57.6

Table 5. Characteristics of the substrate after the disintegration process depending on the technological variant

Type		Carbohydrates [mg $C_6H_{12}O_6/dm^3$]	FOS/TAC	TOC [mg/l]	TC [mg/l]	IC [mg/l]	N [mg/l]	DOC [g/l]	C [% s.m.]	N [% s.m.]	$C_{org.}$ [% s.m.]	$N_{org.}$ [% s.m.]
	K	45.0	5.13	19167	19350	182	3502	45.0	41.09	3.03	38.64	3.13
V1	60s	47.9	5.07	19535	19710	177	3597	45.6	38.36	2.78	38.42	2.89
	120s	48.1	4.93	19603	19800	197	3867	45.2	39.55	2.98	38.70	2.26
	180s	50.33	5.09	19724	19916	192	3794	45.1	39.42	2.89	38.48	2.54
V2	60s	103.23	9.23	15745	15912	167	3007	33.2	41.79	3.26	41.22	3.16
	120s	95.39	9.65	16797	16985	188	3932	35.0	43.01	3.15	42.34	3.12
	180s	98.41	9.32	16785	16959	174	3872	34.6	42.6	3.11	41.86	3.02

amount of obtained biogas was assessed in relation to the applied technological variant. The highest productivity of gaseous metabolites produced by bacteria was determined in variant 1, at the sonification time equal to 180 s. The biogas gain was then 1456.4±0.7 Nml, which was 23.5% more than in the control process (Fig. 1). Quarmby et al. achieved 15% higher productivity of biogas from sludge submitted to sonification than in the control test, where substrate was not conditioned [Quarmby et al. 1999]. The lowest quantities of biogas were noted in both tested variants when the application of ultrasounds lasted for 60 s. However, no statistically significant differences were observed between the amounts of biogas produced at the sonification time of 120 s and 180 s.

Moreover, there were no statistically significant differences in the amount of produced biogas depending on the material submitted to sonification. Sonification of either whole substrate or liquid manure alone resulted in comparable amounts of produced biogas. This finding suggests that there is no need to disintegrate the whole biomass prior to feeding it into a fermentation chamber. For the reasons related to the maintenance of a biogas plant and technological aspects of biogas production, sonification of the liquid phase alone is sufficient.

The content of methane in the biogas obtained, irrespective of the tested process variant, was around 66–69% (Fig. 2). Very similar results were reported by Part et al. who tested the fermentation of *Chlorella vulgaris* microalgal biomass. Disintegration of cells in their experiment was achieved with the help of an STH-750S ultrasonic homogeniser (Sonictopia, Korea) the maximum power of which was 750 W. The ultrasound field applied in the cited study ranged from 5 to

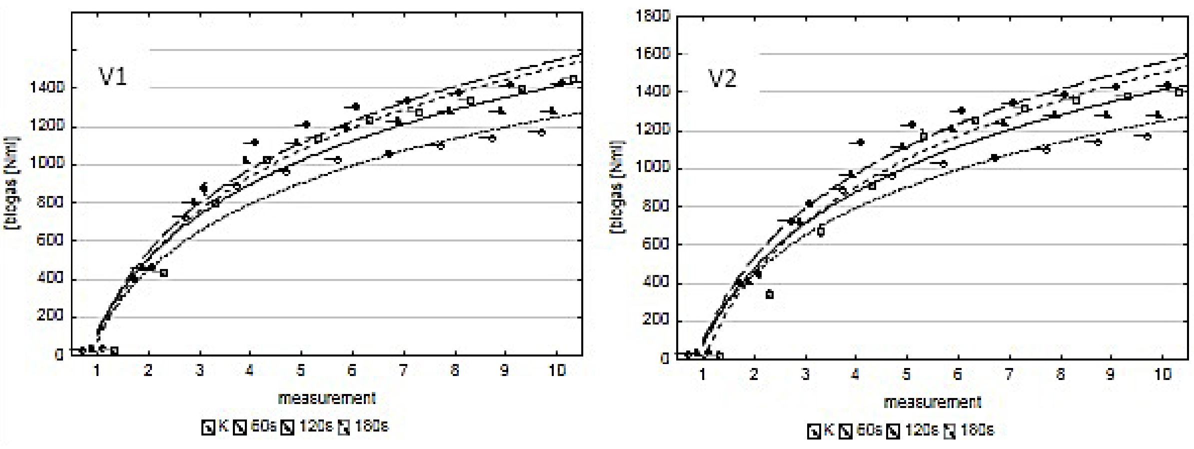

Figure 1. Effectiveness of biogas production in the experiment

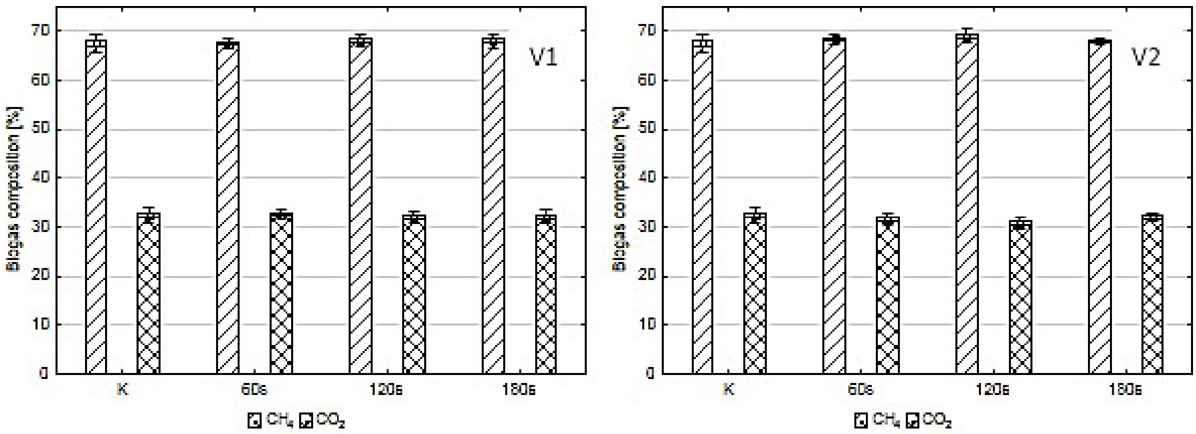

Figure 2. Biogas composition depending on the technological variant.

200 J/ml. The disintegrated biomass was fed to the fermentation chambers which had been inoculated with sludge. The content of methane in the biogas obtained under mesophilic conditions was around [Park et al. 2013].

Alagöz et al. investigated the applicability of preliminary substrate conditioning to improve the productivity of biogas production and to achieve higher biogas production in co-fermentation of pomace from olives and grapes. The use of ultrasonic disintegration enabled the researchers to obtain about 6 000 ml biogas with the methane concentration of 60%. The methane gain per organic matter unit was approximately the same as the one achieved from the fermentation of Virginia fanpetals silage, and equaled *ca* 0.1 Nl/g_{VS} [Alagöz et al. 2018].

The quantities of produced biogas converted per organic matter unit confirmed that the most beneficial disintegration variant was the one where sonification lasted for 120 s. The shortest sonification time only slightly improved the effectiveness of the process, while doubling that time resulted in the production of 1010.67±7.77 Nl/kg_{VS} of biogas (Fig. 3). However, the further extension of the ultrasound pretreatment tested did not lead to significantly higher methane productivity (Fig. 3). The amounts of biogas produced in this experiment are relatively high compared to the previous research on disintegration and fermentation of organic matter. Park et al., mentioned above, who investigated ultrasound disintegration of microalgae *Ch. vulgaris* and their fermentation, reported half the amount of biogas produced in our study, i.e. about 400 ml/g_{VS} [Park 2013]. The productivity of methane for the most productive variant amounted to 687.36±3.18 ml_{CH4}/g_{VS}, which was 30% more methane gain

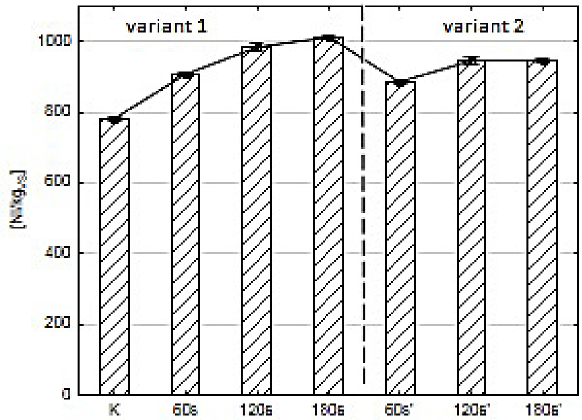

Figure 3. Biogas production depending on the technological variant

than from the control sample, which had not been conditioned prior to fermentation. Similar results were reported by Passos et al., who submitted to sonification mixed biomass of microalage used to pre-treat municipal wastewater and sewage at a secondary settlement tank. The average load of the tanks was about 24 g COD/m^2·d. The biomass of microalgae was exposed to an ultrasound field of the power of 50–70 W. The exposure time was 10, 20 and 30 min. These researchers reported an improvement in the methane productivity ranging from 6 to 33% [Passos et al. 2014].

CONCLUSIONS

The study pertained to the disintegration process applied in two technological variants. The efficiency of biogas and methane production was determined depending on the technological variant used and the time of disintegration. The influence of sonication time on the effectiveness of anaerobic transformation was demonstrated. The highest biogas yield and methane production potential was recorded at 120s. The prolongation of the action time of the ultrasonic field did not significantly increase the biogas production. The use of disintegration of liquid manure as the only medium for the propagation of ultrasonic waves was sufficient to increase the production of gaseous metabolites of anaerobic bacteria. Subjecting the substrate additionally containing mallow silage the process to sonication of did not significantly affect the efficiency of the fermentation process. The percentage of methane in the biogas produced was independent of the pre-treatment conditions of the substrate and was in the range of 66–69%.

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