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Finding the optimal parameters for the steam explosion process of hay

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Abstract

Combining biochemical principles and mathematical methods were defined optimal extrusion (steam explosion) process conditions for pelleted hay resulting in maximal cumulative production of biogas (CBGP). Operating experiments were conducted on a continuous modifiable extruder. Variable parameters, the reaction time, pressure and dry mass were compared and optimized. An assumption that longer reaction time, lower dry mass and higher operating pressure results in higher yields of biogas were confirmed only in defined pressure range. Critical conditions, from where the yield starts to decrease due to inhibitors formations were also modeled. It was found that the optimal process conditions were in the neighborhood of pressure 1.3 MPa, reaction time 7 minutes and 8% of dry mass resulting in 405 m^3 of biogas (52.3% CH_4) per ton of dry mass.

Keywords: extrusion dynamics, process optimization, biogas production.

Busca de los parámetros óptimos para la extrusión de biogás del heno

Resumen

En el presente estudio se compararon las diferentes condiciones de reacción para el proceso de la extrusión (también conocido como explosión de vapor) del heno. Los experimentos de funcionamiento se llevaron a cabo en una extrusora continua y modificable con el fin de encontrar parámetros óptimos. El factor principal de la optimización fue la profundidad de la desintegración de material vegetal comprobada durante 30 días de la producción acumulada de biogás (CBGP). Los parámetros de la extrusión fueron retrasos en la extrusora, hydromodul y presión. La vinculación de los principios bioquímicos y los métodos matemáticos se han encontrado como las condiciones óptimas para el máximo rendimiento de la tecnología del biogás. La suposición de que el proceso de alta presión aumenta los rendimientos de biogás se confirmó sólo en determinadas áreas. Fueron modelados límites, en los que con la presión creciente los rendimientos de la producción de biogás disminyen, debido a la producción de los inhibidores del proceso anaerobio. Se demostró que las condiciones de proceso óptimo paragránulos (*pellets*) del heno se encuentran alrededor de presión 1,3 MPa, de tiempo de residencia en extrusora 7 minutos, 8% de materia seca. Obtenida extrusora crea 405 m^3 biogás (52,3 CH_4%) por tonelada de materia seca.

Palabras clave: dinámica de extrusión, optimización de procesos, producción de biogás.

Introduction

The research is based on the idea of disintegration of the waste phytomass (hay) by waste heat from cogeneration at biogas station. When phytomass is being disintegrated into units with lower molecular weight, special attention must be paid to the formation of fermentation inhibitors. Especially the formation of phenolic compounds from lignin degradation and the formation of furfural and hydroxymethylfurfural (HMF) from sugar degradation should be prevented. Study of the process dynamics is obviously important also for the economic evaluation of the overall technology.

Importance of phytomass disintegration

It is generally known that when any material is disintegrated on smaller particles the surface area increase and most kind of processes are than more intensive with higher yield. Side effect of this procedure is mostly increased density of the material, which has positive impact on logistics. The main dilemma is based on the fact that the energy consumption increases rapidly as the particle sizes decrease slightly [1]. Using knife-milling technology, the specific energy increased from 61 $kWh.t^{-1}$ on 657 $kWh.t^{-1}$ when decreasing the sieve size from 8 mm to 0.25 mm [2].

When discussing the particle size it is also important to know all the relationships between dry mass content, feed rate, costs including consumpted energy related to process conditions and all necessary phytomass properties and specifics.

Most of the phytomass is often described as lignocellulose, which is the most abundant renewable biomass on earth. Lignocellulose is composed mainly of cellulose, hemicelluloses and lignin. Both cellulose and hemicelluloses are polymers, a potential source of fermentable sugars. Lignin can be used for production of other chemicals. Many physio-chemical structural and compositional factors hamper the digestibility of cellulose present in lignocellulosic biomass. Obstacles in pretreatment processes are based on the connective ability of lignin, which is like the ballast binder of cellulose and hemicelluloses. Research is focused on disintegrating phytomass into its constituents in a market competitive and environmentally sustainable way in meaning of minimal use of energy and chemicals.

During the fight against animal kingdom changes in the natural defense of plants evolved such as stiff spines, toxic or antinutritional substances and others. Fixed internal structure of lignocellulose became crucial part of passive defense. Cellulose fibers (hundreds to tens of thousands of crystallized glucose molecules) resemble iron rods, hemicellulose looks like a binding barbed wire and lignin as ballast cement. All together united tough by hydrogen and covalent bonds. Cellulose is a long, unbranched, linear condensation polymer of D-anhydroglukopyranoses (link β-1, 4 bond) with degree of polymerization from 100 to 20 000 [3]. These could be in the 500 to 20 000 range as well [4, 5]. Adjacent cellulose molecules are paired by hydrogen bonds and van der Waals forces, causing parallel crystalline structure, whose nature is a critical cause of the topic.

The research confirmed the importance of reaction temperature, the role of previously mentioned hydrogen bonds and the importance of cellulose-type structure [6]. Coincidentally, it may be noted that the strength of the cellulose structure is also being used by the animal kingdom. For example a group of flagellates (*Dinoflagellata*) use cellulose shields for passive defense of their body [7].

Choosing the correct parameters of disintegration can affect plant matter in such a way that the internal strength (crystallinity) of plant fibers partly disperse. The following hydrolysis is then more effective. Released energy-rich glucose is later broken down to acetic acid. The disintegration of biomass increases the efficiency of hydrolysis of 5 to 25% while it will be expected to reduce the residence time in the digester from 23 to 59% [8]. The goal of any pretreatment technology is to alter or remove structural and compositional impediments to hydrolysis in order to improve the rate of enzyme hydrolysis and increase yields of fermentable sugars from cellulose or hemicellulose. An effective pretreatment is characterized by several criteria. It avoids the need for reducing the size of biomass particles, preserves the pentose (hemicellulose) fractions, limits formation of degradation products that inhibit growth of fermentative microorganism, minimizes energy demands and limits cost. Pretreatment results must be balanced against their impact on the cost of the downstream processing steps and the trade-off between operating costs, capital costs, and biomass costs.

To increase production of constructed BGS plants, operators are offered a wide range of products. Such examples include new varieties of maize, upgraded silage preservatives, single enzymes or even ready enzymatic and microbial mixes to increase BG production, dried sludge or selected strains of microbial cultures and so on. Sometimes, glycerol from bio-diesel production is also used. Effect declared by sales representatives of these products is often hard to statistically prove in the laboratory - worse results are usually achieved in practice. Cellulose recalcitrance based on its crystallinity and low accessible surface area thanks to protection by lignin, and cellulose sheathing by hemicellulose all contribute to its resistance of biomass to enzymatic hydrolysis. It is well known that properly chosen parameters of disintegration can increase the accessibility of cellulose fibers and thereby increase the possibility of degradation of polysaccharides to monosaccharides without significant formations of inhibitors.

Operating experience has shown that neither enzyme mixtures with high activities can efficiently hydrolyze rare phytomass without being disintegrated first. This effect is valid not only in the production of bio-alcohols, but also in biogas plants.

Different disintegration processes are in literature described in terms of the involved mechanisms, its advantages and disadvantages very often including economic assessment. These pretreatment technologies include mostly mechanical and chemical methods in various combinations. Sometimes even biological methods are described. The choice of the disintegration process and its optimum parameters depends very much on the origin of the phytomass, its price and the cost of the pretreatment method.

Once phytomass is disintegrated successfully, hydrolytic enzymes are needed to free the fermentable sugars to start the anaerobic digestion. In most farm BGS reactors, the process generally involves extracellular hydrolytic enzymes produced mainly by wild microorganisms from ruminant digestive tract (live through excrements). Clostridia are able to produce α-amylase, α-glucosidase, β-amylase, β-glucosidase, glukoamylase, pullulanase or amylopullulanase by themselves [9]. The result of this process (hydrolysis) are simpler (shorter), more easily fermentable sugars, that are more useful in subsequent processes (acetogenesis). Anaerobic fermentation is a set of interconnected processes in which the mixed cultures of microorganisms gradually breaks down biodegradable organic material of phytomass starting with the most decomposable, than compose-able, sometimes in well balanced conditions, even less-composeable fractions, everything ideally under anaerobic conditions. Product of one group of anaerobic microorganisms becomes largely substrate for the next group and therefore the failure of one of the group results in disruption of the entire system. The efficiency of hydrolysis is crucial for all the processes. Their poor efficiency is strongly reflected negatively in the overall economy.

The previously achieved results have led to the hypothesis that the disintegration of phytomass is one of the BGS key issues. In phytomass destruction is most effective when acid or alkali thermopressure hydrolysis followed by neutralization and microbial activation is used, but these methods are prohibitively expensive in field of BGS.

It is well known that reducing particle size by mechanical or other disintegration methods leads to a substantial increase of the reaction surface. Here, the term “disintegration” is defined as all the processes leading to the dismantling of existing physical or chemical structure of the material. This includes breakage of used material into particles of smaller size. Not only phytomass structures, but also microorganisms are partially destroyed. Their bodies often take active part in the disintegrated product (extrudate, cell lysate). Not only by mixture of their enzymes but also by mass of their dead bodies.

The key issue of phytomass disintegration is to allow enzymes access to structures which they are able to hydrolyze. Most authors describe the phytomass disintegration as a routine operation, where farmers take care of themselves in the field with tools such as cutter or mower. The phytomass enters the BGS mostly as silage (4-11 mm), or hay-silage. However silage process even more smoothed small parts the phytomass, it is not enough at all. Long time of evolution adapted constitution of most terrestrial plants to be highly resistant to microorganism decomposition.

Extrusion

Extrusion (also known as thermo-pressure preparation, steam explosion, rapid thermal expansion or even inexact autohydrolysis) refers to an uncatalyzed steam explosion biomass pretreatment technique in which lignocellulosic biomass is rapidly heated by high-pressure steam without addition of any chemicals. Extruders can be understood as a separate chapter of disintegration. Since extrusion is a continuous process, it can be advantageously applied to the continuous processing systems. Other researchers consider frequency of the rotating screw and the reactor temperature to be the key parameters of the operation. Other authors discuss the addition parameters like screw speed or the ratio of width and length of the extruder, etc. Higher moisture content in raw material affects gelatination aspects while lower water content results in better swelling of the extrudate. One can state that these parameters are obviously crucial in various food preparations, but after a series of operational tests linked to the production of BG and alcohols I concluded that for this purpose it is crucial to find the optimum extrusion hydromodul, the minimum time delay and cost-effective extrusion pressure. This kind of phytomass disintegration can be described as a high - temperature - short - time - process (HTST) or thermo - pressure - preparation (TPP). The material is exposed to both heat and mechanical energy. The pressure inside of the extrusion reactor is usually achieved by increasing the temperature by hot steam. If the extrusion unit is being built near the BGS heat can be utilized from the cogeneration unit. After the reaction time is over, in the moment of expansion these conditions (explosion caused by a fall of pressure) results in physical and chemical changes in the deeper structure of the extruded material. Extensive shear stresses leads to the increase of particluate specific area and the splitting of complex organic compounds (fats, proteins, polysaccharides) into simpler ones. As a result, faster and more efficient conversion of phytomass into BG is achieved mainly due to acceleration of hydrolysis phase. Unlike mechanical treatments [10, 11] extrusion does not reduce the average particle size so intensively [12], but (using high pressures) it is possible to achieve disintegration of phytomass fibers, ideally to the cellular level [13]. When the parameters are set optimally (it is expected that for different types of phytomass is optimal just another optimum pressure, residence time, hydromodul etc.) the destruction is up to the cell cavitation. This process can be inside the plant cell described by creation of vacuum bubbles or gas from the extracellular environment. Then, subsequent implosion caused by a sudden drop in pressure occurs. The vacuum power will cause the area to diffuse gas in the form of vapor from the surrounding liquid phase. Upon the disappearance of vacuum created by cavitation, the gas bubbles implode to form a pressure wave with a destructive effect on the surrounding material. This phenomenon can be indirectly confirmed for example by decrease in viscosity.

As mentioned above, extrusion can be used on many commodities. However, the dynamic of influence of dry mass content, reaction time and pressure on different kinds of phytomass has not been observed.

Experimental

The aim was to find such simulation procedures, which be reproducible in large quantities and which would allow to measure and describe the depth (efficiency) dynamics of the process by a simple and generally-comparable data. The critical issue, the amount of material in the simulations, was chosen to avoid situations where the examined amount is so small that significantly affect the outcome of the test. On the other hand, the amount had to allow as many simulations as possible within a reasonable time. Simulations of cumulative biogas production were chosen to respect operational conditions.

Methods

The operational tests were executed by varying reaction time (4, 6, 8, 10 and 12 minutes), variable hydro-module allowed to change dry weight in range from 5 to 25% and pressure (0,45, 0,9, 1,35, 1,8, 2,25, and 2,7 MPa, maximum construction limit 3,5MPa). Tests matrix can be understood from Table 1. Extrusion treatments were carried out on modifiable own-designed (CZ 21314 U1, Figure 1) device in work dimensions (300kg of material per hour). The

Table 1. Testing matrix

p [MPa]	t [min]	dm [%]	CBGP	p [MPa]	t [min]	dm [%]	CBGP	p [MPa]	t [min]	dm [%]	CBGP
0.45	4	19.9	263	1.35	4	19.9	260	2.25	4	19.9	133
0.45	4	15.3	265	1.35	4	15.3	266	2.25	4	15.3	130
0.45	4	10.6	262	1.35	4	10.6	270	2.25	4	10.6	135
0.45	4	5.2	264	1.35	4	5.2	281	2.25	4	5.2	122
0.45	6	19.9	265	1.35	6	19.9	392	2.25	6	19.9	64
0.45	6	15.3	266	1.35	6	15.3	394	2.25	6	15.3	60
0.45	6	10.6	263	1.35	6	10.6	397	2.25	6	10.6	53
0.45	6	5.2	264	1.35	6	5.2	404	2.25	6	5.2	50
0.45	8	19.9	261	1.35	8	19.9	396	2.25	8	19.9	62
0.45	8	15.3	269	1.35	8	15.3	398	2.25	8	15.3	51
0.45	8	10.6	265	1.35	8	10.6	403	2.25	8	10.6	49
0.45	8	5.2	264	1.35	8	5.2	403	2.25	8	5.2	48
0.45	10	19.9	263	1.35	10	19.9	390	2.25	10	19.9	64
0.45	10	15.3	265	1.35	10	15.3	395	2.25	10	15.3	56
0.45	10	10.6	266	1.35	10	10.6	394	2.25	10	10.6	53
0.45	10	5.2	264	1.35	10	5.2	395	2.25	10	5.2	47
0.45	12	19.9	263	1.35	12	19.9	388	2.25	12	19.9	59
0.45	12	15.3	264	1.35	12	15.3	392	2.25	12	15.3	53
0.45	12	10.6	265	1.35	12	10.6	390	2.25	12	10.6	54
0.45	12	5.2	265	1.35	12	5.2	387	2.25	12	5.2	46
0.9	4	19.9	284	1.8	4	19.9	203	2.7	4	19.9	135
0.9	4	15.3	296	1.8	4	15.3	188	2.7	4	15.3	133
0.9	4	10.6	297	1.8	4	10.6	175	2.7	4	10.6	138
0.9	4	5.2	299	1.8	4	5.2	167	2.7	4	5.2	95
0.9	6	19.9	287	1.8	6	19.9	188	2.7	6	19.9	63
0.9	6	15.3	296	1.8	6	15.3	165	2.7	6	15.3	56
0.9	6	10.6	298	1.8	6	10.6	153	2.7	6	10.6	51
0.9	6	5.2	303	1.8	6	5.2	101	2.7	6	5.2	49
0.9	8	19.9	290	1.8	8	19.9	185	2.7	8	19.9	57
0.9	8	15.3	293	1.8	8	15.3	162	2.7	8	15.3	51
0.9	8	10.6	299	1.8	8	10.6	161	2.7	8	10.6	50
0.9	8	5.2	301	1.8	8	5.2	93	2.7	8	5.2	44
0.9	10	19.9	288	1.8	10	19.9	82	2.7	10	19.9	73
0.9	10	15.3	290	1.8	10	15.3	83	2.7	10	15.3	67
0.9	10	10.6	288	1.8	10	10.6	79	2.7	10	10.6	67
0.9	10	5.2	290	1.8	10	5.2	79	2.7	10	5.2	64
0.9	12	19.9	272	1.8	12	19.9	82	2.7	12	19.9	71
0.9	12	15.3	270	1.8	12	15.3	82	2.7	12	15.3	65
0.9	12	10.6	266	1.8	12	10.6	81	2.7	12	10.6	68
0.9	12	5.2	268	1.8	12	5.2	77	2.7	12	5.2	63

Description: p [MPa] = pressure [10^6Pascal], t [min] = time t [1 minute], dm [%] = dry matter [per cent], CBGP = cumulative biogas production [$m^3.t_{dw}^{-1}$ per 20 days].

hydromodule was set-up right in the moment processing due to technological limitations. That is why humidity in the operating mass was probed retrospectively. All obtained samples of extrudates were taken into polyethylene container and kept frozen until the CBGP simulations.

The CBGP testing reactors (Figure 2) were placed by 60 in electrically heated bath (38°C). Each single reactor was made from glass bottle (0.5 L) assigned with double-glazed plastic tube outlet into external graduated cylinder. Cylinder was placed into low concentrated HCl (pH = 4.5) to prevent CO_2 dissolution. Loosely mounted cylinders enabled reading the volume of non-pressed biogas. 40 grams of dried extrudate was mixed with 340 mL of water and 20 mL of inoculate. Temperature was set 38°C and the anaerobic digestion took 20 days. The values of gas volume were read in the temperature of 38°C because the volume of biogas changes also by temperature.

A total of 120 different extrusion conditions were examined by CBGP test. Due to the number of examined conditions was each simulation triple. Repeated were only the simulations which have obviously failed. The methane content was analyzed by COMBIMASS DA-m (Binder Group Company).

Mathematica 8 (Wolfram) software was used to evaluate and render the obtained data. The 3D function was chosen by Neural Network 3D Fitting Interface (number of hidden nodes in layer = 6) from ZunZun.com.

Materials

The examined material was pelleted hay of humidity 4.4%, density 1.213 t.m^{-3}. Inoculate was the liquid phase (percolate) from BGS Nedvědice (dry fermentation technology using mainly maize silage and straw-manure). Energy used for running the extruder was supplied by heat exchanger from the BGS co-generation unit. Extruder capacity 0.3 t.h^{-1}, other parameters in Table 1.

Results

Analyses of biogas content showed that the portion of methane was stable (average 52.33%, median 52.31%, dispersion 0.01, standard deviation 0,02). Due to the low information value of this statistic these numbers were not stated. Also the reaction times in extruder have relatively low information values. The time delay of less than 4 minutes is found to be insufficient. Conversely, the delay times 10 or more minutes are too long, resulting in unnecessarily slowing down of the process. If it was not possible to supply the energy to the extruder from the heat exchanger connected on the co-generation unit, the importance of finding the optimum phytomass delay in extruder be matter of greater importance.

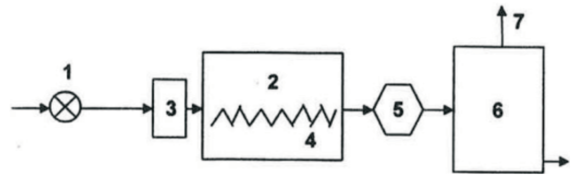

Figure 1. Modifiable extruder CZ 21314 U1. 1: high-pressure screw pump, 2: high-pressure reactor, 3: material heater, 4: internal screw for material transport, 5: expansion tourniquet, 6: expansion tank, 7: steam exhaust.

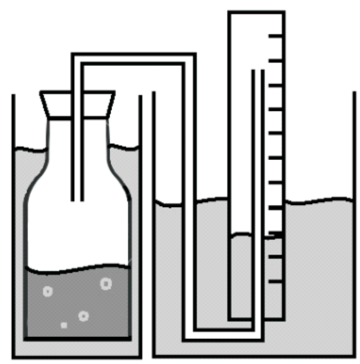

Figure 2. Single reactor.

Mathematical and statistical evaluation of 4 dimensional data is unreasonably robust. From the matrix of data is clear that the optimal reaction time is from the technological point of view in the area of 6 and 8 minutes reaction time. Process dynamics at times 6 and 8 minutes were drawn and evaluated (Figures 3 and 4).

If the reaction time is 6 minutes, maximum of 405.1 m^3 of biogas can be achieved in the area of 1.31 MPa and dry mass of 7.9%.

If the reaction time is 8 minutes, maximum of 404.8m^3 of biogas can be achieved in the area of 1.28 MPa and dry mass of 8%.

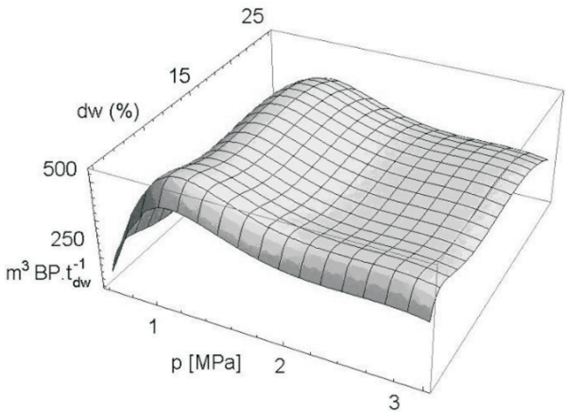

Figure 3. Process dynamics in reaction time of 6 minutes.

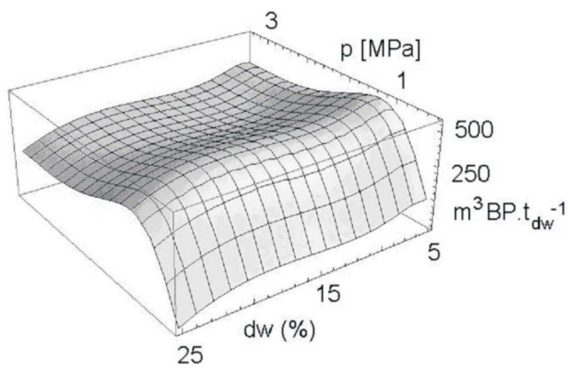

Figure 4. Process dynamics in reaction time of 8 minutes.

Discussion

Results presented indicate that the dynamics of the process is influenced by all factors studied. Most research in the field of phytomass extrusion was carried out with materials suitable for food or feed use, which are not relevant for this topic. Other experimentalists almost always considered the high-pressure acid or alkaline hydrolysis or extrusion followed by enzymatic hydrolysis [14-16]. Also the most common straw treatment methods were performed to produce bio-ethanol and similar bio-alcohols.

Other authors [17] state that the wheat straw effluents from both bio-ethanol and bio-hydrogen processes were further used to produce methane with the yields of 0.324 and 0.381 m^3/kg volatile solids respectively. In comparison with such data, it is clear that the results presented above are roughly half. Their first soaking step was operated at temperature of 80 °C and residence time around 6 min. The pre-soaking wheat straw was then heated up in stage two to approx. 180 °C for 15 min. followed by heating at 190 °C for 3 minutes in a third stage. Hydrothermal pretreatment of wheat straw resulted in a liquid fraction called hydrolysate, containing mainly hemicelluloses and a solid fraction rich in cellulose. Biological methane potentials were evaluated also in batch experiments. The experiments were performed in 118 mL serum glass bottles with working volume of 40 mL consisting of 30 mL of inoculum and 10 mL of substrate. Substrate was diluted with distilled water to attain a substrate concentration of 5, 50 or 100%. The headspace in the bottle was flushed with pure N_2 for 3-5 min. In addition, 2-3 drops of sodium sulphide was added to ensure anaerobic conditions. The experiment bottles were incubated statically at 55 °C. It can be assumed that such perfect results of biogas production are achieved by intensive multi-stage disintegration, also the conditions of thermophilic anaerobic digestion plays an important role in these simulations.

Another point for discussion is the steep fall of biogas production, which occurs at higher pressure. It is expected that according to another works [18], furans formed by sugar degradation and phenol monomers from lignin degradation are important co-factors in inhibition. In the steam explosion processes related to the bio-alcohols production water extraction removes the majority of the hemicellulose fraction, as well as the degradation products, from the cellulose fiber. The steep fall appeared because water removing did not take part in presented biogas production.

In terms of solubilisation thermal pretreatment was better than sonication or ozonation [19]. But, in terms of batch anaerobic biodegradability, best results were obtained with ultrasounds with energy of 6250 or 9350 kJ/kg TS and a thermal treatment at 170 or 190 °C. Moreover, treatments had effects on physico-chemical characteristics of sludge samples: apparent viscosity decreased after all treatments but the reduction was more important with thermal treatment. Median diameter of sludge flocs was reduced after sonication, increased after thermal treatment and did not change after ozonation. Finally, capillary suction time (CST) increased after ozonation, increased highly after sonication and was reduced after thermal treatment.

Conclusions

The results showed that from the technological point of view hay be can considered as a kind of phytomass which is promising for biogas production. In situation when the overproduction turns this commodity priceless is such use interesting even from economical point of view.

If the material is processed according to optimum conditions, over 400 cubic meters of biogas can be expected. Such biogas production represents roughly one-third increase over the levels achieved without steam-explosion pretreatment. Methane content in biogas stays in values of 52.3%, which is slightly more than usual. In comparison with the operational data can be concluded that one ton of dry matter of steam-exploded hay is equivalent to 2 tons of dry matter of frequently used mayze silage.

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References

1. Himmel, M.: "Biomass Recalcitrance: Deconstructing the Plant Cell Wall for Bioenergy", ISBN 978-1-4051-6360-6, 2008.
2. Deines, T., Pei, Z.: "Power Consumption Study in Knife Milliong of Wheat Straw", Transactions of NAMRI/SME, Vol. 38, (2010) p. 191-196.
3. Dale, B., Balan, V.: "Process for producing sugars from cellulosic biomass", 2008.
4. Holtzapple, T., et al.: "Pretreatment of lignocellulosic municipal solid waste by ammonia fiber explosion (AFEX)", Applied Biochemistry and Biotechnology, Vol. 1, is. 34-35 (1992), p. 5-21.
5. Holtzapple, T., et al.: "Calcium hydroxide pretreatment of biomass", patent: 5693296, Sep 6, 1994, Issue date: Dec 2, 1997.
6. Mantanis, G., I., Young, R., A., Rowell, R., M.: "Swelling of compressed cellulose fiber webs in organic liquids", Cellulose, Nº 2, (1995) p. 1-22.
7. Roček, Z.: "Pokryv tila a opìrná soustava", Courses, 1995.
8. Hendriks, Zeeman, G.: "Pretreatments to enhance the digestibility of lignocellulosic biomass", Bioresource Technology, Vol. 100, (2009) p.10-18.
9. Badr, H., R., Toledo, R., Hamdy, M., K.: "Continuous acetone-ethanol-butanol fermentation by immobilized cells of Clostridium acetobutylicum", Biomass and Bioenergy, Vol. 20, (2001) is. 2, s. 119-132.
10. Schell, J., Hardwood, C.: "Milling of lignocellulosic biomass: Results of pilot-scale testing", Appl. Biochem. and Biotechn., Vol. 45-46, Nº 1, (1994) p. 159-168.
11. Williams, A., et al.: "The Effect of Extrusion Conditions on the Fermentability of Wheat Straw and Corn Silage", Journal of the Science of Food and Agriculture, Vol. 74, Nº 1,(1999) p. 117-124.
12. Wasylciw, Wayne: "Methods of straw fiber processing", patent US 6,929,854 B2, Aug. 16, 2005.
13. Lee, S., Teramoto, Y., Endo, T.: "Enzymatic saccharification of woody biomass micro/nanofibrillated by continuous extrusion process: Effect of additives with cellulose affinity", Bioresource Technology, Vol. 100, (2009) i. 1, p. 275-279.
14. Brownell, H. H. and Saddler, J. N.: "Steam pretreatment of lignocellulosic material for enhanced enzymatic hydrolysis", Biotechnology and Bioengineering, Vol. 29, (1987) p. 228–235.
15. Puri, V. P.; Mamers, H.: "Explosive pretreatment of lignocellulosic residues with high-pressure carbon dioxide for the production of fermentation substrates", Biotechnology and bioengineering, Vol. 25, Nº12, (1983) p. 3149-3161.

16. Sun, Y., Cheng, J.: “Hydrolysis of ligno-cellulosic materials for ethanol production: a review”, Bioresource technology, Vol. 83, (2002) p.1-11.

17. Kaparaju, P., et al.: “Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept”, Bioresource Technology, Vol. 100, (2009) i.9, p. 2562-2568.

18. Klinke, H., Thomsen, A., Ahring: “Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass”, Appl. Microbiol. Biotechnol., Vol. 66, (2004) p. 10-26.

19. Bougrier, C., et al.: “Effect of ultrasonic, thermal and ozone pre-treatments on waste activated sludge solubilisation and anaerobic biodegradability”, Chemical Engineering and Processing, Vol. 45, N° 8, (2006) p. 711-718.

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