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Research into Effects of Alcaline Hydrolysis of Animal Waste as Pre-treatment in Biogas Production

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Waste is one of the key issues of the modern civilisation and represents the result of our way of life. After the occurrence of *bovine spongiform encephalopathy* and the ban to feed animals with bone meals, new technologies of animal waste processing are developed, as well as animal waste uses for energy purposes. Certain parts of this waste, such as brains and spinal cord, are deemed high-risk substances and can be infected with prions; their treatment is therefore only possible in strictly controlled conditions. Tests against effects of hydrolysis were conducted on samples of first class animal waste, bovine brains, in circumstances of different temperatures and hydrolisation duration, using the designed and built experimental laboratory reactor for alkaline hydrolysis. Production of hydrolysed substance biogas is monitored using the existing laboratory biogas circuit. Large potentials of hydrolyzates utilisation for energy purposes is featured by the analysis and systematisation of quantified results of the research, and the method used can be successfully applied for the pre-treatment of high-risk biological substances in biogas production.

Istraživanje učinka alkalne hidrolize otpada životinjskog porijekla kao predtretmana proizvodnji bioplina

Izvornoznanstveni članak

Otpad je jedan od ključnih problema moderne civilizacije i predstavlja rezultat našeg načina života. Nakon pojave *goveđe spongiformne encefalopatije*, te zabrane hranjenja životinja mesnim koštanim brašnom razvijaju se nove tehnologije prerade životinjskog otpada i njegova upotreba u energetske svrhe. Neki dijelovi ovog otpada, kao mozak i leđna moždina, visoko su rizične tvari i mogu biti zaraženi prionima, te je njihovo zbrinjavanje moguće isključivo u strogo kontroliranim uvjetima. Pomoću osmišljenog i izgrađenog eksperimentalnog laboratorijskog reaktora za alkalnu hidrolizu provedena su ispitivanja učinaka hidrolize pri različitoj temperaturi i trajanju hidroliziranja na uzorcima prve kategorije otpada životinjskog podrijetla, mozgu goveda. Korištenjem postojećeg laboratorijskog bioplinskog sklopa praćena je proizvodnja bioplina hidroliziranog materijala. Analizom i sistematizacijom kvantificiranih rezultata istraživanja istaknut je velik potencijal energetske upotrebe hidrolizata te se korištena metoda može uspješno primijeniti za predtretman visoko rizičnih bioloških materijala pri proizvodnji bioplina.

1. Introduction

Increased energy needs and issues related to non-renewable energy sources used today have prompted scientists to research into replacement sources of energy over the last three decades [11]. With the use of all forms of renewable sources of energy, energy balance and general quality of environment are improved, dependency on fossil fuels is reduced, and through multiplication their development creates new and sustainable jobs. Each year, several millions of tons of agricultural and animal waste in the world are disposed of or treated in different ways [14]. This waste has high potentials as renewable source of energy and can be transformed into several forms of energy.

Owing to high sustainability criteria, scientific research is conducted and technological solutions for utilisation of waste for producing highly effective energy, while at the same time reducing impact on environment, are developed and enhanced. Therefore, additional research into energy-related utilisation of high-risk categories of animal waste is proposed. Animal waste to the largest extent originates from ungulates and poultry slaughterhouses and its quantity continuously grows due to increasing meat consumption, thus placing burdens onto environment

and increasing production costs [6, 17]. With the existing technological treatment method, animal waste in rendering plants is recycled into meat-bone meal and technical fat [15]. Meat and bone meals were used for producing fodder, but with the occurrence of *bovine spongiform encephalopathy*, ban was introduced on the use of products of animal proteins in feeding animals [2, 3]. Confronted with the ban to use animal proteins in feeding livestock in parallel with a significant increase in meat and meat products consumption, global and European research focused on the new methods of animal waste treatment that could supplement or replace the existing rendering plants' treatments [4]. With the development of new biogas production [13] and alkaline hydrolysis technologies [9] effective possibilities of using animal waste for energy purposes is researched into. Energy-related potential of animal waste is very big and represents a biomass sources for direct production of electric and heating energy by converting biogases into solid, liquid or gas fuels [10]. The EU Regulation 1774/2002, also implemented by Croatia, introduces also new replacement ways of animal waste treatment with the use of alkaline hydrolysis and biogas production. Animal waste is divided into three categories: first category (K1) is a high-risk category which is suspected of infection with *transmissible spongiform encephalopathy* (TSE), second category (K2) is, among other, made of manure and contents of digestive tract, while third category substance (K3) is meat returned from stores for expiration reasons, etc. Animal waste is used in biogas production, but since these are high-risk substances, such process requires sterilisation pre-treatment [7,8]. Alkaline hydrolysis process is particularly applied for the treatment of contaminated K1 and K2 categories tissues, while hydrolysed substance was treated at depots [8]. Hydrolysed substance is fully decontaminated and proteins and lipids mixture can be used for biogas production.

Hydrolysed substance is fully decontaminated and proteins and lipids mixture can be used for biogas production. Thereby, alkaline hydrolysis can be used as pre-treatment to anaerobic fermentation process. With the operation of alkaline mediums, temperature and pressure, larger molecules are degraded into smaller ones, which represents an important step in breaking the links in large protein chains, like prions, into smaller chains [12]. Alkaline hydrolysis can be catalysed with enzymes, metal salts, acids and alkali. In conducting experimental tests, authors used aqueous solution of sodium hydroxide, and effects of alkaline hydrolysis were monitored at different temperatures, including the standard temperature up to $\vartheta 1$=150°C [8].

2. Aim of research

To present the method and effects of alkaline hydrolysis and its contribution to the quality of pre-treatment (sterilisation) of high-risk animal waste (bovine brains) in producing biogas as fuel for energy-related needs, by using the experimental device and experimental tests. Research could be a contribution to an amendment of EU Directive 1774/2002, which defines methods for treating animal waste.

3. Designing of experimental device for alkaline hydrolysis

For the purposes of experimental tests, alkaline hydrolysis reactor with elementary physical and technical parameters presented in table 1 was designed and used.

Laboratory reactor case is made of stainless steel, 175 mm in height and 165 mm in diameter. Ten mm wide space between inner and exterior wall is used for cooling the reactor by circulating plain water of ϑ = 15°C temperature at device's entry point. The needed reaction mixture temperature was maintained through indirect cooling of reactor wall, without measuring water's exit temperature and quantity of heat driven away.

Table 1. Physical and technical parameters of alkaline hydrolysis reactor

Tablica 1. Fizikalno – tehnički parametri reaktora za alkalnu hidrolizu

Parametres	Values of Experimental Alkaline Hydrolysis Reactor
Capacity, m^3	0,001586
Height · diameter, m	0,175 · 0,165
Wall thickness, m	0,001
Pressure, bar	1 - 7
Temperature, °C	do 180
Hydrolysation time, min	120 - 360
Water speed, m/s	0,5 - 2,0
Water entry temperature, °C	≈ 15

Built onto reactor's lid is a manometer with accurate measuring division for continued monitoring of the pressure of reaction mixture, as well as a safety valve for stabilising possibly excessive working pressure during the course of experiment. Reactor is calibrated for 7 bars. At the bottom of the reactor, there is an opening with duct for driving hydrolysed substances away, which significantly facilitates the discharging of the experimental device and reaction mixture. Reactor is mounted on a specifically shaped electric heater with magnetic stirrer, which is equipped with thermometer for permanent measuring and monitoring of the reaction mixture temperature. There is a magnet in the reaction mixture, which stirs reaction mixture with the use of magnetic stirrer (Figures 1, 2).

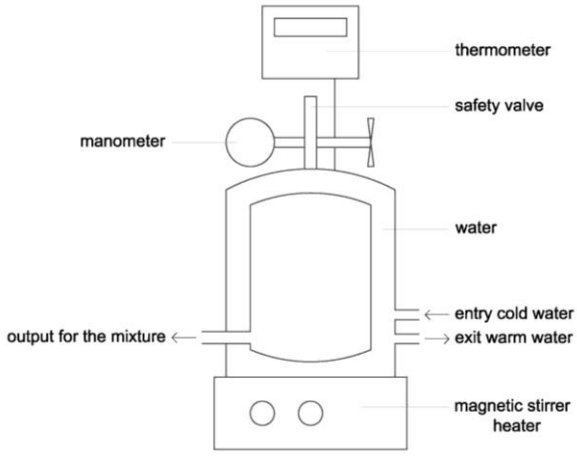

Figure 1. Shematic display of alkaline hydrolysis reactor
Slika 1. Shematski prikaz reaktora za alkalnu hidrolizu

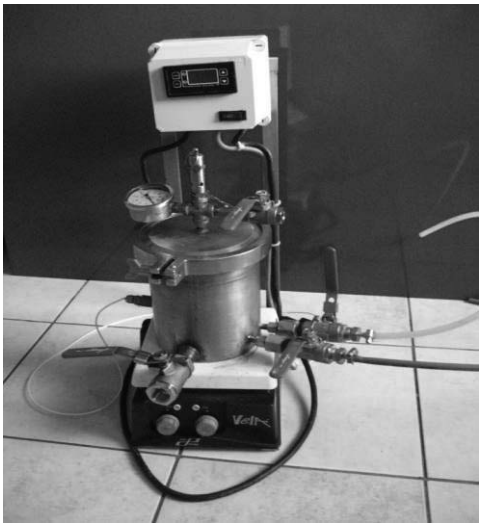

Figure 2. Laboratory alkaline hydrolysis reactor
Slika 2. Laboratorijski reaktor za alkalnu hidrolizu

High-risk animal waste treatment experiments were conducted using the reactor and through hydrolysis process, at standardised temperature ϑ = 150 °C, pre-pressure p = 4 bar and duration t = 2 hours, as well as on temperatures higher or lower than the standardised (given).

4. Materials and methods

Samples of high-risk first category of animal waste were used, i.e. bovine brains for the purposes of the experiment and research into efficiency of alkaline hydrolysis method at different temperatures and hydrolysation duration. Hand blender was used for mixing and chopping up the needed quantity of waste until the mixture became homogenous. Homogenous mixture of waste was placed in the reactor, and 45% NaOH solution and water were added. The obtained reaction mixture contained 400 grams of experimental brains sample, 30 ml of 45 % NaOH and 600 ml of water. Reaction mixture of the same characteristics was prepared for three experimental cycles, under the testing conditions. Homogeneity of reaction mixture during the running of each experiment was obtained by using a magnetic stirrer and through continuous stirring. Prior to starting to heat up the reaction mixture, the reactor is hermetically closed and there is no contact with environment. Maintaining of experimental temperature of the reaction mixture was obtained through continuous supply of cold water between double walls and by driving away the heat with the same quantity of water from the cooled surface area of the reactor. Hydrolysis was conducted under the following testing conditions: hydrolysation duration t_1 = 2 hours, t_2 = 3 hours and t_3 = 6 hours, temperature ϑ_1 = 135 ºC, ϑ_2 = 150 ºC and ϑ_3 = 153 ºC and pre-pressure p_1 = 2,75 bars, p_2 = 4,78 bar and p_3 = 5,20 bars, as shown in Table 2.

After each experiment under certain temperature, pressure and duration values, hydrolysed material was separated, cooled to room temperature and then stored in refrigerator at -20 °C until the overall experiment is completed. The total of 27 experimental samples was received through alkaline hydrolysis during the total time of 200 hours of the experiment. All 27 samples were used for production of biogas, i.e. in the process of anaerobic fermentation. Anaerobic fermentation of one sample took 30 days and was done over the course of one year. Nine samples were fermented simultaneously.

Statistical analysis took place in accordance with randomly selected experimental design, i.e., three replications and values that represent arithmetic mean value ±SD of three replications were calculated.

5. Anaerobic fermentation of animal waste

Anaerobic fermentation took place in reactors of laboratory biogas facility, with water and inoculums added, i.e., active mesophilic sludge [5]. The process of anaerobic fermentation took place in mesophilic conditions [10]. Reduction of high weight molecular organic compounds into low weight molecular compounds in the hydrolysed substance is the first degree of fermentation. In the second degree of fermentation, methane bacteria that degrade products of metabolism and products of further splitting develop.

Table 2. Experimental parameters of hydrolysation process
Tablica 2. Pokusni parametri hidroliziranja

Parameters set for Hydrolysation					
Sample 1	Sample 2	Sample 3	Temperature, ºC	Duration of hydrolysis, min	Pre-pressure, bar
A1	B1	C1	135	120	2,75
A2	B2	C2	150	120	4,78
A3	B3	C3	153	120	5,20
A4	B4	C4	135	180	2,75
A5	B5	C5	150	180	4,78
A6	B6	C6	153	180	5,20
A7	B7	C7	135	360	2,75
A8	B8	C8	150	360	4,78
A9	B9	C9	153	360	5,20

Methane bacteria live through intramolecular breathing, i.e., oxygen needed for breathing is drawn from molecules and other substances and are therefore capable of degrading organic substances into the smallest gas molecular forms, such as methane (CH_4) and carbon dioxide (CO_2). Further degrading of hydrolysed substance results in the creation of methane and carbon dioxide. Majority of methane is obtained through fermentation of acids and alcohol and a smaller part is obtained from hydrogen and CO_2.

The process of formation of methane took place according to the following chemical equations:

$$2\,HCO_2 \longrightarrow 2\,CO_2 + H_2$$
$$4\,H_2 + CO_2 \longrightarrow 2\,H_2O + CH_4$$
$$CH_3COOH \longrightarrow CH_4 + CO_2$$

6. Analysis of results

Using the selected high-risk samples of animal waste and after a series of laboratory experiments, efficiency and applicability of alkaline hydrolysis method as pre-treatment to production of biogas are tested. Samples of hydrolysed substance are prepared at temperatures $\vartheta_1 = 135$ ºC, $\vartheta_2 = 150$ ºC and $\vartheta_3 = 153$ ºC; pre-pressure $p_1 = 2{,}75$ bars, $p_2 = 4{,}78$ bar and $p_3 = 5{,}20$ bars and hydrolysation duration $t_1 = 2$ hours, $t_2 = 3$ hours and $t_3 = 6$ hours. Visual check shows that all samples of hydrolysed substance are of light brown colour with strong odour reminiscent of soap. After mixing with water, there is no heat or gases released and no subsequent chemical reaction or change of colour is observed.

All samples prepared were subjected to anaerobic fermentation. Biogas production begins with the fifth day of fermentation and achieves its maximum value on day 30, after which it drops, which is usual for such mixtures. Cumulative biogas production started only with the fifth day of fermentation, identically as in case of anaerobic fermentation of all other samples of hydrolysed substance. All analysed samples are shown in the Table 3 and what stand out are very similar values of obtained biogas on fermentation day 30.
Figure 3. shows cumulative growth of produced biogas from hydrolysed animal waste, and Table 4. shows the composition of received biogas.

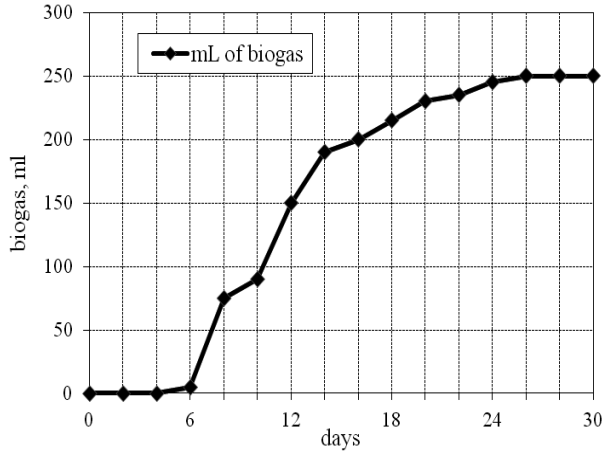

Figure 3. Cumulative growth of biogas from hydrolysed animal waste in mesophilic process of anaerobic fermentation

Slika 3. Kumulativni prirast bioplina iz hidroliziranog otpada životinjskog porijekla u mezofilnom procesu anaerobne fermentacije

Analysis of all samples of hydrolysed substance shows that conditions – hydrolysation duration, temperature and pressure – do not affect hydrolysed material to the extent that biogas production should change significantly. Therefore, besides standard (standardised) method, it is possible to use the method at lower temperature and pressure under the condition that animal waste is not infected with transmissible encephalopathy.

Table 3. The mean values of biogas production on fermentation day 30 for all analysed samples of hydrolysed animal waste

Tablica 3. Srednje vrijednosti proizvodnje bioplina tridesetog dana fermentacije za sve analizirane uzorke otpada životinjskog porijekla

The mean values of biogas production on fermentation day 30 for all analysed samples of hydrolysed animal waste					
Sample	Biogas, ml	Sample	Biogas, ml	Sample	Biogas, ml
A1	250.1±0.03	B1	249.9±0.08	C1	250.3±0.26
A2	250.2±0.07	B2	250.0±0.02	C2	250.0±0.04
A3	250.3±0.17	B3	249.8±0.18	C3	250.2±0.16
A4	250.0±0.13	B4	249.9±0.08	C4	249.8±0.24
A5	250.0±0.13	B5	250.0±0.02	C5	250.1±0.06
A6	250.1±0.03	B6	250.2±0.22	C6	250.1±0.06
A7	250.2±0.07	B7	250.1±0.12	C7	250.0±0.04
A8	250.1±0.03	B8	250.0±0.02	C8	249.9±0.14
A9	250.2±0.07	B9	250.0±0.02	C9	250.0±0.04

Table 4. Mean values of biogas composition expressed in % for hydrolysed substance

Tablica 4. Srednje vrijednosti sadržaja bioplina izražene u % za hidrolizirani materijal

Samples	**Methane (CH_4), %**	**Carbon dioxide (CO_2), %**	**Other, %**
ΔA	63	33	4
ΔB	62,5	33,5	4
ΔC	63,2	33,2	3,6

Composition of biogas was also identified using gas chromatograph. Obtained results are shown in Table 4 and represent a mean value of all analysed samples.

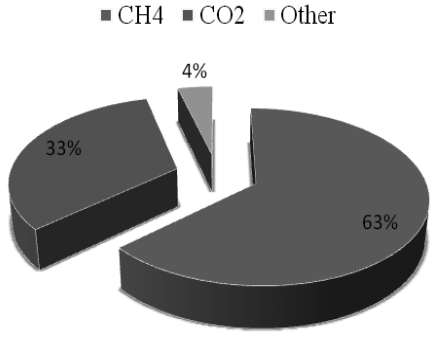

Figure 4. Composition of biogas received through anaerobic fermentation of hydrolysed animal waste in the mesophilic process

Slika 4. Sastav bioplina dobiven anaerobnom fermentacijom hidroliziranog otpada životinjskog porijekla u mezofilnom procesu

Received biogas has the usual composition with values for methane CH_4 around 63 %, carbon monoxide CO_2 around 33 %, while the rest is mixture of gases like hydrogen sulphide, water vapour, etc. [1].

7. Conclusion

There is a growing quantity of animal waste in the world caused by increased consumption of meat. Such trend is true for Croatia as well. Data available for 2010 indicates that 100.000 tons of animal waste was collected and treated.

The importance of alkaline hydrolysis method and its efficiency depend upon conditions under which the alkaline hydrolysis takes place. Standard prescribed method of alkaline hydrolysis is as follows: temperature ϑ = 150 ºC, pressure p = 4 bar and hydrolysation duration t = 3 hours. Researching into different conditions of alkaline hydrolysis, and under assumption that samples of treated substances are not infected with prions, the method proves the same results on lower temperatures, i.e., at temperature ϑ = 135 ºC. Higher temperature and shorter duration of hydrolysation prove also similar results, which indicates that hydrolysation method can be applied also at temperatures lower than standard (standardised), while obtaining similar or identical results. This is particularly important in biogas production when alkaline hydrolyses is used as pre-treatment, i.e. for sterilisation and homogenisation of reaction mixture of high-risk animal waste and contributes to the more rational use of heat.

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