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Theevaluation of the biogas potential of lignocellulosic wastes subjected to the enzymatic hydrolysis

A Dereszewska[1,*] and S Cytawa[2]

[1] Faculty of Entrepreneurship and Quality Science, Gdynia Maritime University, 87 Morska Street, Gdynia, 81-225 Gdynia, Poland
[2] 'Swarzewo' Wastewater Treatment Plant, 84 Wladyslawowska Street, Swarzewo, 84-100 Wladyslawowo, Poland

E-mail: a.dereszewska@wpit.am.gdynia.pl

Abstract. The aim of the study was to analyze the influence of enzyme preparation in the solution of solid wastes with different degree of affinity and resistance on hydrolysis (grass, straw, sawdust) as well as on the increase of biogas production. The lignocellulosic wastes were pre-treated using commercial preparation with cellulolytic enzymes or using enzyme from moldy bread. The study was carried out in the 50 L fermentation chamber model, the volume of biogas production was measured for 72 hours after the feedstock was introduced. The changes in some parameters of solutions of lignocellulosic pulp (LCW) and fermentation sludge (pH, COD,TS,VS) were also analyzed. The test results showed that 4-50% increase of COD and lower pH in waste solutions was obtained after the enzyme addition. The amount of biogas obtained per 1 kg VS of pure LCW was 10-975 L, yet after the enzyme application, it increased by as much as 130%. The largest biogas potential was obtained for grass- wastes but the influence of enzyme on this substrate was the lowest. The cyclic introduction of the investigated lignocellulosic pulp into the fermentation chamber had neither negative effect on the fermentation process nor significantly altered the fermentative sludge parameters. Moreover, enzymatic pre-treatment improves the degree of sludge mineralization. During 3 months of the study, the content of VS in the dry matter decreased from 71.8% to 39.3%.

1. Introduction

In the age of high demand of renewable energy, cheap waste-friendly raw materials are needed for its production. In municipal sewage treatment plants the production of energy is obtained through the fermentation of primary and excess sludge as well as biogas burning. One of the intensification methods of this production is the implementation of co-fermentation, in which the sludge is mixed with organic wastes before digestive chambers [1,2]. As a result, the production of biogas is increased which allows it to cover energy demand consumed in the sewage treatment plant and can lead to self-sufficiency of energy [3].

Thebeneficial effect of co-fermentation is, among others, the optimization of nutrient balance in the substrate mixture. Nitrogen-rich substrates can be co-fermented with carbon-rich substrates. The quality of fermented material is important for methanogenesis and its efficiency. Microorganisms in digestive chamber require certain specific substances, both those which serve as substrates for the production of methane and those needed to maintain optimal conditions for the development of fermenting bacteria (eg micronutrients)[4,5]. The ratio of carbon to nitrogen (C/N) is particularly relevant for the fermentation mixture. Optimal results during fermentation are obtained when the C/N ratio is in the range of 10 to 30. When this proportion is too low, the excessive ammonia production is observed which, in high concentrations, is toxic to methanogenic bacteria [6,7].

Inthe case of co-fermentation in the sewage treatment plant, the nitrogen content of mixed wastes with sewage sludge should be monitored particularly as the digestion effluent is recycled back to the biological part of the treatment plant. Thus, in the case of high nitrogen concentrations, the digestion of fermentative lechate requires high energy inputs. The removal of nitrogen compounds from wastewater is essential in wastewater treatment plants due to the processes of eutrophication of water bodies to which wastewater is discharged. The cost of solid wastes is an important factor in the substrate selection, so many common wastes products are used (e.g. brewery wastes, oil). Wastes from animal production are characterized by high methanogenic potential as well as high nitrogen content. Wastes from plants are characterized by favorable ratio of C/N, are also a source of micronutrients which deficiency is observed in fermented sludge [8]. Low-nitrogen wastes includes, among others, lignocellulosic wastes (LCW). Commonly available lignocellulosic substrates (grass, straw, sawdust, vegetables) are cheap but relatively small in use. The advantage of lignocellulosic wastes is the low cost and the full availability of the raw material during the whole year. The disadvantage is the difficulty of the availability of organic carbon for methanogenic bacteria [9].

Obtaining simple, high energy organic compounds requires pre-treatment and therefore the costs increase. The most commonly used LCW pre-treatment methods include:

Mechanical treatment is one of the physical methods of lignocellulosic biomass pre-treatment. It includes milling, grinding, extruding, and other processes to reduce substrate size, destroy lignocellulosic structures and improve the availability of specific surface area for bioactive substances. For example, together with the decrease of size of straw particles from 2-4 cm to 53-149 μm, the 39% increase in glucose after hydrolysis was observed [1,10,11].

Chemical treatment is usually carried out using acids or bases. One of the most popular methods of chemical treatment of biomass is the hydrolysis of sulphuric acid. Sodium hydroxide is used for basic hydrolysis. Both methods are accompanied by hemicellulose decomposition, however, the alkaline method results in less carbohydrates degradation than acid treatment. For specific substrates, specific pre-treatment methods such as ozonolysis or wet-oxidation are also used [1,10,12]

Biological treatment. Compared to other methods, biological biomass pre-treatment is significantly less energy intensive and does not require expensive equipment. In addition, pollution generated by this method is generally less burdensome for the environment. Biomass pre-treatment uses microorganisms that break down lignin and cellulose (e.g. rot fungi) [11,13]. Fungal pre-treatment is effective at the long run of both pre-treatment and hydrolysis. Biological methods are also included in acidic fermentation at the pre-treatment stage. Such processed raw materials include wastes used in agricultural biogas plants (maize, beet pulp) [10,11]

Enzymatic treatment. A specific pre-treatment method is the hydrolysis of enzyme-isolated use. It is most commonly used for the further processing of lignocellulosic raw materials for bioethanol, as this process requires the use of selective enzymes [14,15]. The major groups of enzymes that interact with cellulose and hemicellulose are: endo-glucanases, exo-glucanases and β-glucosidases. These enzymes act synergistically to hydrolyse different bonds in lignocellulosic compounds. [16-19]. Enzymes can also improve the efficiency of raw materials for biogas. For example, this effect is obtained for beet pulp that is subjected to endoglucanase, xylanase or pectinase interaction [20]

The paper presents the results of research aimed at determining the most advantageous composition of the co-fermentation mixture containing lignocellulosic waste. The plants with different degree of affinity and resistance on hydrolysis (grass, straw, sawdust) were selected for the study. Each type of waste part was subjected to an enzymatic pre-treatment in order to determine the extent of pre-treatment effects of this type to the biogas production. In case of co-fermentation, very selective selection of enzymes is not required. Therefore, in the research part of this work, it was decided to apply a commercial preparation containing cellulolytic enzymes to the pre-treatment of lignocellulosic waste. Such preparations are used for feed improvement and in the food industry e.g. maceration of fruit and vegetable pulp [16,17].

Lignocellulosic wastes, especially wood-wastes, is also very susceptible to the enzymes produced by some strains of mold. The hydrolytic effect of mold fungus on cellulose is extensively characterized in the literature [5,11,13]. Some of these fungi (e.g. Aspergillus) may also develop on baker's wastes [21]. Previous studies have shown that the use of bakery wastes co-fermented with primary sludge has a beneficial effect on the intensification of biogas production [22,23]. For this reason, lignocellulosic wastes mixed with moldy (to a large extent) bread wastes and pre-treated with enzymes contained in this mixture are also used.

2. Materials and methods

2.1. Materials

The research material was lignocellulosic wastes (LCW) with varying degrees of hydrolysis resistance: sawdust (Sd), straw (S) and grass (G). Sawdust was characterized by a particle size of 0.5-1 mm whereas grass and straw were cut into 1 cm lengths. Some of the test samples were pretreated by adding an enzyme containing cellulases (BrennZymeVR Plus, Brenntag) at 0.1 ml per 100 g dry matter or by mixing with a solution of moldy bread (B) in a 1: 2 weight ratio. The bread solution was prepared by diluting 1 kg of moldy bread in 5 liters of water. The characteristics of the fermented wastes are presented in Table1.

Table 1. Selected parameters of fermented mixtures.

Feedstock	Symbol	Total solids TS (%)	Volatile solids VS (%TS)	pH
Primary sludge	**PS**	2.3	73.1	7.6
Moldy bread	**B**	14.3	96.2	3.8
Sawdust	**Sd**	94.7	98.3	5.6
Sawdust+enzyme	**SdE**	94.7	98.3	5.6
Sawdust+moldy bread	**SdB**	94.9	98.9	4.9
Straw	**S**	93.5	97.6	6.5
Straw+enzyme	**SE**	93.5	98.2	6.5
Straw+moldy bread	**SB**	93.7	99.0	6.0
Grass	**G**	35.2	92.3	6.3
Grass+enzyme	**GE**	35.2	92.3	6.3
Grass+moldy bread	**GB**	35.8	92.8	5.3

2.2. Methane fermentation

Methane fermentation was carried out in a model reactor with a capacity of 50L, filled with sludge from the fermentation chamber of biological sewage treatment plant in Swarzewo. The total dry solids (TS) of the sludge was 2.28%. During the study, the temperature was maintained at 36 ± 1 °C and the pH was 7.3 ± 0.2. In the chamber a cyclic mode of mixing was used: stirring for 30 minutes/ 30 minutes break. Measurement of produced biogas volume was done in a system consisting of a graduated (up to 0.5L) tank filled with water and a reservoir tank. The system worked on the principle of connected vessels and the produced biogas pushed the excess of water into the reservoir tank.

To measure the biogas potential of lignocellulosic wastes, a mixture of 50g of wastes/500g of water (in the co-fermentation study 50g of wastes/500g of primary sludge) was added to the chamber. The volume of produced biogas was measured after 24 hours and after 72 hours of fermentation. After 3 days the gas production was negligible and it was assumed that the fermentation process had run to the end. The amount of biogas generated for 500g of the pure primary sludge (PS), and the same amount of PS pre-treated as the LCW wastes, were also measured. The obtained biogas volumes were calculated as the amount of biogas produced by 1kg of volatile organic matter (L/kg VS) of the analyzed wastes

2.3. Measurement

For LCW / water solutions (1/10), dissolved chemical oxygen demand (COD_s) was tested for 14 days. COD_s was determined colorimetrically (Vega Spectrophotometer from Merck Spectroquant® 1.14541). The measurements of pH were carried out using a WTW Multi-720 pehameter. The total solids (TS) and volatile organic matter(VS) were determined using the standard methods. The drying temperature for the TS assays was 105°C, the VS were determined using heating ramp from room temperature to 550°C

3. Results and discussion

3.1. COD_s measurement

For the aqueous solutions of the tested wastes and the solutions of wastes after pre- treatment, the dissolved chemical oxygen demand was measured for three weeks. COD_s value indirectly characterizes the content of soluble organic matter in wastes and indicates their usefulness in co-fermentation [24]. The study was conducted to determine whether enzymatic hydrolysis increased the content of COD_s in the solution, which may indicate the breakdown of lignocellulosic compounds.

The highest values of COD showed waste solutions mixed with moldy bread. Obtained values were respectively 150g O_2/kgVS for SdB, 167gO_2/kgVS for SB and 1330g O_2/kgVS for GB. Among the pure lignocellulose examined wastes, the highest value was noted for grass 227 g O_2/kgVS . The value of COD_s for straw was 28g O_2/kgVS and for sawdust 8.5g O_2/kgVS. The initial values of COD_s of wastes mixed with the enzyme preparation were the same as for pure LCW.

Percentage changes of COD_s over time for LCW without pretreatment (Sd; S; G) after enzymatic treatment (SdE; SE) and after mixing with moldy bread (SdB; SB; GB) are shown in Figure 1.

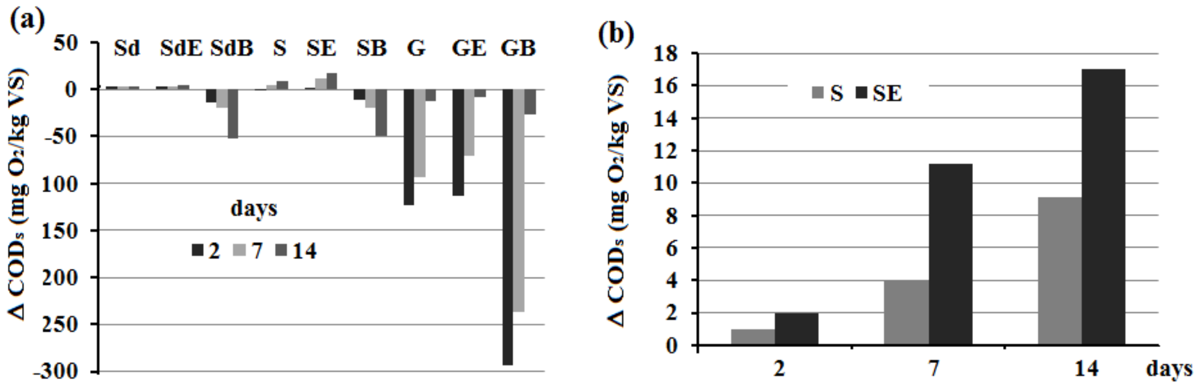

Figure 1. The increment value of COD_s: (a) in solution of lignocellulosic wastes after 2, 7 and 14 days (b) for straw waste (S) and straw waste after cellulase treatment (SE).

For all LCWs, a greater increase in COD_s showed wastes treated with cellulolytic enzymes. The biggest difference was observed for straw (S)after a week. For sawdust and grass solutions the differences are insignificant, while the COD_s of straw solution after application is twice as high as in solution without cellulases.

The COD_s values for grass solution significantly decreased in the early days of the experiment, rapid fermentation of the solution and a significant decrease in pH (from 6.3 to 4.2) was observed. After 7 days the straw solution also underwent acidic fermentation (pH decrease from 6.5 to 4.8), whereas in the sawdust solution this process was observed after 14 days (decrease pH from 5.6 to 5.2).

In solutions of sawdust and straw, acid fermentation processes cause a significant increase in the value of CODs. For all wastes containing added moldy bread, systematic decline of CODs occurred. This may be due to the activity of mold and the use of substrates contained in the solution for metabolic processes.

3.2. Biogas production

Methane fermentation for each kind of wastes were carried out three times. Averaged biogas volume measurement and biogas potential for fermented wastes are shown in Table 2.

Table 2. Biogas potential for investigated wastes.

Fermented wastes	VS [g]	Biogas volume 24 h [L]	Biogas volume 72h [L]	Biogas potential 24h [L/kgVS]	Biogas potential 72h [L/kgVS]
FSE	887	4	8.5	4.5	9.6
PS	8.25	3.5	4	424.2	484.8
B	13.7	4	7	292.0	510.9
Sb	44.5	4.5	5	101.1	112.4
SbE	44.5	5.5	8.5	123.6	191.0
Sb/PS	52.8	12.5	18	236.7	340.9
SbE/PS	52.8	14.5	21.5	274.6	407.2
SbB	58.2	22	31	378.0	532.6
S	45.7	5.5	6.5	120.4	142.2
SE	45.7	6	15	131.3	328.2
SB	59.1	19	25.5	321.5	431.5
G	16.3	9.5	13.5	582.8	828.2
GE	16.3	9	13.5	552.1	828.2
G/PS	24.6	19	24.0	772.4	975.6
GE/PS	24.6	17	23.5	691.1	955.3
GB	30.0	19	28.0	633.3	933.3

In the primary sludge (PS samples), a large quantity of undigested cellulose compounds is constantly being fed to the wastewater treatment plant. They are mostly transferred with PS to the fermentation sludge. Therefore, the amount of biogas obtained during the fermentation of the tested wastes samples may be influenced by previously added substrates. Adding the enzyme directly to the fermentation sludge in the chamber (FSE sample) resulted in the production of 4.5 L of biogas within 1 day. The process ended after 36 hours, and the maximum volume of biogas was 8.5 liters. Frequent addition of the enzyme to the fermentation sludge does not causes such effects. Probably the accumulation of unreleased cellulose in it requires a longer period of supply of the fermentation chamber with waste.

During the study, the enzyme preparation was added to the chamber together with the samples of wastes which significantly influenced the degree of mineralization of the fermentation sludge in the model chamber. At the start of the test, total solids (TS) was 2.28% and contained 71.8% volatile solids (VS). After 6 weeks TS content decreased to 2.20% versus VS to 45.4%. After 10 weeks TS accounted for 2.0%, including 40.1% VS, and after 3 months these two values decreased respectively to 1.85% and 39.9%. In further planned investigations it would be necessary to confirm whether this process is reproducible for full scale digestive chambers.

The highest biogas potential among the examined lignocellulosic wastes was grass (828 L/kg VS). Even more biogas was obtained during co-fermentation of grass mixed with primary sludge (975 L/kg VS). On the other hand, the effects of enzymatic pre-treatment on the production of biogas were not observed for all tested grass-based feedstocks.The cause may be a collection of grass at an early stage of its growth (April-May), when the plant is characterized by high levels of nutrients and a low degree of its wood- affinity. The early cutting of grass has, according to many researchers, the greatest influence on the amount of biogas production. The research conducted in Germany has shown that for grass cut in May 929 L/kg VS of biogas can be obtained, while in the cutting period from June to February, the biogas potential decreased linearly from 541 L/kg VS to 299 L/kg VS [8,25]. The reasons for the decrease in biogas potential in subsequent months of grass vegetation are seen in the increasing content of hemicellulose and lignin, which are resistant to anaerobic digestion.

The highest increase in biogas production due to the enzymatic pre-treatment of wastes was noted for straw (130%). In the case of sawdust the increase was 70%. Despite such significant increases, the biogas potential of both raw materials is less than the biogas potential of the primary sludge. Thus, the fermentation of straw or sawdust together with primary sludge will not intensify biogas production. The amount of biogas obtained from the straw (142 L/kgVS) is relatively low, which could have been influenced by the low degree of sample fragmentation [1,11].

For all waste mixed with moldy bread waste, high biogas potential values were obtained. This also applies to moldy bread itself, which indicates that this waste, like grass, contributes to the intensification of biogas production in the fermenting chambers of the wastewater treatment plant. In order to determine its usefulness as a cellulases source in the process of enzymatic pre-treatment of cellulosic substrates, it is necessary to carry out a wider scale of long-term studies

3.3. Economic impact

Fermentation tests carried out for straw and sawdust have shown a beneficial effect of the enzyme preparation containing cellulase on biogas release. Enzyme addition resulted in a 70% to 130% increase in biogas production. However, at the present stage of the study, the cost of pre-treatment of lignocellulosic wastes with BrennZymeVR Plus has not been calculated, because, despite of the increase, the amount of biogas volume was too low.Thus the addition of LCW would not contribute to the intensification of energy production in the treatment plant. On the other hand, the studies have showed a positive influence of the cellulase containing enzyme preparation on undigested cellulose compounds present in the fermentation chamber.Adding the enzyme directly to the fermentation sludge resulted in extra biogas production and an increase the sludge mineralization. For this reason, the cost that would be incurred by wastewater treatment plant in the case of using fermentation aid with BrennZymeVR Plus was estimated.

The wastewater treatment plant in Swarzewo conducts the fermentation of the primary and excess sludge in 2 reactors, with a volume of 3.600m^3 each. In the chambers there are 216 Mg of TS, with retention time of 20 days. Enzyme preparation should be dosed at 200 g/Mg of cellulose in organic wastes and its cost is € 14 per kg. Assuming that the cellulose content in the fermentation sludge is no more than 20% [17] and the enzyme should be added every 20 days, then the annual cost of purchasing the enzyme is approximately € 1000. If the tendency observed in the studies were maintained, the enzyme support of fermentation would allow an annual increase in the production of 37,000 m^3 of biogas, which, after conversion to electricity, would generate an additional profit of around € 8500 per year (1m^3 biogas = 3 kWh).

Benefits for the treatment plant are also connected with the increase of mineralization of the fermentation sludge. As a result, less post-fermentation sludge production reduces the cost of its management.The high biogas potential obtained for all LCW wastes mixed with moldy bread as well as for the moldy bread itself indicates that enzymatic processing from enzymes obtained in this way also results in an increase in biogas production.The cost of collection of such substrate is reduced to the cost of collection and transport, as its further processing does not require any separate technology or additional equipment. The high values of COD_s obtained for wastes mixed with moldy bread indicate a high content of organic carbon in this wastes and their usefulness in co-fermentation.

4. Summary

In the sewage treatment plant, the fermentation chambers are fed with primary sludge and with excess sludge (after the biological wastewater treatment stage). The study showed that only grass, from the tested lignocellulosic waste, is characterized by higher biogas potential than the primary sludge.In the case of straw and sawdust this value is four times lower than that of the primary sludge. The efficiency of biogas production from grass is not influenced by enzymatic treatment, so it is not profitable to apply it to this waste.

The most effective enzyme treatment with cellulases was found in the case of straw. Enzymes resulted in both biogas production doubling and COD_s doubling release. The values obtained could be even higher if more fragmented wastes were used in the studies. On the other hand, straw proved to be the most burdensome substrate. Quickly swells, makes it difficult to feeding and mixing with fermentation sludge in the chamber.

Obtaining straw with very high fineness would also require financial inputs as its elasticity makes it difficult to grind in macerators. On the contrary sawdust is very convenient for dispensing co-substrate. The low biogas potential of this substrate obtained in the studies could be due to short time - both enzymatic pre-treatment and fermentation in the chamber.

It has been shown that the addition of an enzyme preparation containing cellulases into the digestion chamber decomposes the remaining cellulose nutrients in the sludge. Thus, both the increase of biogas production and the increase of sludge mineralization are beneficial.

It can be assumed that it facilitates the availability of cellulosic components in fermentation sludge and in primary sludge. The enzyme dosage into the fermenting chambers is profitable when it comes once for a period of time characterizing the sludge retention time. From the biogas extracted in this way, the energy can be obtained that yields 8.5 times the cost of buying the enzyme preparation. It would also be advisable to carry out research on a broader scale that would cover a wider range of cellulolytic preparations available on the market.

Mixing with lignocellulosic wastes of moldy bread was found to be very favourable for increasing the production of biogas. Although the effects of enzymes produced by molds on LCW were not demonstrated directly, the addition of moldy bread significantly improved the biogas potential of straw and sawdust waste. In addition, the molds produced during molding may be beneficial in the fermentation of other wastes. However, it should be noted that molds in metabolic processes consume significant amounts of organic carbon, as evidenced by the decrease in CODs observed for all LWC solutions mixed with moldy bread.

Studies show that in case of fermentation of lignocellulosic wastes in sewage treatment plants, each type of LCW requires an individually selected recipe. The efficiency of biogas production is influenced by various factors, such as fragmentation, availability of organic matter, biological pre-treatment etc. The use of lignocellulosic co-substrates does not compromise the performance of the fermentation chamber, it increases the diversity of nutrients and, in combination with the enzymatic effect of cellulases, improves the mineralization of the sludge.

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