The impact of mechanical pretreatment on biogas production from waste materials of the chemical and brewing industries

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Abstract
Respirometric tests, carried out in OxiTop system, were used to determine biogas production (BP) from two waste materials, willow bark residue (W) from the chemical industry and brewer’s spent grain (BSG) from the brewing industry. Moreover, the kinetics of BP and the loss of organic compounds (expressed as COD) were investigated. In this investigation, W and BSG were used both in their unchanged forms and after mechanical pretreatment (grinding to a diameter of 1 mm) (W_G and BSG_G). The initial organic load in the bioreactors was 4 kg OM/m³. The BP from W was 154.1 dm³/kg DM (166.6 dm³/kg OM), and from BSG, it was 536.9 dm³/kg DM (559.5 dm³/kg OM). This probably resulted from the fact that the content of lignin that was hard to biodegrade was higher in W than in BSG. Mechanical pretreatment increased BP from W_G to 186.7 dm³/kg DM (201.9 dm³/kg OM), and from BSG_G to 564.0 dm³/kg DM (588.7 dm³/kg OM). The net biogas yield from W and BSG increased by 17% (35 dm³/kg OM) and 5% (29 dm³/kg OM), respectively. The kinetic coefficient of BP (kB) and the rate of BP (r_B) of W were lower than those of BSG. Mechanical pretreatment increased the kB and r_B of biogas production from both waste materials.

Keywords: willow bark residue, brewer’s spent grain, pretreatment step, kinetics of biogas production and loss of COD, lignocellulosic biomass
1. Introduction

A side effect of economic development is the depletion of conventional energy sources and the production of a large amount of waste, including biodegradable waste. The anaerobic methane fermentation of biodegradable waste is of great importance; this is due to not only the decomposition of organic matter but also the production of biogas and digestate that can be used environmentally (De Bere et al., 2000; Nichols et al., 2004; Zhong et al., 2011). Biogas is a gas mixture consisting of 48–65% methane, 36–41% carbon dioxide, up to 17% nitrogen, <1% oxygen, and 32–169 ppm of hydrogen sulphide (Rasi et al., 2007). The common direction of digestate utilisation is its use as a fertilizer. It should be emphasised that physicochemical properties of digestate should be taken into consideration.

In recent years, many biogas plants have been implemented in EU countries, and a wide range of substrates (such as corn or silage, wheat, sugar cane, and agricultural waste) was used for feedstock preparation. In the context of using a mixture of substrates, co-fermentation is advantageous and enables achieving more favourable C:N:P ratios, which results in good conditions for the growth of microorganisms and an increase in biogas production. Moreover, the addition of pH-regulating substances or external nutrients can be avoided (Dias et al., 2014).

According to Directive 2009/28/EC, agricultural waste or residues of biological origin are called biomass. The term biomass refers to the biodegradable part of products, waste or residues of biological origin from agriculture (including plant and animal substances), forestry and related industries, including fishery and aquaculture, as well as the biodegradable part of industrial and municipal waste. The amount of global biomass production, which is a promising resource for fermentation, exceeds 220 billion tons/year (Yoshida et al., 2009). Ladicsh et al. (1983), Lechner and Papinutti (2006) indicated that biomass is highly abundant and the cost of obtaining it is low. Energy from biomass accounted for 14% of global energy consumption, while in developing countries, this share was even higher and amounted to 38% (Saxena et al., 2009). Lignocellulosic biomass (e.g. corn straw (Zhu et al., 2010) or grass mixtures (Frigon et al., 2012)) was successfully used as substrates for methane production due to its availability and low costs (Monlau et al., 2013).

Lignocellulosic materials can be classified into four groups depending on their origin: (1) forestry waste, (2) the organic fraction of municipal solid waste, (3) paper waste, and (4) crop residues. Lignocellulosic biomass consists of 48% carbon, 45% oxygen, 6% hydrogen and a small amount of inorganic substances (Buranov et al., 2006). The main component of lignocellulosic biomass (30–60% dry matter) is cellulose, a linear glucose polymer with additional hydrogen bonds. The crystalline nature of cellulose increases its resistance to biological degradation (Frigon et al., 2012; Karimi et al., 2016). Hemicellulose, constituting 20–40% of the dry matter of the raw material, is a short polymer made up of various monomers, such as pentoses and hexoses. The amorphous structure and lower degree of polymerisation of hemicellulose means that it has a greater ability to hydrolyse than cellulose (Li et al., 2015). Lignin, made of aromatic polymers consisting of phenylpropanoid precursors, accounts for 15–25% of the dry matter; it fills the space between cellulose and hemicellulose in lignocellulosic biomass, hindering biomass degradation, which limits its use in the methane fermentation process (Thomsen et al., 2014).

The presence of cellulose, hemicellulose, and lignin in lignocellulosic biomass, which are not easily degradable by microorganisms, may limit the potential for methane production. Therefore, the pretreatment of biomass is recommended. The objective of pretreatment is to increase the surface area of biomass and its biodegradability by removing lignin and reducing the length of polymer chains. Physical (mechanical and irradiation), chemical (using acids or bases), physicochemical (extrusion, hydrothermal, steam explosion) and biological (microbial or enzyme pretreatment) methods can be used as a pretreatment step in the case of lignocellulosic biomass (Abraham et al., 2020).
Biogas plants, especially those with a large capacity, remain open to receiving substrates that are the organic components of feedstock. Thus, new substrates for methane fermentation are still desirable. Most of the studies concerning biogas production from lignocellulosic biomass have focused on such plants as corn, lucerne and grass, as well as other agricultural waste. These plants are easily available and possess a high growth yield. A variety of waste materials are generated by different industries and are thus available on the market. One of these materials from the chemical industry is willow bark residue following the extraction of salicylates. There is a lack of data concerning biogas production from willow bark residue. The issue of limited data also applies to that relating to brewer’s spent grain. Moreover, there is no information on how the pretreatment step affects the production of biogas from these waste materials. It is known that the brewer’s spent grain has a high content of fibre and protein of around 60% and 20-26% of dry matter, respectively, and a very low fat content. The high content of organic and dry matter enables the use of brewer’s spent grain as an additive substrate which increases the biogas yield but also causes thickening of liquid feedstocks in biogas plants.

In the present study, two waste materials (willow bark residue from the chemical industry and brewer’s spent grain from the brewing industry) were tested as potential substrates for methane fermentation. The aim of the study was to determine biogas production from these two waste materials both in their untreated forms and following the use of mechanical pretreatment. Moreover, the kinetics of biogas production and the loss of organic (expressed as chemical oxygen demand (COD)) content were determined.

2. Materials and methods

2.1. Waste materials used in the experiment

Willow bark residue (Salix alba) (W) used in the experiment was a waste product of the extraction of salicylate in the chemical industry. Brewer’s spent grain (BSG) was a waste of the brewing industry. The moisture of willow bark residue was merely 7.5%; by contrast, the moisture content in brewer’s spent grain was higher than 80%. The content of organic matter in both substrates was high, amounting to more than 90% of dry matter (DM). The characteristics of the substrates are presented in Table 1.

| Parameter                  | Waste materials |
|----------------------------|-----------------|
| dry matter (DM), %         | 92.5            |
| moisture, %                | 7.5             |
| dry organic matter (OM), % | 92.4            |
| ash, %                     | 7.6             |
| pH                         | 8.1             |

Willow bark (W) and brewer’s spent grain (BSG) were used as substrates for biogas production both in their unchanged form and following grinding (W_G and BSG_G). The waste materials were ground in a Retsch SM 100 mill to a grain diameter of less than 1 mm.

2.2. Biogas production measurement procedure

Biogas production was determined with the use of the respirometric (GP21) test in accordance with the methodology given by Heerenklage and Stegmann (2005), in the OxiTop® control system. The system consisted of glass measuring vessels...
(bioreactors) with a capacity of approx. 600 ml and measuring heads, each with a built-in pressure sensor. The initial organic load (OL) in the bioreactors was 4 kg OM/m³. To ensure anaerobic conditions, the space above the sample was flushed with nitrogen gas. Samples in triplicate were incubated for twenty-one days under mesophilic conditions at a temperature of 37±2°C. Pressure values obtained from respirometric measurements enabled the volume of produced biogas to be determined.

Approximately 100 g of inoculum and the appropriate dose of substrate was introduced into the OxiTop measuring bioreactors to achieve the starting OL. Biogas production from the inoculum only was also determined. Biogas production from the substrate itself was determined by identifying the difference between the amount of biogas generated in the inoculum vessels and the substrate, and the amount of biogas generated from the inoculum only.

As the inoculum (I), fermented sludge from a closed mesophilic digester chamber at the Municipal Wastewater Treatment Plant using activated sludge method (Poland) was used. The dry matter (DM) content of the inoculum was 2.20%, whereas organic matter (OM) constituted 66.80% of the DM. After the introduction of the substrates, the content of the DM increased to 2.75% (W) and 2.35% (BSG), and the OM was around 71% of the DM.

To enable sampling for COD analyses without interrupting the production of biogas in the OxiTop bioreactors, additional glass bottles with the same volume as the OxiTop bottles were prepared for each substrate (using the same dose of inoculum and substrate) and incubated together. Both biogas production and the loss of COD content proceeded with the first-order kinetic model. Kinetic coefficients were determined with the use of nonlinear regression analysis using the Statistica software program, version 13.3 (StatSoft).

Moisture content, dry matter (DM), organic dry matter (OM; % DM) in the waste materials, and in mixtures of the substrates and inoculum supplied to the OxiTop measuring bioreactors before and after the measurements of biogas production (BP) (after twenty-one days), and COD concentration in the supernatants was determined according to Polish standards. Dry matter (DM) is determined by drying the sample at 105°C to ensure a constant weight. Organic dry matter (OM) is determined by ignition of the sample for at least 4 h at 550°C. The pH was measured in water extracted from the waste materials using a Schott titroline system.

2.3. Results and discussion

The average volumes of biogas generated over the twenty-one days of respirometric measurements from both inoculum only and from inoculum with waste materials are shown in Fig. 1. The difference constitutes the volume of biogas produced by the substrates alone. The cumulative biogas volumes from brewer’s spent grain (both without pretreatment and following the pretreatment step) were almost twice as high (ca. 0.42 dm³) than those from willow bark residue (ca. 0.21 dm³).

Biogas production in dm³/kg DM and dm³/kg OM was calculated on the basis on the biogas volume. In Fig. 2, the curves of biogas production in dm³/kg OM are shown. Biogas production from willow bark residue was lower than that produced from brewer’s spent grain. Biogas production from willow bark residue (W) without pretreatment was 154.1 dm³/kg DM (166.6 dm³/kg OM), whereas from brewer’s spent grain (BSG), this value was 536.9 dm³/kg DM (559.5 dm³/kg OM).

Higher production of biogas is probably the result of the composition of the used waste materials. It is known that brewer’s spent grain contains less lignin than willow bark residue. The lignocellulose composition of the waste materials was not investigated in the present study; however, this subject has been studied extensively in the past. For example, Robertson et al. (2010) showed that the content of lignin in barley grains was 14.5%, whereas polysaccharides and proteins constituted 38.5% and 18.6%, respectively. A lower content of
lignin, 8.13% was found by Tišma et al. (2018) in brewers' spent grain (cellulose and hemicellulose constituted 16% and 20%, respectively). Wikberg (2017) characterised brewer's spent grain with the content of carbohydrates, lignin and protein being 43.2%, 16.1% and 24.4%, respectively; however, in the case of willow, these values were 48.5%, 27.2% and 2.4%, respectively. This means that the content of lignin, being hard to degrade by microorganisms, was almost twice as high in willow than in barley grain. Krzyżaniak et al. (2014) confirmed that willow has a high content of lignin (25.5%).

Biogas production from the waste materials tested in the present study was compared to literature data concerning biogas production from other lignocellulosic biomass. Kowalska (2017) found that biogas production from rape residues after the extraction is 516 dm³/kg DM (633 dm³/kg OM). The author also reports that biogas production from beet and rye stock is 70 dm³/kg DM (550-600 dm³/kg OM) and 170-220 dm³/kg DM (550–680 dm³/kg OM), respectively. Because of its high availability, maize has been one of the most commonly used crops for studies of biogas production. Oslaj et al. (2010) showed that biogas production from maize ranged from 515 to 568 dm³/kg VS (methane content of 56–57%) and found that biogas production is strongly dependent on the composition of the maize, and increased with the maturity of the grain (the content of crude protein). Gao et al. (2012) reported similar methane production, 213.94-313.63, 195.88-334.81 dm³/kg VS, from fresh and silage maize, respectively.

As previously mentioned, the pretreatment step usually increases the biogas production from organic substrates, especially from lignocellulosic biomass. The most commonly applied pretreatment step is mechanical treatment (milling and grinding), which is used to reduce the size of lignocellulosic biomass particles. The fragmentation degree of lignocellulosic biomass is in the range of 10-30 mm for the chopping process (Kumar et al., 2009) and 0.2-2.0 mm after the milling or grinding process (Bruni et al., 2010). The use of mechanical pretreatment increases the digestion surface area of the available raw material and reduces the crystallinity and degree of cellulose polymerisation (Kratky et al., 2011). According to Mshandete et al. (2006), fibre degradation improves methane production efficiency as particle size decreases from 100 mm to 2 mm. Maceration can support the process of mechanical pretreatment.

In the present study, mechanical pretreatment caused an increase in the volume of biogas produced from both substrates used in the present study. The volume of biogas produced from W was 0.0709 dm³. After mechanical pretreatment, the volume of biogas produced from W_G increased by 0.015 dm³. The volume of biogas produced from BSG and BSG_G was 0.2259 dm³ and 0.2377 dm³, respectively. Mechanical pretreatment increased biogas production from both W_G and BSG_G to 186.7 dm³/kg DM, (201.9 dm³/kg OM) and 564.0 dm³/kg DM (588.7 dm³/kg OM), respectively. The net biogas yield increased by around 17% (35 dm³/kg OM) for willow bark residue and around 5% (29 dm³/kg OM) for brewer’s spent grain.
Angelidaki and Ahring (2000) showed that the maceration of manure combined with the mechanical grinding of plant fibres increased biogas production potential by 16% from particles with a size of 2 mm. Biogas production efficiency increased by 20% at a particle size of 0.35 mm. For some types of cellulosic biomass, excessive fragmentation may result in the secretion of biogas inhibitors (De la Rubia et al., 2011). However, in most cases, the use of mechanical pretreatment of lignocellulosic biomass results in an increase in biogas production by up to 25% (Hartmann et al., 2000). Pakarinen et al. (2009) compared the efficiency of biogas production from grass silage (timothy and meadow fescue) without pretreatment and following alkaline pretreatment with 1% NaOH at 20°C for twenty-four hours. Biogas production from fresh silage without pretreatment was 431 dm³/kg VS which, following hydrolysis increased to 703 dm³/kg VS. Tsapekos et al. (2015) studied the effect of pretreatment on the anaerobic digestion of meadow grass, obtaining a 25% increase in methane production compared to the untreated substrate. Sugar beet pulp was ground to 2.5-mm particles and resulted in the cumulative biogas production of 617.2 dm³/kg VS. The value was 20.2% higher in comparison with non-pretreated substrate (Ziemiński et al., 2017).

In the present study, biogas production during anaerobic digestion was accompanied by the removal of organic compounds from the substrate, for example, in the form of dissolved organic compounds, i.e. COD content in the supernatant. The initial intensive decrease in COD content in the supernatant correlated with high daily biogas production (in dm³/(kg OM d)) (Fig. 3). In waste materials both without pretreatment and following mechanical pretreatment, during the first days of measurement of biogas production, a rapid increase in the daily biogas production to the maximal value was observed. Daily biogas production
then gradually decreased. During the first three days of the measurements, the amount of biogas produced was around 95 and 115 dm³/kg OM in W and W_G, respectively. These values were much higher in BSG and BSG_G, around 326 and 353 dm³/kg OM, respectively. In these cases, the amount of biogas produced over three days constituted around 60% of the total biogas production. During this time, the decrease of COD content in the supernatant was around 50% of the total loss of COD concentration. Over fourteen days of the measurement, 90% of the cumulative biogas production from W and W_G was achieved. For BSG, 90% of the biogas was produced within twelve days. The shortest time for 90% of biogas production was obtained in BSG_G (up to the tenth day). It should be emphasised that in samples with BSG (without and following pretreatment), the initial COD contents in the supernatant were higher than in samples with W. Moreover, mechanical pretreatment caused a slight increase in the initial COD content in the supernatant of both waste materials (Fig. 3). The final values of COD content in the supernatant of all variants of the experiment were similar to this value in inoculum only. This means that all available soluble organics (as COD concentration) were converted into biogas.

As previously mentioned, the content of organic matter that included soluble and suspended organic compounds in W and BSG samples introduced into the OxiTop measuring bioreactors was around 71%. After measurement of BP, the content of OM decreased to the value determined in the samples with the inoculum only (64-66% of DM). This means that the organics (organic matter) were used by the microorganisms during anaerobic transformation into biogas.

Changes of the biogas production and COD content in the supernatant were described with first-order kinetic models. The kinetic coefficients of biogas production (k_B) of willow bark residue were lower than that of brewer’s spent grain. Similarly, higher rates of biogas production (r_B) were achieved in the case of fermentation of brewer’s spent grain. Mechanical pretreatment increased both k_B and r_B. The kinetic coefficients of the loss of COD content (k_COD) ranged from 0.21 to 0.25 1; however, slightly higher values were observed when mechanical pretreatment was applied for W and BSG before anaerobic measurement of biogas production. As a result, the rates of the loss of COD content were around 1.13 times higher following mechanical pretreatment (Table 2).

| Kinetic parameters | Unit       | W       | W_G     | BSG     | BSG_G    |
|--------------------|------------|---------|---------|---------|----------|
| C0_B               | L/kg OM    | 167.0   | 202.0   | 559.5   | 588.7    |
| k_B                | d⁻¹        | 0.19    | 0.22    | 0.26    | 0.30     |
| r_B                | L/(kg OM·d)| 33.4    | 44.4    | 145.5   | 176.6    |
| C0_COD             | kg COD/m³  | 730.0   | 766.0   | 855.0   | 878.0    |
| k_COD              | d⁻¹        | 0.23    | 0.25    | 0.21    | 0.23     |
| r_COD              | kg COD/(m³·d)| 167.9  | 191.5   | 179.6   | 201.9    |

* the initial rate of biogas production rBiogas = C0_B·kB; the initial rate of COD loss rCOD= C0_COD·kCOD

Data concerning the impact of mechanical pretreatment on the kinetic parameters of biogas production from lignocellulosic biomass are scarce. Kinetics of biogas production can be affected by the increase of the content of lignocellulosic materials. Bernat et al. (2019a) determined biogas productivity and kinetics during methane fermentation and co-fermentation of food (FW) and green waste (GW) with different proportions of FW and GW. Although FW and GW had similarly high contents of organic matter (88.4–93.1% TS), the content of lignocellulosic materials was around 25% and 63%, respectively. Biogas production increased when the share of FW in the mixtures increased.
because the content of fibrous materials decreased. The kinetic coefficients of biogas production and of organic matter content loss during fermentation also increased when the share of FW in the mixtures increased.

Bernat et al. (2019b) also investigated the kinetics of biogas production from sewage sludge both without and following pretreatment with homogenisation or ultrasound disintegration. They found that biogas production from sewage sludge after pretreatment with ultrasound disintegration was up to 16% higher than without pretreatment. Homogenisation affected neither kinetic coefficients of biogas production ($k_b$) nor the methane content in the biogas which was similar as in the case of non-pretreated sludge. Ultrasound disintegration increased kinetic parameters of the biogas production from 1.18 to 1.39 times depending on the conditions of the pretreatment.

3. Conclusions

The study showed that waste materials, for example, willow bark residue and brewer’s spent grain, can be used as alternative substrates or co-substrates for methane fermentation. The biogas production of willow bark residue was 154.1 dm$^3$/kg DM (166.6 dm$^3$/kg OM) and was almost three times lower than that of brewer’s spent grain (536.9 dm$^3$/kg DM, 559.5 dm$^3$/kg OM). This indicates that brewer’s spent grain is more favourable for methane production. The mechanical pretreatment step not only increased the kinetic coefficient of biogas production but also the rate of biogas production. This resulted in an increase of biogas production from willow bark residue and brewer’s spent grain by 17% and 5%, respectively.

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Wpływ wstępnego mechanicznego przetwarzania na produkcję biogazu z materiałów odpadowych z przemysłu chemicznego i browarniczego

Streszczenie
Wykorzystano testy respirometryczne (bioreaktory OxiTop) do określenia produkcji biogazu (BP) z materiałów odpadowych tj. pozostałości kory wierzby (W) oraz młota (BSG). Ponadto wyznaczono kinetykę BP i usuwania związków organicznych (ChZT). W i BSG stosowano w formie niezmienionej oraz po mechanicznej obróbce wstępnej (rozdrobnienie do średnicy 1 mm) (W_G, BSG_G). Początkowe obciążenie ładunkiem związków organicznych w bioreaktorach wynosiło 4 kg s.m.o./m³. Produkcja biogazu z W oraz BSG wynosiła odpowiednio 154,1 dm³/kg s.m. (166,6 dm³/kg s.m.o.) i 536,9 dm³/kg s.m. (559,5 dm³/kg s.m.o.). Prawdopodobnie było to wynikiem wyższej zawartości trudno biodegradowalnej ligniny w pozostałości kory wierzby. Po mechanicznym rozdrobnieniu, produkcja biogazu z W_G oraz z BSG_G zwiększyła się do 186,7 dm³/kg s.m. (201,9 dm³/kg s.m.o.) i 564,0 dm³/kg s.m. (588,7 dm³/kg s.m.o.). Wydajność biogazu netto wzrosła odpowiednio o 17% (35 dm³/kg s.m.o.) i 5% (29 dm³/kg s.m.o.). Współczynniki kinetyczne BP (k_B) oraz szybkości produkcji biogazu (r_B) były niższe gdy substratem była W. Po mechanicznym rozdrobnieniu parametry kinetyczne BP były wyższe.

Słowa kluczowe: pozostałości kory wierzby, młoto, wstępne przygotowanie, kinetyka produkcji biogazu i usuwania ChZT, biomasa lignocelulozowa