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Research paper

Pretreatment of energy crops with sodium hydroxide and cellulolytic enzymes to increase biogas production

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ABSTRACT

This work presents the influence of alkali pretreatment on the enzymatic hydrolysis and efficiency of anaerobic digestion of lignocellulosic biomass pretreated both in a one- (chemical or enzymatic) and two-step (chemical and enzymatic) process. In this study two species of energy crops were used *Miscanthus giganteus* and *Sida hermaphrodita*. The aim of this work was to compare biogas production and methane yield during fermentation of pretreated and untreated energy crops. The results show that alkali pretreatment is necessary for the effective biogas generation from plant material due to high delignification level and significant hemicellulose degradation. The two-step hydrolysis process consisting on the alkali and enzymatic step leads to the release of high concentrations of glucose (about 20 g L^{-1}). The best results were achieved for *M. giganteus* with biogas production yield of 421.5 Ndm^3 kg TS^{-1} and with methane production yield of 257 Ndm^3 kg TS^{-1}.

1. Introduction

Anaerobic digestion is one of the methods for renewable energy generation [1]. In practice its successful use depends mostly on the type of the feedstock converted into biogas during the fermentation process. It consists of four major steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis [2]. Each one of them involves specific bacteria species responsible for the conversion of high molecular structures into simpler ones, which in consequence leads to biogas production with high methane content [1].

Typical substrates for the anaerobic digestion processes are organic wastes, both municipal and industrial, sewage sludge, wastewater, agriculture residuals and energy crops [2–6]. Dependent on the kind of substrate used in the fermentation, different technologies are applied. In the case, in which lignocellulosic biomass is used, a conventional process is extended with the additional pretreatment steps, most often a chemical step [7,8]. This procedure is performed due to the complex chemical structure of the plant material, which makes biological hydrolysis hardly possible [5,9,10]. The three major components: lignin, hemicellulose and cellulose are not soluble in water, so their degradation into simpler compounds, as well as further biogas production is retarded [4,11,12].

There are many methods for the chemical pretreatment of lignocellulosic biomass like acid hydrolysis [13–15], Advanced Oxidation Processes (AOPs) [16–19]. For several years alkali hydrolysis with the use of sodium hydroxide or calcium hydroxide has occurred to be very promising [1,10,20].

The main advantage of the alkali pretreatment is the decomposition of lignin, the structure that limits enzymatic actions during the anaerobic digestion process [21,22]. If lignin is not destroyed, the availability of cellulose for the bacterial metabolism is significantly hindered [23]. Alkalis break ester bonds cross-linking lignin and xylan [6,7]. Hemicellulose is also partially decomposed during the pretreatment process [3,7]. All these facts indicate that the pretreated material is more useful for the anaerobic degradation and enzyme action and access to cellulose is facilitated. However, it is still not known, in what way the alkali hydrolysis influence further biogas production, whether there is a necessity to separate liquid and solid phase before anaerobic digestion after the alkali pretreatment and what disadvantages of this chemical pretreatment method of energy crops referred to fermentation process are.

The aim of this work was to determine the efficiency of the alkali hydrolysis with the use of sodium hydroxide on the degradation level of two different species of energy crops: *Miscanthus giganteus*

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and *Sida hermaphrodita*. Moreover, the influence of the alkali hydrolysis on the further enzymatic step and on biogas production was investigated. Optimum conditions were determined for the efficient chemical pretreatment and following biogas production. The comparison between single and two-step hydrolysis was studied.

2. Materials and methods

2.1. Preparation of raw plant material

Two species of energy crops were used in this study: *M. giganteus* and *S. hermaphrodita*. They represent different groups of energy crops (fast-growing grass and perennial, respectively) and belong to the group of non-food renewable energy sources. Raw plant material was ground in the mechanical mill to obtain particles of dimensions from 0.1 to 1 mm. Extraction with 96% ethanol was conducted according to Polish Standard Method PN-92/P-50092 in order to remove chlorophyll disturbing in the spectrophotometric measurements. Biomass prepared in that way was then rinsed with distilled water until pH of filtrate was neutral and then dried in a dryer at constant temperature of 45 °C.

2.2. Alkali pretreatment

A 2.5 g of biomass prepared as described in Section 2.1 was suspended in 50 cm^3 of NaOH solution in Erlenmeyer flasks. Concentrations of sodium hydroxide ranged from 0.25% to 5.0%. Then the sample was placed in the autoclave and the process of hydrolysis was conducted at 121 °C for 30 min. In order to complete the pretreatment step, the sample was cooled in water bath and separated by centrifugation into two phases: supernatant and pretreated biomass. Thereafter, the obtained liquid and solid phases were analyzed by means of methods described below. The experiments were conducted in duplicate.

2.3. Enzymatic hydrolysis

A 2.5 g of raw or chemically hydrolyzed plant biomass was suspended in 50 cm^3 of 50 mM citrate buffer solution at pH of 4.8. Two enzymes cellulase (Celluclast 1.5L) and cellobiase (Novozyme 188) were added to the sample. The loading of cellulase was 160 EGU/g of solids, and the loading of cellobiase was 17.2 CBU/g of solids. The flask with the sample was placed in a shaker at constant temperature of 50 °C for 24 h. The obtained samples were then used as a feedstock in the anaerobic digestion process carried out in shake cultures. The experiments were conducted in duplicate.

2.4. Anaerobic digestion

Pretreated in one- or two-step process plant biomass with the 540 cm^3 of obtained supernatant was mixed with 360 cm^3 of inoculum. The inoculum culture was obtained from the sludge digestion chamber located in Wastewater Treatment Plant in Lodz, Poland, where biological stabilization of the excessive sludge takes place. The inoculum contained the consortium of various anaerobic bacteria. The pH of mixture was adjusted to 7.2 with the use of either $NaHCO_3$ or H_2SO_4. Then the sample was placed in the shake flasks at mesophilic conditions (constant temperature of 37 °C). Volumes of biogas produced were measured by liquid displacement method with the use of 33% NaCl solution. Biogas composition was measured by the gas chromatography analysis. Anaerobic digestion was made once for each sample.

2.5. Analytical methods

In raw plant material the following parameters were determined:

- Total Solids (TS, Polish Standard Method PN-92/P-50092, sample dried at constant temperature of 105 °C),
- Volatile Solids (VS, Polish Standard Method PN-92/P-50092, mineralization in an oven at constant temperature of 550 °C),
- ash (Polish Standard Method PN-92/P-50092, subtraction TS-VS),
- soluble and insoluble lignin content (Polish Standard Method PN-92/P-50092, removal of holocellulose from biomass with the use of sulfuric acid),
- holocellulose content (Polish Standard Method PN-92/P-50092, delignification of the plant material with the use of sodium chlorite and ice-cold acetic acid),
- cellulose content [24], hemicellulose content (subtraction holocellulose-cellulose),
- Chemical Oxygen Demand (COD, Polish Standard Method PN−74/C−0457, oxidation with potassium dichromate),
- elemental composition (C, H, N, S; Elemental Analyzer NA 2500, CE Instruments, incineration in pure oxygen and chromatographic analysis of gases).

The following parameters were determined in the hydrolysates obtained after the process: volatile fatty acids (VFA, water vapor distillation, BÜCHI), Chemical Oxygen Demand (COD, mineralization and spectrophotometric analysis in DR 5000 apparatus, method no. LCK 514), Total Organic Carbon (TOC) and total nitrogen (TN, HACH-LANGE), Total Phenolic Content (TPC, Follin Ciocalteou's Method [25], UV-VIS T80 + PG Instruments Limited spectrophotometer, $\lambda = 765$ nm), ammonia nitrogen ($N{-}NH_4^+$, water vapor distillation and subsequent absorption in boric acid solution, BÜCHI), glucose and xylose concentration (Waters HPLC equipped with Bio-rad Aminex HPX-87H column, Waters 410 RI Detector and Waters 717 + sampler; column temperature 60 °C, mobile phase 0.01 N sulfuric acid at the flow rate of 0.6 mL min^{-1}, USA).

Biogas composition was measured by GC analysis (Gas Chromatograph SRI 8610C, SRI Instruments, Inc, USA equipped with TCD Detector; column temperature 60 °C, detector temperature 150 °C, carrier gas helium at a flow rate 10 mL min^{-1}).

The measurement of data dispersion was made by determining the standard deviation based on three replicates or the absolute error for biogas measurements. This measurement corresponded to the accuracy of the analytical methods only.

3. Results and discussion

3.1. Alkali hydrolysis

After the alkali pretreatment of *Miscanthus* and *Sida* biomass several parameters like COD, VFA, TOC, N and TPC in the obtained supernatants were measured. Moreover, the concentrations of monosaccharides were determined. The results achieved for various plant materials and at various concentrations of NaOH solution are shown in Tables 1 and 2.

The observed high values of measured indicators (VFA, TPC, COD, TOC) showed that some part of each polymeric structure building the plant material was degraded. The presence of phenolic compounds in the obtained supernatants indicated that delignification of biomass took place. Hemicellulose was transformed mainly into volatile fatty acids and cellulose was partially converted into glucose and then fast into simpler compounds, what was proved by high COD and TOC of supernatants.