BIOCHEMICAL METHANE POTENTIAL OF BIOLOGICALLY AND CHEMICALLY PRETREATED SAWDUST AND STRAW

Lauma Laurinovica[1], Janis Jasko[2,3], Eriks Skripsts[2,3], Vilis Dubrovskis[3]
[1]University of Latvia; [2]Institute of Biomechanics and Physical Research, Latvia ; [3]Latvia University of Agriculture
lauma.laurinovica@gmail.com

Abstract. Biochemical methane potential from biodegradable waste (hardwood sawdust and rape straw biomass) was investigated. Pilot biological (fungal) and chemical (alkali) pre-treatment was performed for both substrates. Anaerobic fermentation was performed in 100 ml serum bottles for 30 days. The highest biogas yield reached in this study was from alkali pretreated straw biomass 301 $l \cdot kg_{TS}^{-1}$ or 316 $l \cdot kg_{VS}^{-1}$. The results showed that alkali pretreatment increases the biogas yield. At the same time selection of microbial and fungal strains and proper growth conditions are significant for efficient cellulose containing substrate biodegradation.

Keywords: biomass, pretreatment, anaerobic fermentation, biogas.

Introduction

Biomass is an organic material derived from plants as a matter that obtains energy from solar power via photosynthesis. The energy is stored in chemical bonds of biomass. The major components of plant biomass are cellulose, lignin and hemicellulose. The quantity of the polymers differs with the age and the species of plants [1]. The composition of the main plant polymers in various substrates is shown in Table 1.

Table 1

Percentage composition of major polymer in vascular plants [1]

Source	Cellulose, %	Hemicellulose, %	Lignin, %
Softwood	45-50	25-35	25-35
Hardwood	45-55	24-40	18-25
Grasses	25-40	25-50	10-30

These three polymers are bind together in vascular plant tissues and are degraded in habitat by bacteria and fungi. Biomass is used as one of the main sources for bioenergy production. Primary bioenergy products from biomass are power, heat and fuel [1-3].

Biogas production actuality has increased in recent years due to the expanding greenhouse gas pollution and rapid use of fossil energy resources. For biogas production different substrates can be used. The main goal nowadays is to use agricultural residues or crops that minimally compete with energetic crops. A significant advantage of biologically pretreated organic substrates is that processing residuals and by-products can be used as substrates further [2; 4].

Biological pretreatment has taken a significant role in biomass processing. The sugars released during plant polymer degradation are taken up by many bacteria. Pre-treatment of lignocellulose and hemicellulose make the cellulose easy accessible to hydrolytic enzymes [1; 2].

Materials and methods

1. Description of raw material

Fine fraction (< 0.5 mm) of deciduous tree saw dust was collected from a sawmill in Riga, Latvia. Rape straw in bales was obtained from a farmer in Zemgale region, Latvia. Long straw was milled in the industrial hammer mill till fraction 0.5-5 mm reached. Cultivation of three fungal strains was done on part of sawdust and straw biomass. Before chemical hydrolysis the raw material was analysed for total (TS) and volatile solids (VS), the data are shown in Table 2.

Table 2

Content of total and volatile solids in substrates

Substrate	TS, g·kg^{-1}	VS, g·kg^{-1}
Rape straw	889.4	848.1
Hardwood sawdust	894.9	888.5
Straw with *Paecilomyces* sp.	251.8	220.8

2. Fungal pre-treatment

Fungal pre-treatment was carried out as recommended by Zhong et al. [5]. Three different, frequently tested fungi strains were used: *Aspergillus niger* L., *Trichoderma viride* L. and *Phanerochaete* L. sp. The strain selection was based on their extracellular enzyme diverse qualities. *Phanerochaete* species produce enzyme complex that decomposes all three major components of the plant cell walls. *Trichoderma* species produce cellulose degrading enzymes (*β*-glucosidases, endoglucanases, celobiohydrolases). *Aspergillus* sp. does not produce effective celluloses but enzymes have high hemicellulose disrupting qualities [1; 6].

Seven days old mycelia cultures from potato dextrose agar (PDA) media were suspended in nutrient solution (10 ml). The solution was composed with 1 % KH_2PO_4, 1 % NH_4Cl, 0.2 % urea, 1 % $NaHCO_3$ and boiled tap water. The nutrient solution pH was 7.4 ± 0.2.

To 10.0 g of substrate (sawdust or straw) 5 ml of suspension and 30 ml of nutrient solution was added in covered up, aerated 100 ml plastic boxes. Incubation (7 days) was performed in room temperature (18 ± 1 ºC) and each sample had one repetition.

Growth of the selected fungal strains was not observed due to wild fungal flora (*Paecilomyces* sp.) that started to grow on the substrates. Wild fungus pre-treated straws were used for methane fermentation under mesophilic conditions to determine the natural flora pre-treatment efficiency.

3. Alkali treatment

To 0.5 g of substrate 15 ml of sodium hydroxide or distilled water was added in serum bottles. The serum bottles were incubated at 38 ± 1 ^{o}C for 18 hours. The alkali was neutralised with 135 µl of concentrated HCl. In addition, 0.08 g of $NaHCO_3$ was added to raise the buffering capacity. Substrate treated in distilled water was used as control to avoid possible unspecific (swelling etc.) effects on biogas formation.

4. Experiment methodology

Biochemical methane test was performed as recommended by Angelidaki et al. [7]. Each sample had three repetitions. 0.5 g of substrate, 15 ml of sodium hydroxide (distilled water was used instead for untreated samples and reference), 30 ml of nutrient media (as proposed in [7]) and 40 ml of seed material was filled up in serum bottles with exception for the reference were no substrate was added. Seed material addition was done in anaerobic environment; after nutrient media addition each bottle was flushed with N_2 gas to provide anaerobic conditions. Anaerobic fermentation was carried out for 30 days.

The seed material was obtained from 50 litre anaerobic continuously stirred tank reactor (CSTR) from own laboratory with plant biomass mixture as substrate. The reactor was operated at 50 days HRT, 37 ± 1 ºC with stable pH 7.8 ± 0.2 without pH control. The content of methane in biogas was 55 ± 1 %.

5. Analytical methods

Daily methane volume was determined using a method developed in own laboratory. A 60 ml medical syringe (producer BD Plastipak^{TM}, Ireland) with a needle and closure valve was filled with 10 ml of 10 % NaOH solution. The rubber stopper of the serum bottle was punctured with the needle to transfer gas from the serum bottle headspace to the syringe. When gas has stopped bubbling, the valve was closed, syringe shaken and left for 30 minutes to react. Sodium hydroxide reacts with CO_2 and H_2S – two main components besides methane in biogas, leaving only methane in gas phase.

Total solids (TS) were determined by drying the sample in the laboratory oven (60/300 LFN, Snol) at 105 ºC till constant mass reached. The sample weight was determined by the laboratory balance (GF-3000, A&D). Volatile solids (VS) were determined by keeping the dried samples in a laboratory furnace (8.2/1100, Snol) at constant temperature of 550 ± 5 ºC during the period of 0.5 hours.

Results and discussion

Rape straw biomass was anaerobically fermented to determine the effect of different treatment on biogas production. Alkali treatment was preformed 18 hours. The results in Fig.1 show that on day 30 the methane yield was 316 ± 3.3; 222 ±16.4; 267 ± 1.3 $l \cdot kg_{VS}^{-1}$ for alkali treatment, fungal treatment and without treatment respectively. Methane production is rapid on the first 15 days and becomes little slower afterwards. The methane yield for rape straw has been increased by 18 % by alkali treatment comparing to untreated straw biomass.

Biogas yields from fungus pretreated straw biomass are lower than from straw biomass without treatment. As it is only the first such experiment several explanations can be suggested: (i) fungus can have low cellulolytic enzyme activity; (ii) fungus could possibly have used easy degradable sugar monomers for own biomass development reducing total BMP of substrate; (iii) wild fungus could have produced biologically active molecules that inhibit growth and metabolism of microorganisms involved in biogas production. Sterilization of substrate and nutrient media optimization are needed to avoid the natural strain oppressing effect on preferred strain growth [8].

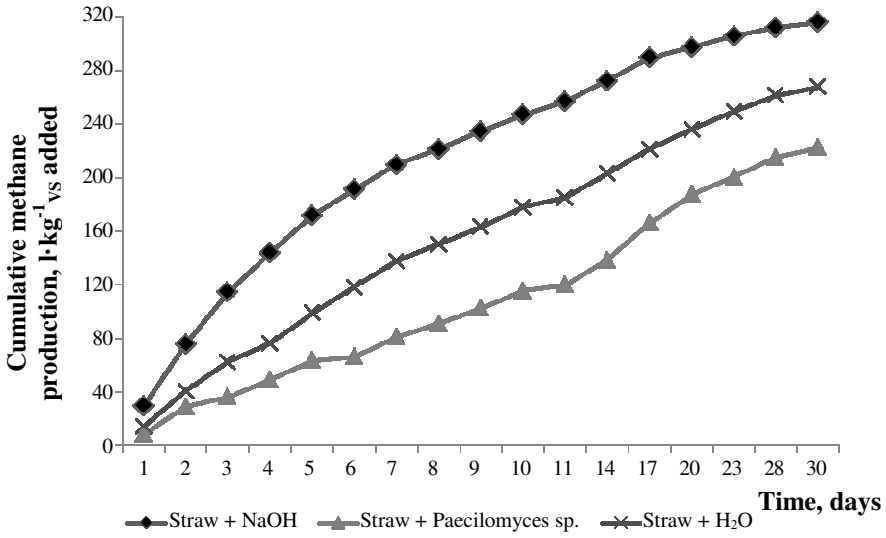

Fig. 1. **Cumulative methane yield from straw biomass**

As shown in Fig. 2, hardwood sawdust alkali treatment has a similar positive effect on the methane yield as for straw treatment. At the same time gas production has a different slope type – the first 7 days biogas production is very rapid and on day 8 the biogas production rate decreases thus slowing the cumulative biogas yield growth. The results in Fig. 2 show that on day 30 the methane yield was 315 ± 20.7 and 252 ± 8.8 $l \cdot kg_{VS}^{-1}$ for alkali treatment and without treatment respectively. The methane yield for saw dust has been increased by 25 % by alkali treatment.

The lignin degradation occurs in secondary metabolism. Only a special set of organisms produce secondary metabolites through specialized pathways, mostly during the stationary phase [1; 9]. Biogas from sawdust biomass should accumulate slower comparing to straw substrate. This relevance appears to untreated substrates (with distilled water). As shown in Fig. 2, biogas production increment from untreated sawdust starts on day 5, it is three days later comparing to untreated straw biomass (Fig. 1).

The experimental results are consistent with the studies reported in [5; 10], the cumulative methane yield after alkali treatment had a positive effect. These studies represented results that by NaOH pretreated biomass 29 % and 49.2 % higher biogas production is obtained than untreated or by urea treated substrates respectively.

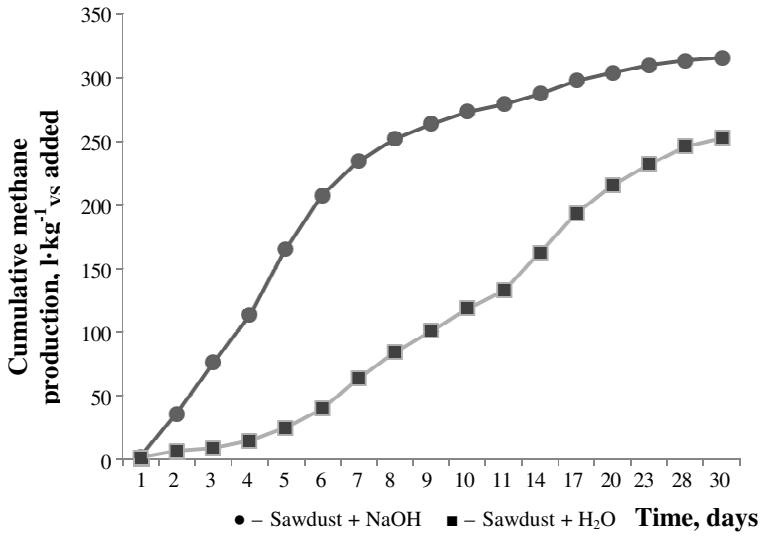

Fig. 2. **Cumulative methane yield from sawdust biomass**

Conclusions

1. A simple alkali pretreatment process was confirmed to be effective for improving the biochemical methane potential in mesophilic anaerobic fermentation of straw and sawdust biomasses.
2. The fungal pretreatment method needs to be optimized to achieve a positive effect on the methane yield.
3. The amount of lignin in biomass has a noticeable effect on the biogas formation curve.

Acknowledgments

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