### ANAEROBIC FERMENTATION OF BIOLOGICALLY PRETREATED SAWDUST FOR ENERGY APPLICATIONS

# Janis Jasko<sup>1,2</sup>, Eriks Skripsts<sup>1,2</sup>, Vilis Dubrovskis<sup>1</sup>, Laila Dubova<sup>1</sup>, Ina Alsina<sup>1</sup>

<sup>1</sup>Latvia University of Agriculture; <sup>2</sup>Institute of Biomechanics and Physical Research, Latvia janis.jasko@gmail.com

**Abstract.** During the study fermentation of biologically pretreated saw dust was carried out in pilot scale 50 1 bioreactors. Deciduous tree sawdust had been pretreated with mycelium of *Pleurotus ostreatus* for more than three months before fermentation. Biogas production was stable with average methane composition about 50 %. The experiment was carried out for 71 days resulting with no major problems in reactor operation. It can be concluded that biologically pretreated sawdust can be used as the substrate for anaerobic fermentation.

Keywords: anaerobic fermentation, biogas, saw dust, fungi, mushroom.

### Introduction

Usage of anaerobic fermentation process to produce renewable energy becomes more widespread in the European Union and also in Latvia. According to the Latvian Biogas Association in the mid of 2012 there are 36 biogas stations in operation in Latvia. A large part of biomass used as a substrate for biogas production is energy crops, mainly maize and grass silage. At the same time, according to the latest renewable energy policy trends in the EU, cultivation of energy crops should not compete with food production for arable land. To replace energy crops with other biomass that would generate an equivalent biogas amount, research on available biomass biochemical methane potential (BMP) is needed.

Lignocellulosic biomass is considered as the most abundant renewable resource with the potential of making a substantial difference in the supply of biofuel [1]. Plant biomass is composed primarily of cellulose, hemicelluloses and lignin in varying amounts in the different parts of the plant and they are intimately associated to form the structural framework of the plant cell wall [2]. Latvia has high potential for lignocellulosic biomass usage for anaerobic fermentation. Approximately 550 thousand tons of straw (grain and rape) per year would be technically available for energy applications in Latvia [3]. Forests cover 3365.4 thousand hectares in Latvia corresponding to 52 % of the territory [4]. 5.38 million m3 of bark, wood chips and sawdust are produced yearly [5].

Due to the chemical and mechanical structure of lignocellulosic biomass (straw, saw dust etc.) it has substantially lower biogas yield per volatile solids in conventional anaerobic fermentation compared to starch, lipids or protein rich biomass. Different thermal, chemical and biological pretreatment methods have been studied to enhance anaerobic fermentation of lignocellulosic biomass and increase the gas yield [1; 2; 6-9].

Fungal pretreatment has been reported as an effective process to increase gas yields for anaerobic fermentation [1; 7; 10]. Different species of fungus and mushrooms produce a set of enzymes for lignocellulosic biomass brake down. Compared to the chemical and thermal process, fungal pretreatment has low energy or reagent requirements. Oyster mushroom can be grown easily and profitably on locally available lignocellulosic biomass with high yield potential [11].

## Materials and methods

## 1. Reactors

Two continuously stirred tank reactors with gas holders (BR-100, OPTILAS) were used in the study. The total volume of the reactors is 50 liters and the working volume 45 liters. Schematic diagram of the system is presented in Fig. 1. The reactors were operated at temperature  $37 \pm 1$  °C. The volume of the produced gas was measured by the water displacement method in the gas holder. The automatic control system releases and burns the collected biogas when the gas holder has reached its full capacity. One emptying cycle refers to 112 liters of biogas.



Fig. 1. Schematic diagram of bioreactor: 1 – reactor part; 2 – gas holder part; 3 – mixer motor;
4 – feeding pipe; 5 – mixer axis; 6 – heater; 7 – mixer blades; 8 – emptying valve; 9 – sample valves;
10 – digestate pipe; 11 – gas transportation pipe; 12 – gas valve controller; 13 – gas; 14 – gas holder stationary part; 15 – water; 16 – weight; 17 – gas holder moving part

#### 2. Sawdust pretreatment

Fine fraction (< 0.5 mm) of deciduous tree saw dust was collected from a sawmill in Riga, Latvia. Oyster mushroom was selected for fungal treatment as the Institute of Soil and Plant Sciences, Latvia University of Agriculture had experience with this species cultivation. Sawdust was further prepared for ouster mushroom cultivation as follows. Fresh sawdust was mixed with boiling water 1:1 by mass. When temperature of wet sawdust had decreased below 30 °C the mass was inoculated with mycelium of *Pleurotus ostreatus*, stacked in plastics bags and left in darkness for 20 days. On day 21 small holes were cut in plastic to allow fruiting bodies to form outside of the sacks. Luminescent light and air humidifier were switched on in the room for the next 3 months. Fruiting bodies were collected and weighed as they got ready. After 3 months active fruiting body formation ended and sawdust with ingrown mycelium was used for anaerobic fermentation. During the anaerobic fermentation experiment pretreated sawdust was kept in the refrigerator at 4 °C.

Table 1

Substrate	Total solids, g·kg <sup>-1</sup>	Volatile solids, g·kg <sup>-1</sup>
Pretreated saw dust	477	418
Plant biomass pellets	925	798

Characteristics of substrates used in this study

# 3. Seed material

The seed material initially was obtained from cow manure and cultivated in 50 liter reactors for several months prior the experiments. Pelleted biomass mixture was used as substrate. The reactors were operated at 37 °C with stable pH 7.8  $\pm$  0.1 without pH control. The content of methane in biogas was 55 % at average.

### 4. Experiment methodology

Before start of the experiment one half of the process substrate was interchanged between the reactors to reach equilibrium of microorganisms.

During the study each reactor was fed with 880 g of tap water and 100 g of substrate – either biologically pretreated sawdust or pelleted grass biomass mixture per day. Feeding and digestate removal was done manually once per day. The volume of the produced gas as well as pH and ORP were measured daily. Temperature was set to  $37 \pm 1$  °C, stirring was performed periodically for 1 minute after each 30 min at 45 rpm.

The organic loading rate for grass biomass was 1.77 kg $\cdot$ m<sup>-3</sup>·day<sup>-1</sup>and 0.93 kg $\cdot$ m<sup>-3</sup>·day<sup>-1</sup> for sawdust.

#### 5. Analytical methods

The gas composition was determined by the gas analyzer (Gasboard-3200L, Wuhan Cubic Optoelectronics).

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

The level of pH was monitored by pH meter (PH-208, Lutron; electrode PE-05T Lutron). The oxidation-reduction potential (ORP) was measured by a multi-meter (Create ORP 651, electrode Create CT 201).

#### **Results and discussion**

100 0

The experiment was carried out for 71 days during which no major failure or biogas production problems were detected. During literature study no similar experiments with continuous pilot scale fungal- pretreated saw dust were found what increases the value of the positive data acquired.

Biogas production in both reactors gradually increased and stabilized at approximately day 50 as can be concluded from Fig. 2. Volumetric biogas production rate after day 50 reached 0.82  $\pm 0.01 \ 1^{-1} \cdot day^{-1}$  for grass biomass and  $0.51 \pm 0.02 \ 1^{-1} \cdot day^{-1}$  for sawdust.



![](_page_2_Figure_11.jpeg)

80

Fig. 3. Biogas yield per kg of added biomass volatile solids

In Fig. 3 biogas production data are expressed to the volatile solids being fed in reactors. After day 50 the biogas yield from grass pellets was 512  $\pm$ 7 l·kg<sup>-1</sup><sub>VS</sub> biomass and 610  $\pm$  23 l·kg<sup>-1</sup><sub>VS</sub> from

sawdust. The methane content in biogas was  $55 \pm 2\%$  and  $55 \pm 5\%$  for plant biomass and pretreated sawdust respectively. Biochemical methane potential for the same sawdust without treatment was  $252 \pm 9.1 \text{ kg}_{\text{VS}}^{-1}$  for 30 days (unpublished data). Zhong et al. reported biogas yield 319  $1 \text{ kg}^{-1}_{\text{VS}}$  from corn stalk biomass pretreated by fungus *Pleurotus florida* for 60 days. This indicates that the results obtained in this experiment are in line with our previous research on lignocelullosic biomass fermentation as well as the cresults from other researchers.

As can be seen in Fig. 4 pH in sawdust fermentation decreased during the study till 7.3. At the same time pH for grass pellet fermentation did not change substantially and was at average 7.8.

![](_page_3_Figure_4.jpeg)

Fig. 4. pH in reactors

As represented in Fig. 5 ORP in sawdust fermentation increased during the study till  $-380 \pm 21$ . At the same time ORP for grass pellet fermentation did not change substantially and was at average  $-464 \pm 7$ . Lowering of pH and rise of ORP represents either accumulation of organic acids or reduction of ammonia produced (amonia makes process substrate more alkali). As plant biomass pellets have relatively high percentage of proteins ammonia production needs to be taken into account and controlled during further studies.

![](_page_3_Figure_7.jpeg)

Fig. 5. ORP in reactors

As it was the first approach to use fungi pretreated sawdust for continuous biogas production only limited yet promising data have been acquired. Further study with untreated sawdust control, macro element monitoring and longer duration of experiment would be necessary to draw substantiated conclusions on the rate of effectiveness and economy of such method. At the same time oyster mushroom growers can use the presented data to calculate the potential value of their used sawdust to be used as substrate for biogas production.

## Conclusion

Continuous pilot scale fungal-pretreated saw dust anaerobic fermentation has been successfully demonstrated. It can be concluded:

- 1. Pleurotus ostreatus pretreated saw dust is perspective substrate for biogas production with sufficient biogas potential;
- 2. Further experiments with controlled mass-nutrient balance need to be carried out to calculate the effectiveness of fungal pretreatment.

## Acknowledgements

This study was supported by ERDF project "New technology for bio-hydrogen production and separation in anaerobic digestion process", Contract Nr: 2010/0326/2DP/2.1.1.1/10/APIA/VIAA/132.

# References

- 1. Zhong W., Zhang Z., Qiao W., Fu P., Liu M. Comparison of chemical and biological pretreatment of corn straw for biogas production by anaerobic digestion. Renewable Energy 36, 2011, pp. 1875-1879.
- 2. Jorgensen H., Kristensen J.B., Felby C. Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities. Biofuels, Bioproducts and Biorefining 1, 2007, pp. 119-134.
- Adamovičs A., Dubrovskis V., Plūme I., Jansons Ā., Lazdiņa D., Lazdiņš A., Kārkliņš G. 2009. Biomasas izmantošanas ilgtspējības kritēriju pielietošana un pasākumu izstrāde. Valsts SIA Vides projekti. Rīga. (in Latvian).
- 4. United Nations Food and Agriculture Organization statistics website. [online] [09.03.2013]. Available at:http://faostat.fao.org/site/377/DesktopDefault.aspx?PageID=377#ancor.
- Koksnes biomasas izmantošana enerģijas ieguvē Attīstības tendenču un iespēju novērtējums (in Latvian). [online] [09.03.2013.]. Available at:
  - http://www.zm.gov.lv/doc\_upl/2\_pielikums\_prezentacija.pdf
- 6. Cheng J., Su H., Zhou J., Song W., Cen K. Microwave-assisted alkali pretreatment of rice straw to promote enzymatic hydrolysis and hydrogen production in dark- and photo-fermentation. International Journal of Hydrogen Energy 36, 2011, pp. 2093-2101.
- 7. Cheng X.Y. and Liu C.Z. Fungal pretreatment enhances hydrogen production via thermophilic fermentation of cornstalk. Applied Energy 91, 2012, pp. 1-6.
- 8. Jurcoane S., Radoi-Matei F., Toma R., Stelian P., Vintiloiu A., Diguta C. Hydrolysis of agricultural biomass by combined pretreatment and enzymatic methods in order to produce biofuels (ethanol, biogas). Zootehnie si Biotehnologii, 2009, pp. 58-63.
- 9. Zhoa R., Zhang Z., Zhang R., Li M., Lei Z., Utsumi M., Sugiura N. Methane production from rice straw pretreated by a mixture of acetic–propionic acid. Bioresource Technology 101, 2010, pp. 990-994.
- 10. Jayachandra T., Venugopal C., Anu Appaiah K.A. Utilization of phytotoxic agro waste— Coffee cherry husk through pretreatment by the ascomycetes fungi Mycotypha for biomethanation. Energy for Sustainable Development 15, 2011, pp. 104-108.
- 11. Basak M.K., Chanda S., Bhaduri S.K., Mondal S.B., Nandi, R. Recycling of jute waste for edible mushroom production. Industrial Crops and Products 5, 1996, pp. 173-176.