University of Sopron

Theses of PhD dissertation

Study of anaerobic fermentation of lignocelluloses

Makk Ádám Nándor

Sopron

2017

University of Sopron
Faculty of Forestry
Kitaibel Pál Doctoral School of Environmental Science
Program of Bioenvironmental Science

Supervisors:
Dr. habil. Rétfalvi Tamás
Dr. habil. Hofmann Tamás

INTRODUCTION

Nowadays the most promising research fields are the investigations of the renewable energies. The consumption of biomass in Hungary was limited to the production heat. The disadvantage of this on the one hand is the poor efficiency of the converted electricity, on the other hand the deficiency of the storage. The anaerobic fermentation of lignocelluloses (being the largest amount of biomass) is an alternative possibility, but the industrial application involves several questions to answer.

The present dissertation focused on the opportunities to produce biogas from forestry wastes. Woody waste biomass has been less explored than other biomass types in the literature, so the knowledge is poor.

Fermentation of typical Hungarian wood industrial by-products was investigated, involving the wood of elderberry and the bark of oak. The recovered biogas yields were tested under thermophilic conditions and compared with literature data. Biogas yield enhancement was carried out with physical, chemical and biological pretreatment methods. The using of different rot-fungi as biological pretreatments was found to be the most efficient method in several aspects. In order to evidence that the increased methane yield was the reason of rot-fungi degradation, more examinations were carried out on the pretreated wood samples.

1. RESEARCH GOALS

- **Examination of sample pretreatment possibilities to find the optimal method.**

The fermentation of lignocelluloses and the slow hydrolysis process of macro components are closely related. The pretreatment methods were compared to redound the digestion of the lignocellulosic systems thereby enhanced the efficiency of enzymes. Industrial application and cost-effectiveness are important factors in the comparison.

- **Testing the biogas production of pretreated samples**

The anaerobic fermentation of samples was tested with different pretreatments. The efficiency of different pretreatments were compared with biogas yields. Respecting efficiency and biogas production, the main goal was to select the most suitable method.

- **Investigation of the pretreated wood with the optimum method**

After selecting the optimum process for the pretreatment, it was necessary to accomplish different examinations to understand the key of efficiency. In order to demonstrate that the biogas yield increased as an effect of the pretreatment, the structure and chemical composition of the wood, as well as the composition of biogas and biogas sludge were investigated.

2. MATERIALS AND METHODS

Important aspect for the choice of the substrates, used for experiments was that they should be industrially valueless or by-products of processing: the bark of oak (*Quercus petraea* (Matt.) Liebl.) is a waste product and the wood of elderberry (*Sambucus nigra* L.) is an industrially valueless wood. Furthermore the wood of elderberry represents a mixed lignocellulosic by-product because of the average chemical composition.

After sample collection, samples were immediately processed, ground and sieved (mesh size < 4 mm). Prepared samples were stored in freezer until use.

2.1. PRETREATMENT METHODS

The close bonds between lignocellulosic polymers hinder the hydrolysis of polysaccharides during fermentation. The following pretreatment methods were applied to facilitate this process.

Dilute acid pretreatment: Dilute acid pretreatment of elderberry wood was done using 4% acetic acid. The mass ratio of wood: acid was 1:3 so 100 g dried wood was mixed with 300 g of acetic acid. Mixtures were left at room temperature for 24 hours (Phaiboonsilpa és Saka 2011).

Extraction pretreatment: The goal of the oak bark extraction pretreatment was to dissolve the inhibitory components. 100 g of dried oak bark was mixed with 800 ml distilled water. Mixtures were left at room temperature for 24 hours (Makk *et al.* 2013).

Microwave pretreatment: The microwave pretreatment was applied to wood of elderberry and oak bark too. 250 g of each sample was pretreated with a household microwave oven with 700 Watt microwave energy for 2 x 2 minutes (Jackowiak *et al.* 2011/a, Makk *et al.* 2013). The substrates were stored in freezer until final utilization.

Hydrothermal pretreatment: The hydrothermal pretreatment is a heat treatment in distilled water with microwave energy. The wood of elderberry was treated with Michem MD6 device for 2 x 2 minutes. The mass rate of the wood:distilled water is 1:3, 100 g of dried wood was mixed with 300 g of distilled water. The samples were stored in freezer until utilization.

Physical and chemical pretreatment*:* Wood of elderberry was treated physical- and chemical method in a same time with Michem MD6 device. The samples were pretreated with 700 Watt microwave energy at 150°C and at 210°C for 2 x 2 minutes. The ground samples were treated with constant microwave energy, different temperatures and in 2% and 4% acetic acid. The ratio of the wood samples:acid was 1:3 so to 100 g dried wood was added to 300 g acetic acid.

Biological pretreatment: Wood of elderberry was treated with two different rot-fungi species. One was the brown-rot cellar fungus (*Coniophora Puteana* (Schumach.) P. Karst) and the another was the white-rot oyster mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm.). Agar/agar-malt was the growth medium in a 20 x 20 cm thermostable box. Different times (14, 21, 28 days) were applied during the pretreatment period. The combination of the two rot-fungi species were also used in the experiments. 150-200 g of each samples and the thermostable boxes were sterilized with 120 °C stream for 20 minutes. The samples were stored in dark climate chamber at room temperature during the pretreatment periods.

2.2. INVESTIGATION OF WOOD PRETREATED WITH ROT-FUNGI

The following tests were performed to demonstrate that the different changes of elderberry wood are the reason of the rot-fungal degradation.

Determination of total organic matter content: Measurements of the total organic matter content were started with the milled wood samples accurate analytical mass measurement and after that were incinerated at 600 °C. After cooling in the desiccator from the remained inorganic matter mass the total organic matter content was calculated.

Determination of cellulose content: Cellulose content was measured with Kürschner-Hoffer method. (Kürschner and Hoffer 1931).

Determination of total soluble carbohydrate content: Extraction: to 0.25 g milled dried wood was mixed with 25 ml 4:1 - methanol:water and stirred with a magnetic stirrer for 6 hours (Visiné Rajczi 2008). The extracts were filtered using a Whatman G2 glass fiber syringe filter.

Total soluble carbohydrate contents were measured with Dubois method (Dubois *et al.* 1956).

Determination of total phenolic content: Extracts were prepared with the same technique as in case of total soluble carbohydrate content. The total phenolic contents were measured with Folin-Ciocalteu method (Singleton and Rossi 1965).

Measurements with HPLC-MS/MS: To 0.75 g of dried, rot-fungal pretreated elderberry wood samples were added 25 ml water and stirred with a magnetic device for 6 hours. The extracts were filtered with 0.45 µm porosity cellulose acetate filter.

The three parallel HPLC-MS/MS measurements in each sample were done using a Shimadzu LC-20 liquid chromatograph and an AB Sciex 3200 Qtrap linear ion trap/triple quadrupole mass spectrometer.

Electron microscopic images: The electron microscopic images were made with Hitachi S-3400N scanning electron microscope at 25 kV acceleration voltage, 70 kPa pressure, 200 times magnification, and in BSE (Back Scattering Electron) mode.

Determination of dry matter content: After the analytical precision weighing the milled wood of elderberry samples were dried at 105°C to constant weight. The samples were cooled in desiccator and then from the weighing of dry wood mass the dry matter contents were calculated. Three measurements were prepared in each sample.

2.3. BIOGAS PRODUCTION AND MEASUREMENT OF BIOGAS YIELD

Biogas production: The production of biogas was carried out in a 2500 ml volume brown bottle with thread neck. Biogas sludge occupied about 1000 ml from the total volume of the bottle. Graft material for the fermentation experiments was obtained from the biogas plant of the Magyar Cukor Zrt., Kaposvár (Hungary) and was specialized to the fermentation of plant biomass. The anaerobic digestion experiments were run for 30-60 days, in semi-continuous flow and thermophilic (55ºC) environment. Produced gas was collected into Tedlar® bags. Measurement of the gas volumes was carried out using a 500 ml Hamilton syringe. The temperature was maintained with a Memmert WNB 14 Basic water bath.

Determination of biogas composition: The composition of the biogas was monitored using Ecoprobe 5-IR type equipment calibrated for CH_4, CO_2, O_2 compounds. Calibration gas mixture comprised of 60% methane (v/v), 30% CO_2 (v/v) and 10 % O_2 (v/v) and had a purity of 99.995 % (v/v).

Genomics investigations: The genomic compounds of different sludges were discovered with metagenomics sequencing and bioinformatical analysis.

Monitoring of biogas sludge: The chemical parameters (pH, COD, total phosphorus, ammonium) were monitored with standard methods (Rétfalvi *et al*. 2016).

3. THESES OF THE PHD DISSERTATION

1. Results of the investigations were demonstrated that the forestry lignocelluloses such as the oak bark (*Quercus petraea* (Matt.) Liebl.) and the wood of elderberry (*Sambucus nigra* L.) adaptable to biogas production in thermophilic and anaerobic environments. The specific methane yields of these substrates were 20-35% of the yields gained from most commonly used agricultural by-products.

2. The semi-continuous, thermophilic and anaerobic laboratory fermentation results proved that the specific methane yield of the milled oak bark (*Quercus petraea* (Matt.) Liebl.) (67 ml/ g dry volatile solid) decreased by 20% with physical (microwave) pretreatment. Chemical (aqueous extraction) and combined (microwave and extraction) pretreatments did not affect specific methane yields significantly.

3. The semi-continuous, thermophilic and anaerobic laboratory fermentation of elderberry (*Sambucus nigra* L.) wood resulted a relatively low specific methane yield (87 ml/ g dry volatile solid) which increased by the applied chemical, physical, physical-chemical and biological pretreatment methods. The most favourable effects were found using the hydrothermal (38% increase) and biological (30% increase) pretreatments.

4. The semi-continuous, thermophilic and anaerobic laboratory fermentations of the wood of elderberry (*Sambucus nigra* L.) testified that the biological pretreatments with white rot- (*Pleurotus ostreatus* (Jacq.) P. Kumm.) and brown rot-fungi (*Coniophora Puteana* (Schumach.) P. Karst) increased the specific methane yield significantly. The duration of the pretreatment had significant effect on the methane yield. The most effective (30% increase) pretreatment was evidenced by applying 28 days. The period of the pretreatment was shortened by 7 days with the simultaneous application of both types of rot-fungi.

4. SUGGESTIONS, FUTURE AIMS

Laboratory fermentation experiments demonstrated that the wood of elderberry and oak bark were adaptable for biogas production and the methane yield was increased with the appropriate pretreatment methods. This dissertation represents a significant basis for the applied materials or other forestry and wood industrially by-products to biogas utilization even for an industrial production. While the research goals were answered several new questions and goals were raised, which require further research in the future.

Future research possibility:

- Application of other wood species (oak, black locust, pine, poplar) for biogas production.
- Application of other rot-fungi species for biological pretreatment.
- Examination of oak bark biological pretreatment.
- Testing of high volumes for industrial application.
- Technological development for industrial implementation.

5. PUBLICATIONS

5.1. PUBLICATIONS CONNECTED TO THE DISSERTATION

Article:

Makk, Á.N., Rétfalvi, T., Hofmann, T. (2017) *Utilization of oak bark (Quercus petraea (Matt.) Liebl.) for anaerobic digested biogas production.* Acta Silvatica & Lignaria Hungarica, (Vol. 13 (2), in press)

Proceedings:

Makk, Á., Bak, M., Hofmann T., Németh, R., Rétfalvi T. (2014) *Effect of fungal pretreatment on anaerob fermentation of bourtree (Sambucus Nigra).* Eco-efficient Resource Wood with special focus on hardwoods: IAWS Plenary Meeting 2014.09.15-18- Sopron (Hungary) In: Róbert Németh, Alfred Teischinger, Uwe Schmitt (szerk.), (ISBN:978-963-334-191-9), pp. 23-24.

Makk, Á., Rétfalvi, T., Hofmann, T., Németh, R., Bak. M. (2014) *Fekete bodza (Sambucus nigra L.) faanyag fehér- (Pleurotus ostreatus L.) és vöröskorhasztó (Coniophora puteana L.) gombákkal való kezelése*. Tavaszi Szél Konferencia, Debrecen 2014. 03.21-23, (ISBN: 978-963-89560-9-5), pp. 289-299

Makk, Á.N., Rétfalvi, T., Hofmann, T., Farkas, B. (2013) *Lignocellulózok anaerob fermentációja - Az előkezelés hatása*. Kémia, környezettudomány, fenntarthatóság: Kémiai Intézet Tudományos Ülése, 2013.08.29. Sopron, In: Albert L, Szabó P (szerk.) (ISBN:978-963-334-147-6), pp. 71-76

Conference and poster presentations:

Makk, Á., Bak, M., Hofmann T., Németh R., Rétfalvi T. (2014) *Effect of fungal pretreatment on anaerob fermentation of bourtree* (*Sambucus nigra* L.). Poszter előadás. Eco-efficient Resource Wood with special focus on hardwoods: IAWS Plenary Meeting 2014, Bécs, Sopron, 2014. szeptember 15-.18.

Makk Á, Rétfalvi T, Hofmann T, Németh R, Bak M (2014) *Fekete bodza (Sambucus nigra L.) faanyag fehér- (Pleurotus ostreatus L.) és vöröskorhasztó (Coniophora puteana L.) gombákkal való kezelése*. Konferencia előadás. Tavaszi Szél Konferencia, Debrecen, 2014. március 21-23.

Makk, Á.N., Rétfalvi, T., Hofmann, T., Farkas, B., (2013) *Lignocellulózok anaerob fermentációja - Az előkezelés hatása.* Konferencia előadás. Kémia, környezettudomány, fenntarthatóság: Kémiai Intézet Tudományos Ülése, Sopron, 2013. augusztus 29.

Makk, Á., Hofmann, T., Rétfalvi, T. (2012) *Lignocellulóz rendszerek előkezelési lehetőségei az anaerob fermentáció elősegítésére*. Konferencia előadás. MTA, Természetes Polimerek Munkabizottsági ülés, Sopron 2012.05.22.

5.2. OTHER PUBLICATIONS

Article:

Makk Á.N., Hofmann T., Rétfalvi T. (2013) *A (+)-catechin kinyerése tölgyek kérgéből.* Faipar, 61 (2), 16-26

Conference and poster presentations:

Hofmann, T., Makk, Á.N., Rétfalvi T., Albert, L. (2009) *Antioxidáns polifenolok kinyerése tölgyek kérgéből*. Poszter előadás. IX. Környezetvédelmi analitikai és technológiai konferencia, Környezetvédelem és élelmiszerminőség a III. évezredben, Sopron, 2009. október 7-9.

Albert, L., Hofmann, T,, Rajczi E., Csepregi, I., Makk, Á. (2007) *Polifenolok kinyerése: Extrakciós eljárások hatékonyságának vizsgálata.* Poszter előadás. Nyugat-magyarországi Egyetem, Erdőmérnöki Kar Konferencia, Sopron, 2007

6. REFERENCES

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F. (1956) *Colorimetric method for determination of sugars and related substances.* Anal. Chem., 28, 350- 356

Jackowiak, D., Bassard, D., Pauss, A., Ribeiro, T. (2011) *Optimisation of a microwave pretreatment of wheat straw for methane production.* Bioresource Technol. 102, 6750–6756

Kürschner, K., Hoffer, A. (1931) *Eine neue quantitative Cellulosebestimmung.* Chem. Ztg. 17, 161-168

Makk, Á.N., Hofmann, T., Rétfalvi, T. (2013) *A (+)-catechin kinyerése tölgyek kérgéből*. Faipar, 61, 2, 16-26

Phaiboonsilpa, N., Saka, S. (2011) *Effect of acetic acid addition on chemical conversion of woods as treated by semi-flow hot-compressed water*. Holzforschung 65, 667-672

Rétfalvi, T., Szabó, P., Tukacs-Hájos, A., Albert, L., Kovács, A., Milics, G., Neményi, M., Lakatos, E., Ördög, V. (2016) *Effect of co-substrate feeding on methane yield of anaerobic digestion of Chlorella vulgaris.* J. Appl. Phycol. 28(5), 2741–2752

Singleton, V.L., Rossi, J.A. (1965) *Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents.* Am. J. Enol. Vitic. 16, 144–158

Visiné Rajczi, E. (2008) *Bükk (Fagus sylvatica L.) extraktanyagok képződése akkumulációja és megoszlása.* PhD disszertáció, Nyugat-Magyarországi Egyetem