BIOGAS PRODUCTION FROM AGROINDUSTRIAL WASTE PRE-TREATED WITH LIGNOLYTIC FUNGI

Csilla Szűcs[1], Etelka Kovács[1], Zoltán Bagi[1], Gábor Rákhely[1,2,3], Kornél L. Kovács[1,4,5]

[1]Department of Biotechnology, University of Szeged, Szeged, Hungary
[2]Institute of Environmental and Technological Sciences, University of Szeged, Hungary
[3]Institute of Biophysics, BRC, Szeged, Hungary
[4]Department of Oral Biology and Experimental Dentistry, University of Szeged, Szeged, Hungary
[5]Hungarian Biogas Association

Abstract

Renewable energy was never more important than in a century when energy consumption is unprecedented. Biogas is considered to be one of the most important natural energy sources. We aim to enhance biogas production through the pre-treatment of substrates, that are normally hard to digest because of their high content of cellulose, hemi- and lignocellulose. As part of fungal pre-treatment we used *Aspergillus nidulans* and *Trichoderma reesei.* Both of these filamentous fungi are well known for their ability to synthetise various enzymes – including cellulases. During our experiments *A. nidulans* and *T. reesei* filamentous fungi's endoglucanase activities were measured by spectrophotometer and methane-producing was monitorized by gas chromatography.

Introduction

Our society's hunger for energy is growing rapidly. Solving the situation should include environmentally friendly energy sources, such as plant biomass, which is produced in large amounts every day – in both natural and artificial ways. [1] Agroindustry generates unbelievable quantities of corn stalk and wheat straw each year, and they cannot be used in further processes. Organic waste and by-products are processed and valuable energy (biogas) is gained by anaerobic digestion. [2] Filamentous fungi are known as inhabitant of lignocellulose-rich plant tissues and also as unique enzyme producers. [3,4] Combining these two properties suggests that filamentous fungi can successfully enhance biogas production.

Experimental

Filamentous fungi *A. nidulans* was isolated from cattle rumen previously. *T. reesei* was used from our strain collection. (Department of Biotechnology, University of Szeged, Szeged, Hungary) The fungal colonies were maintained on Czapek-Dox-agar plates. [5] Plates contained 3 g/l $NaNO_3$, 1 g/l K_2HPO_4, 0.5 g/l $MgSO_4$, 0.5 g/l KCl, 0.5 g/l $FeSO_4$, 15 g/l agar and either corn stalk or wheat straw as source of carbohydrate. Corn stalk and wheat straw were inoculated with the filamentous fungi as pre-treatment. The steriled substrates were added 20 ml of sterile distilled water and 10^7 spores of either *A. nidulans* or *T. reesei* in the different samples.

Endo-(1,4)-β-D-glucanase activity was measured in every three day during the pre-treatment using 3,5-dinitrosalicylic acid (DNSA) method [6]. The absorbance of the samples were measured spectrophotometrically at 550 nm using a GENESYS UV-Visible scanning Spectrophotometer (ThermoFisher Scientific, Wilmington, DE, USA). After 10 days of fungal pre-treatment the samples were inoculated with sludge. (Zöldforrás Biogas Plant, Szeged, Hungary)

The negative controls contained only sludge, the positive controls contained only α-cellulose as substrate. All anaerobic digestion experiments were carried out under mesophilic conditions. Methane-concentration of the produced biogas was measured daily via gas chromatography (Agilent 6890N Gas Chromatograph, Agilent Technologies, Santa Clara, CA, USA).

Results and discussion

Scanning electron microscopy pictures (SEM) were taken of the plant tissues during pre-treatment. *A. nidulans* filamentous fungus obviously inhabited the substrate wheat straw (Fig. 1-2.).

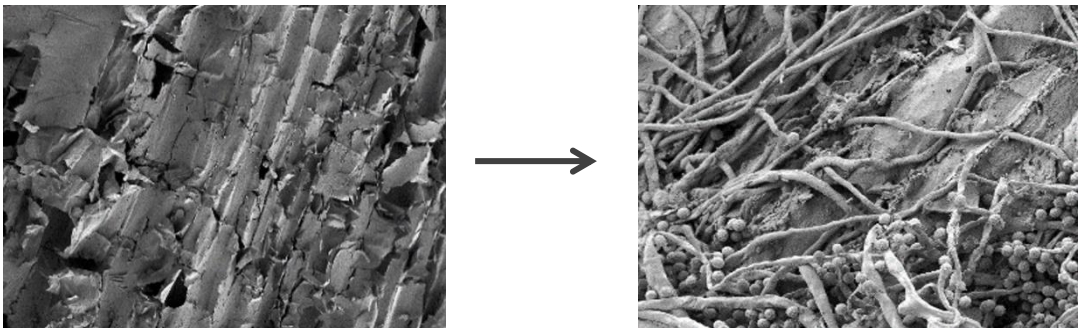

Figure 1. and 2.

Endoglucanase activity

It was observed that endo-(1,4)-β-D-glucanase activity depends mostly on the applied substrate and fungus. As it was expected *A. nidulans* proved to be a stunning enzyme producer. It preferred corn stalk as a substrate rather than wheat straw. Figure 3. shows that endoglucanase activity was outstanding when corn stalk was applied as a source of carbohydrate for *A. nidulans.*

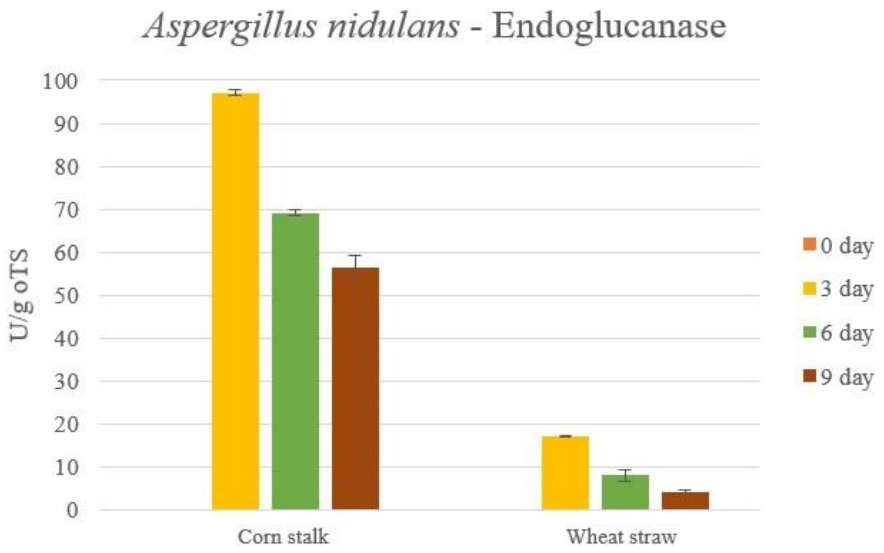

Figure 3.

The endoglucanase activity of *T. reesei* was lower on both substrates, however this fungus was also more successful on corn stalk. (Fig. 4.) A descending activity was observed in every sample, which refers to the diminishing of nutrients in the solutions.

Methane production

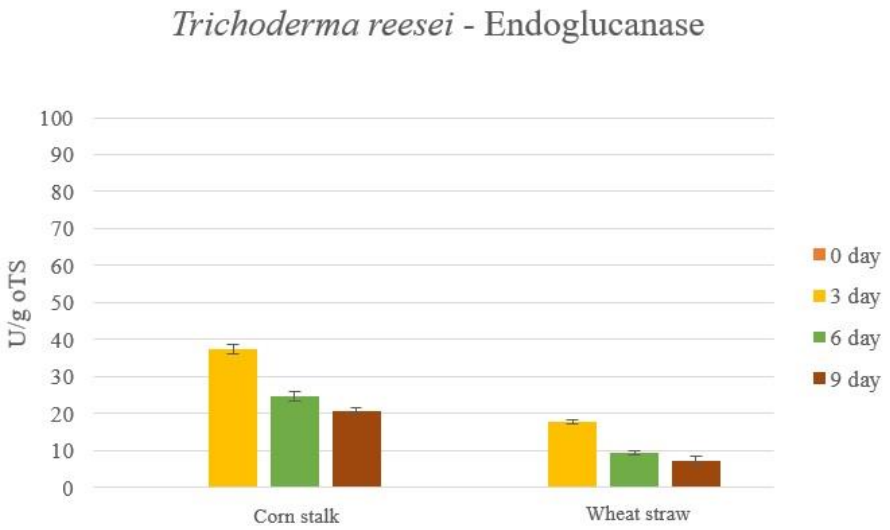

Figure 4.

On Fig. 5. it is shown that fungal pretreatment increased methane yield. The results of methane production is expressed as percentage of the non pre-treated substrates. As it was expected after the enzyme activity assays, corn stalk combined with *A. nidulans* had the highest methane yield, pre-treatment of substrate with *T. reesei* also enhanced biogas production.

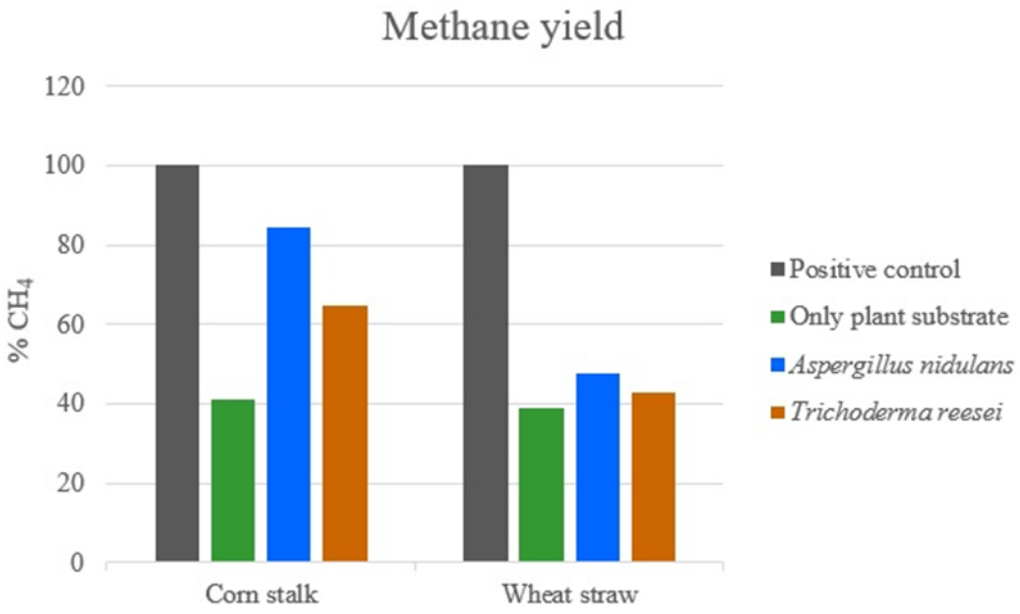

Figure 5.

Conclusion

As demonstrated above both *A. nidulans* and *T. reesei* have significant enzyme producing capability in cellulose-rich environment. Therefore, it is understandable that pre-treatment of plant substrate before anaerobic digestion positively affected biogas production. Furthermore, it is not only beneficial as renewable energy source, but with this method we could decrease the amount of organic waste in a more profitable way.

Acknowledgements

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