IMPROVING METHANE PRODUCTION FROM LIGNOCELLULOSIC BIOMASS PRE-TREATED WITH ANAEROBIC FUNGI

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Abstract
Two anaerobic fungi (AF) strains, isolated from faeces of an elephant and of a sheep, were assessed for their ability to degrade lignocellulosic biomass. The effects on biogas production of anaerobic fungi from both animal species were determined in two step batch experiments. The hydrolysis process during the AF incubation led to an initial increase of biogas production, an accelerated degradation of dry matter and an increased concentration of volatile fatty acids. Thus, a separate hydrolytic pre-treatment phase with anaerobic fungi, represents a feasible strategy to improve biogas production from lignocellulosic substrates.

Introduction
Degradation of lignocellulose-rich material into biogas is an attractive strategy to face growing energy demands and moderate greenhouse gas emissions from the exploitation of fossil energy resources. Lignocellulosic residues (e.g. crop residues, green waste, mill waste) are highly frequent (1), they are easily accessible, cheap and do not require additional land to grow on in this way do not trigger “food or fuel” conflicts. This biomass is composed of interwoven cellulose and hemicellulose, coated by recalcitrant lignin (2). This is the explanation why bacteria and archaea in the biogas reactor are not efficient in disintegration of the lignin, leaving a considerable portion of the more easily convertible sugars untouched. Current strategies to release this carbon rely on expensive enzyme cocktails and physicochemical pre-treatment, producing inhibitory compounds that hinder subsequent microbial bioproduction. Microbial pre-treatment utilizing the fibre degrading potentials of aerobic fungi may be a much cheaper alternative but there are some drawbacks e.g. loss of carbohydrates by respiration and biomass build-up and the requirement of long pre-treatment periods (3). Anaerobic fungi (AF) from the phylum Neocallimastigomycota are natural inhabitants of the digestive tract of herbivorous animals (4), which decompose a big share of the ingested forage. The AF attach to the plant material and crack the fibres mechanically by growth and expansion of their rhizoids or bulbous holdfasts (5). In addition, AF possess cellulosomes which contain a multitude of lignocellulolytic enzymes. These fungi are an appealing solution as they hydrolyze crude, untreated biomass at ambient conditions into sugars that can be converted into value-added products by partner organisms.

Experimental
The objective of this study was the application of two newly isolated strains to the hydrolysis phase in order to improve hydrolysis of lignocellulosic biomass. The applied isolates were obtained from animals living on a high fibre diet, namely sheep (*Ovis aries*) (Fig.1.) and Asian elephant (*Elephas maximus*). The effects on biogas production of anaerobic fungi from both

animal species were assessed in two step batch experiments, comprised by a hydrolytic/acidogenic stage, followed by a methane production stage. Checking the enzyme activity in hydrolytic stage, beta-glucosidase activity was measured by p-nitrophenyl-β-D-glucopyranoside, for the endoglucanase concentration DNSA-method was used (3,5-dinitrosalicylic acid). The produced organic acids were measured by HPLC. Additionally, gas composition was analysed by GC during the methane production stage.

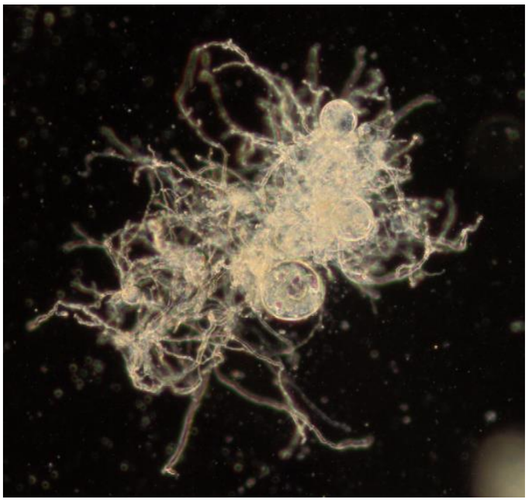

Figure 1. Anaerobic fungi on soluble carbon substrate from sheep fecal sample.

Results and discussion

In this study, treatment with anaerobic fungi cultures increased the total biomethane yield during the experimental period of 20 days. After the pretreatment the medium was taken apart into liquid and solid phases, which were treated separately. Columns show the difference between the pretreated and non-treated data (Fig. 2.).

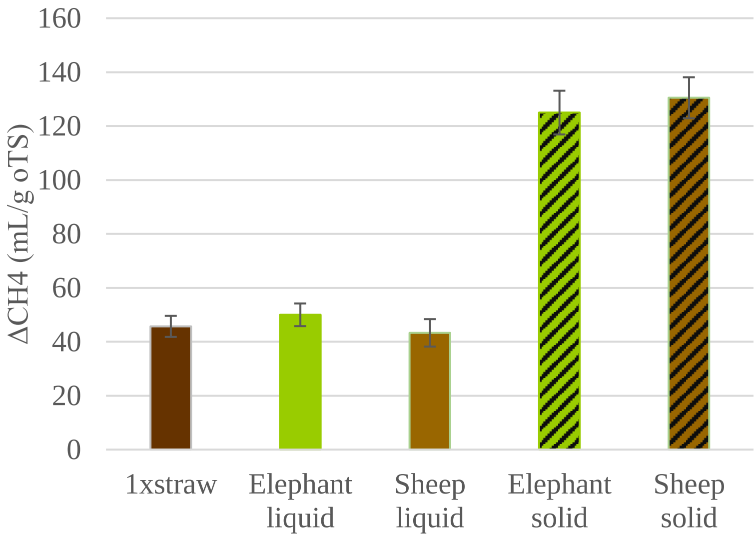

Figure 2. Biomethane yield after the anaerobic fungi pre-treatment.

Pretreatment with anaerobic fungi significantly improved the degradability of straw. This is evidenced by the fact that about 2.5 times more biomethane was produced from the elephant solid and sheep solid samples during the experiment than from the untreated straw (Fig. 2.). The results for the elephant fluid and sheep fluid samples correlated well with the organic acid concentrations measured by HPLC (Fig.4.) and the high endoglucanase and beta-glucosidase results measured during the tests (Fig. 3). From these it can be concluded that the anaerobic fungi degraded the substrate efficiently during the 15-day long treatment. As a result of the pre-treatment, acetic acid, lactic acid, glucose and cellobiose were produced in the highest amounts. These products could be used by methane-producing Archea producing methane during biogas fermentation. The efficiency of biogas fermentation was well characterized by the amount of biomethane produced as well as the concentrations of organic acid measured by HPLC (Fig.4.), which show that methanogens with otherwise slow metabolism were able to use the products of anaerobic fungi from the solution, so inhibition did not occur.

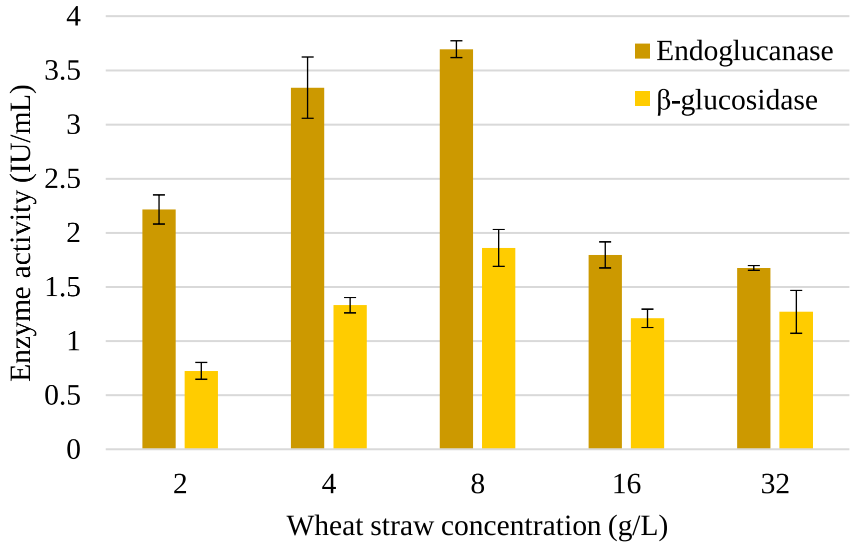

Figure 3. Enzyme activities during wheat straw fermentation using sheep-AF isolate.

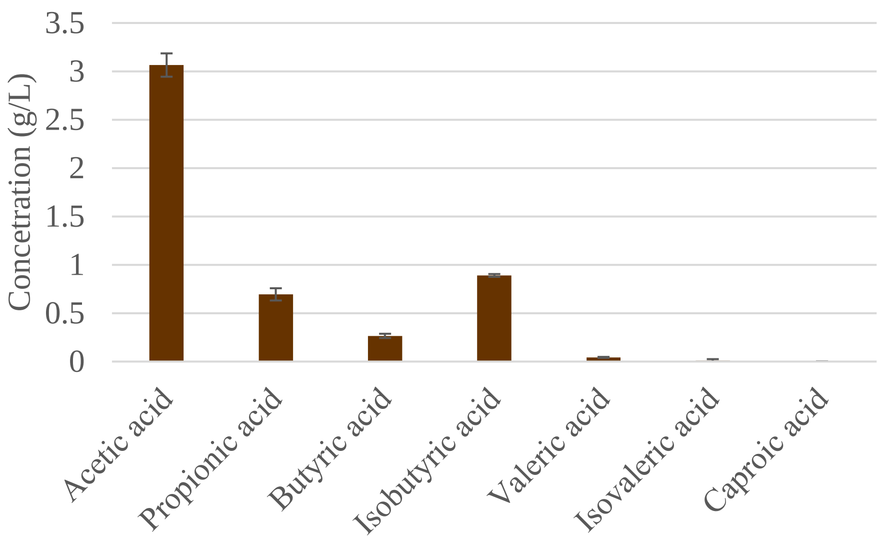

Figure 4. Organic acid concentration at the end of the biogas fermentation using anaerobic fungi from sheep.

Conclusion

Anaerobic fungi isolates from sheep and elephant are excellent candidates for the conversion of agricultural waste products to biofuels. The two tested isolates efficiently pretreated the hard-to-degrade straw substrate to produce significant amounts of acetic acid, lactic acid, glucose, and cellobiose. The kinetics of the degradation were optimal for the slow metabolism of methanogenic microbes, which were thus able to efficiently utilize the aforementioned by-products and make biomethane from them.
Based on these results AF isolates were effective in enhancing cellulose degradation and successfully increased biogas production.

Acknowledgements

This study has been supported by Hungarian NKFIH fund NKFI-PD 128345. We kindly thank the ZOO in Szeged and Körösvölgyi Állatpark for providing the needed feces for the isolation. We thank Prof. Michael Theodorou for introducing us into the cultivation secrets of anaerobic fungi.

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