HAPPY MARRIAGE ACROSS THE EVOLUTION: CO-OPERATION OF METHANOGENIC ARCHEA AND ANAEROBIC FUNGI

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Abstract

Four anaerobic fungi (AF) strains, isolated from faeces of anoa, giraffe, bison and moose, were assessed for their ability to degrade lignocellulosic biomass. The effects on biogas production of anaerobic fungi from these 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 four 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 anoa (*Bubalus depressicornis*) (Fig.1.) and giraffe (*Giraffa camelopardalis rothschildi*), bison (*Bos bonasus*) and moose *(Alces alces)*. The effects on biogas production of anaerobic fungi from these 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 anoa fecal sample.

Results and discussion

In this study, treatment with anaerobic fungi cultures (14-day) increased the total biomethane yield during the experimental period of 20 days.

Methane yield from the same amount of organic total solids (oTS) of black locust and fresh wheatgrass with and without AF+M co-culture pre-treatment was presented in Figure 2. Black locust is a lignin-rich substrate that is difficult for biogas fermenter microbes to degrade. As a result of the AF+M pretreatment, the degradability of black locust increased in the case of the use of both isolates. When 3g of substrate were used, the amount of methane did not increase proportionally, which may indicate inhibition.

In the case of using fresh wheatgrass, the AF+M pretreatment significantly increased the degradability of the substrate both when 0.5g and 1g of substrate were used, no inhibition occured (Fig. 2.).

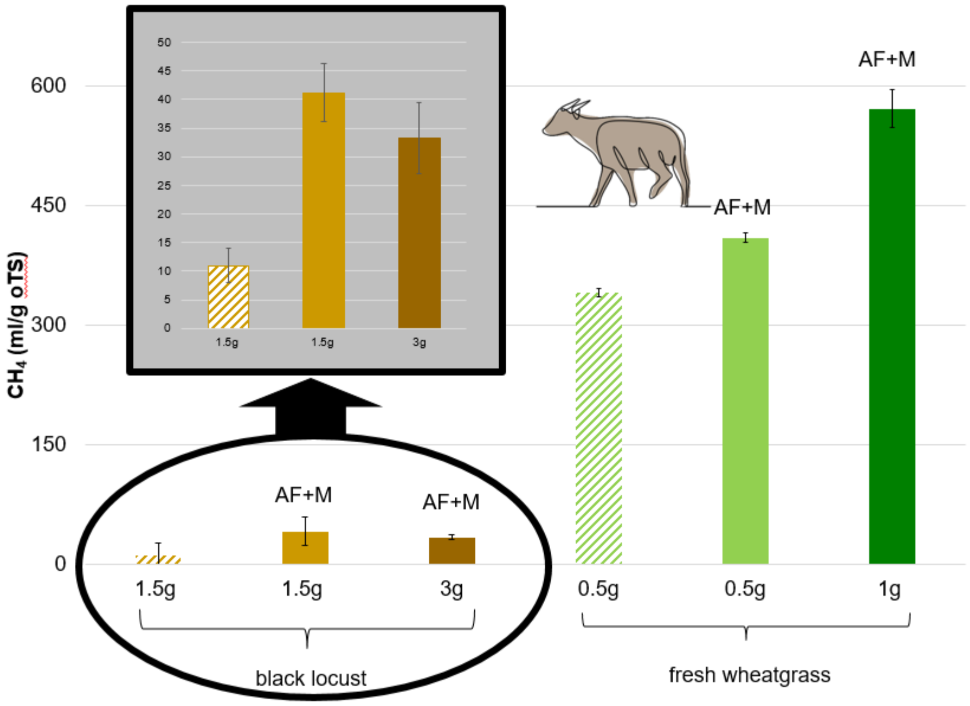

Figure 2. Biomethane yield after the anaerobic fungi pre-treatment using anoa isolate.

Pretreatment with anaerobic fungi significantly improved the degradability of substrates. The results for AF biomethane yields correlated well with the organic acid concentrations measured by HPLC (Fig.3.). From these it can be concluded that the anaerobic fungi degraded the substrate efficiently during the 14-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.3.), 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.

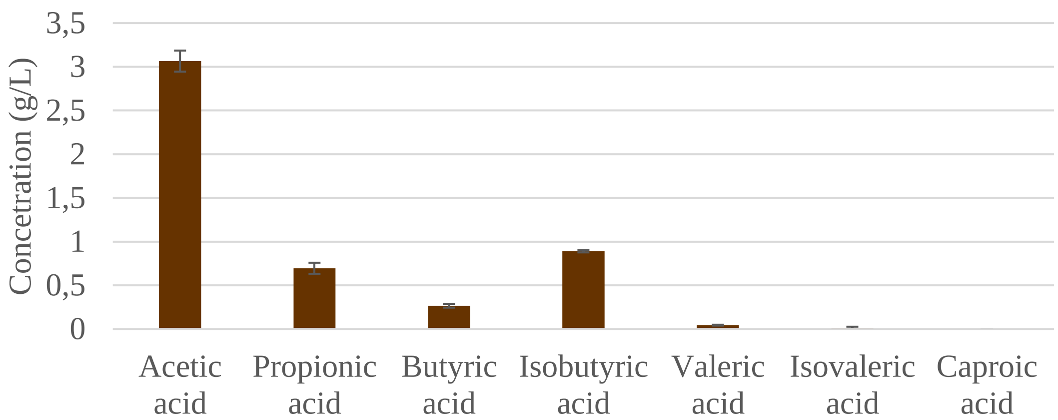

Figure 3. Organic acid concentration at the end of the biogas fermentation using anaerobic fungi from anoa.

Conclusion

Anaerobic fungi isolates from anoa, giraffe, bison and moose are excellent candidates for the conversion of agricultural waste products to biofuels. The four 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.

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References

1 Williams, L., Gallagher, J., Bryant, D., Ravella, S.R. 2016. Anaerobic digestion and the use of pre-treatments on lignocellulosic feedstocks to improve biogas production and process economics. Advances in biofeedstocks and biofuels, Volume One: Biofeedstocks and their processing, pp. 121.

2 Rodriguez, C., A. Alaswad, K.Y. Benyounis, A.G. Olabi 2017. Pretreatment techniques used in biogas production from grass. Renew. Sust. Energy Rev., 68 (Part 2) (2017), pp. 1193-1204.

3 Isroi, M., Ria, Syamsiah, S., Niklasson, C., Cahyanto, M.N., Ludquist, K., Taherzadeh, M.J., 2011. Biological pretreatment of lignocelluloses with white-rot fungi and its applications: a review. BioResources 6(4), 5224-5259.

4 Liggenstoffer, A.S., Youssef, N.H., Couger, M.B., Elshahed, M.S. 2010.Phylogenetic diversity and community structure of anaerobic gut fungi (phylum Neocallimastigomycota) in ruminant and non-ruminant herbivores. ISME J., 4 (10) (2010), pp. 1225-1235

5 Akin, D.E., Borneman, W.S. 1990. Role of rumen fungi in fiber degradation. J. Dairy Sci., 73 (10) (1990), pp. 3023-3032