Improved biogas production from lignocellulosic substrates using bioaugmentation and pretreatment

Ph.D. thesis summary

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Introduction

Modern society depends on energy to a great extent; therefore, energy crisis has serious effect on the economy. In recent years, energy-requirement has grown rapidly due to rising world population and affluence. The world's energy consumption has more than doubled between 1990 and 2016, i.e., from 10901.84 TWh to 23106.86 TWh (IEA – International Energy Agency World Energy Balances 2018 - https://webstore.iea.org/world-energy-balances-2018). More than 84% of the global energy demand is supplied by non-renewable fossil resources such as coal, oil and natural gas. These resources are not only limited in supply but also have adverse effects on the environment due to the emission of greenhouse gases into the atmosphere upon their excavation and utilization, leading to disastrous climate changes unless they are replaced soon with alternatives (Sawatdeenarunat et al., 2015). Large scale exploitation of nuclear and renewable energy does not contribute to global climate change. Although nuclear energy does not pollute the air, the fission fuel sources are limited and the safe disposal of radioactive waste is difficult to accomplish. Hence a worldwide interest has developed for new, clean and renewable energy supplies. Over the last decades, research efforts focused mainly on wind power, photovoltaics

(PV) and biomass, i.e., bioethanol and biodiesel production. The biomass-based technologies developed in this time period have yielded the first-generation biofuels, which are made from food crops such as maize, sugar cane, and palm oil. However, they have indirectly caused an increase in food prices and thus contributed to the global food crisis. Therefore, production of second generation biofuels by the conversion of whole plant biomass to biofuels, is essential to significantly increase the proportion of sustainable, renewable energy (Guo et al., 2010). Biogas, which is generated during the anaerobic decomposition (AD) of renewable raw organic materials, is one of the highly promising alternatives to fossil energy. When biogas is purified to biomethane, it can be fed into the natural gas grid, compressed or condensed biomethane can be used as fuel in every area where natural gas is used today (Holmes & Smith, 2016).

Lignocellulosic biomass, i.e., agricultural residues and energy plants, receive more attention as suitable materials to produce energy and various products recently. The chemical energy in biogas ultimately originates from the solar energy fixed during photosynthesis. Consequently, biogas can be considered carbon neutral. Lignocellulosic biomass contains cellulose, hemicellulose and lignin, these components are arranged in a very complex and recalcitrant structure. This assembly efficiently protects the plant cells but the hydrolysis of lignocellulose frequently becomes the rate limiting step in AD. During AD, a complex microbial community, including Bacteria, Fungi, Protozoa and Archaea, transforms organic matter to essentially a mixture of methane (CH_4) and carbon dioxide (CO_2) in several metabolic steps.

The general goal of my Thesis work was to study the AD of lignocellulose-rich biomass in details and to increase the efficacy of its conversion to biogas.

Methods

To achieve these goals, I adapted a thermophilic natural biogas producing consortium to cellulose substrate in 0.5 litre fed-batch reactors. The volume of the produced biogas was monitored with water displacement method and the methane content of the biogas was measured with gas chromatograph. The pH of the fermentation mixture was recorded periodically. β-glucosidase or exoglucanase enzyme activities were measured using spectrophotometric assays. Volatile fatty acid concentration changes were followed by high pressure liquid chromatography (HPLC).

I then employed the enriched cellulose degrading consortia in bioaugmentation experiments in AD of lignocellulose-rich agricultural by-product corn stover, which is generated in vast amounts every year. The composition of the enriched cellulose degrading mixed cultures were determined by next generation sequencing and metagenomic methods. The preservation stability of the adapted cellulolytic cultures in time was tested by repeated sequencing of community DNA. The bioaugmentation capacities of the enriched cellulose degrading communities were compared to that of the pure bacterial cultures identified as most abundant Bacteria members of the enriched communities. The biomethane potential of an additional agricultural waste, sunflower stalk, was determined beside corn stover, under mesophilic and thermophilic conditions. Finally, combining my biogas results with statistical and literature data on yearly biomass yields, the amount of conceivable biogas production from corn stover and sunflower stalk as renewable energy biomass resources were estimated for Hungary.

Results

1. I successfully enriched two anaerobic, thermophilic microbial communities on α-cellulose. The efficiency of cellulose hydrolysis was followed by β-glucosidase enzyme activity. I demonstrated that the enriched consortia showed superior lignocellulose decomposition ability by comparing them to an outstanding cellulolytic strain (*Caldicellulosiruptor saccharolyticus*) and established that the enriched cultures bioaugmented the biogas production from lignocellulose-rich biomass in about the same degree (14%) as the *C. saccharolyticus* (11%) did. In the second stage of the adaptation process the biogas production from 1-gram organic dry matter increased by 52% compared to the first stage.
2. I identified the most abundant members of the two enriched cellulose degrading consortia by next generation DNA sequencing using the MG-RAST bioinformatics software package and the NCBI M5nR database. Representing orders of the consortia were *Thermoanaerobacterales* and *Clostridiales.* The 4 most abundant strains were identified as *Thermoanaerobacterium thermosaccharolyticum, Caldanaerobacter subterraneus, Thermoanaerobacter pseudethanolicus* and *Ruminiclostridium cellulolyticum.*

The stability of the composition of the enriched communities was tested by re-sequencing them after 4 years of repeated utilization and storage at -80°C. The read-based evaluation was performed this time using the more up-to-date Kraken software and the RefSeq database. The raw sequencing data from the first sequencing were also re-evaluated with this method. The predominating orders were *Thermoanaerobacterales* and *Clostridiales*, and in accordance with the earlier results, the most abundant strain was *Thermoanaerobacterium thermosaccharolyticum*. Strains with lower abundances were *Thermoanaerobacterium xylanolyticum*, *Hungateiclostridium clariflavum*, *Hungateiclostridium thermocellum* and *Thermoclostridium stercorarium*. To validate these results, a genome-based taxonomic classification was also carried out. 10 bins were successfully constructed, among them were the *T. thermosaccharolyticum*, *H. clariflavum* and *H. thermocellum*. The two approaches verified each other and proved that the consortia can be sustained stably.

3. In addition to corn stover, I determined the biomethane potential of sunflower stalk, another agricultural byproduct, under both mesophilic and thermophilic conditions. The specific methane yields were higher for corn stover than for

sunflower stalk. To facilitate methane production, I compared the effects of pretreatment and bioaugmentation with mesophilic and thermophilic cellulose degrading bacteria using sunflower stalk and corn stover as substrates. No significant difference between the two methods was observed with these substrates: under mesophilic condition methane yields from corn stover increased by 6-9% employing bioaugmentation or pretreatment, respectively. In case of sunflower stalk these values were 9-12%, respectively. Under thermophilic conditions, the methane yields increased by 8-12% in case of corn stover and by 26% and 21% in case of sunflower stalk.

4. I used the enriched cellulose degrading consortia in biogas bioaugmentation experiments using corn stover as substrate, which is a lignocellulose-rich agricultural by-product. The consortia, added to the non-adapted natural biogas producing microbial community, improved the biogas and methane yields (22-24%) in a degree that exceeded the yields measured upon the adaptation process. This indicated that the lengthy adaptation can be substituted with bioaugmentation when corn stover substrate is employed.
5. The most abundant strains identified in the enriched consortia were grown in pure cultures in order to compare their individual and combined bioaugmentation ability with

the performance of the enriched two mixed cultures. The efficiency of the enriched consortia (22-24%) exceeded that of the pure cultures (11%) suggesting that the microbes present in the enrichments in low abundance contributes to the biological activity.

6. I calculated the annually produced and potentially available amounts of corn stover and sunflower stalk for biogas production in Hungary. The cumulative methane production and related energy contents (13 084 GWh) of these substrates would be able to cover the annul energy consumption of Hungarian households. Thus, biogas production from these two agricultural by-products could contribute to the national renewable energy production to a large extent.

Publications

Publications, which served as basis for the doctoral procedure:

1) Orsolya Strang, Norbert Ács, Roland Wirth, Gergely Maróti, Zoltán Bagi, Gábor Rákhely, Kornél L Kovács: Bioaugmentation of the thermophilic anaerobic biodegradation of cellulose and corn stover, Anaerobe 46 pp. 104-113., 10 p. (2017) IF: 2,742

2) Zoltán Bagi, Norbert Ács, Tamás Böjti, Balázs Kakuk, Gábor Rákhely, Orsolya Strang, Márk Szuhaj, Roland Wirth, Kornél L Kovács: Biomethane: The energy storage, platform chemical and greenhouse gas mitigation target, Anaerobe 46 pp. 13-22., 10 p. (2017) IF: 2,742

Papers published in journals:

1) Orsolya Strang, Norbert Ács, Roland Wirth, Gergely Maróti, Zoltán Bagi, Gábor Rákhely, Kornél L Kovács: Bioaugmentation of the thermophilic anaerobic biodegradation of cellulose and corn stover, Anaerobe 46 pp. 104-113., 10 p. (2017) IF: 2,742

2) Zoltán Bagi, Norbert Ács, Tamás Böjti, Balázs Kakuk, Gábor Rákhely, Orsolya Strang, Márk Szuhaj, Roland Wirth, Kornél L Kovács: Biomethane: The energy storage, platform chemical

and greenhouse gas mitigation target, Anaerobe 46 pp. 13-22., 10 p. (2017) IF: 2,742

3) Kornél L Kovács, Norbert Ács, Etelka Kovács, Roland Wirth, Gábor Rákhely, Orsolya Strang, Zsófia Herbel, Zoltán Bagi: Improvement of Biogas Production by Bioaugmentation, BioMed Research International 2013 Paper: 482653, 7 p. (2013) IF: 2,880

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2) Orsolya Strang, Zoltán Bagi, Gábor Rákhely, Kornél L Kovács: Bioaugmentation of the anaerobic degradation of corn stover and sunflower stalk with cellulolytic microorganisms, Acta Microbiologica et Immunologica Hungarica 64: Suppl. 1. pp. 169-170., 2 p. (2017)

3) Orsolya Strang, Norbert Ács, Roland Wirth, Zoltán Bagi, Gábor Rákhely, Kornél L Kovács: Enhancement of the thermophilic anaerobic biodegradation of lignocellulose-rich substrates, In: Biogas, Science 2016 (eds.) Biogas Science 2016 Conference (2016) pp. 66-66., 1 p.

4) Orsolya Strang, Norbert Ács, Roland Wirth, Gergely Maróti, Zoltán Bagi, Gábor Rákhely, Kornél L Kovács: Potential candidates for improvement of anaerobic degradation of lignocellulosic biomass pp. 153-154., In: Jörgen Held, Frank Scholwin (eds.) Conference Proceedings 2nd International Conference on Renewable Energy Gas Technology, Lund, Svédország: Renewable Energy Technology International AB, (2015) p. 164

5) Orsolya Strang, Norbert Ács, Roland Wirth, Gergely Maróti, Zoltán Bagi, Kornél L Kovács: Potential candidates for improvement of anaerobic degradation of lignocellulosic

biomass, Acta Microbiologica et Immunologica Hungarica 62: Suppl. 1. pp. 101-102., 2 p. (2015)

6) Kornél L Kovács, Norbert Ács, Tamás Böjti, Etelka Kovács, Orsolya Strang, Roland Wirth, Zoltán Bagi: Biogas Producing Microbes and Biomolecules, In: Xuefeng Lu (eds.) Biofuels: From Microbes to Molecules, London, Egyesült Királyság / Anglia: Caister Academic Press, (2014) pp. 47-92., 46 p.

7) Zoltán Bagi, Norbert Ács, Tamás Böjti, Balázs Kakuk, Etelka Kovács, Orsolya Strang, Roland Wirth, Kornél L Kovács: Example Strategy: Smart specialisation in the Field of Agribusiness/biomass (2013), Mission of the Four Motors within the Framework of the EU Strategy for the Danube Region, konferencia előadás, 2013.07.02. Novi Sad,

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9) Balázs Kakuk, Orsolya Strang, Norbert Ács, Etelka Kovács, Gábor Rákhely, Kornél L Kovács, Zoltán Bagi: The impact of bacterial pretreatment on corn stover for biogas production,

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14) Orsolya Strang, Zoltán Bagi, Kornél L Kovács: Biogas production from cellulose rich substrates, In: Conference on Advances in Environmental Sciences (2013) pp. 83-87., 5 p.

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