# Molecular biological investigation of biogas producing systems

Ph.D. Thesis

Written by: Norbert Ács

Supervisors: Prof. Kornél L. Kovács
Dr. Zoltán Bagi

Doctoral School in Biology
University of Szeged
Faculty of Science and Informatics
Department of Biotechnology

Szeged, 2014

# Introduction

A clear determinant of the present Society is our energy demand. Overall energy need is increasing along with the population growth. Numerous studies have demonstrated that soon the currently used fossil energy carriers will not be able to meet these high demands. Our aim must be focused towards to the renewable energy, not just because they are environmentally friendly, but because their unlimited nature. Recognizing this, most developed countries have launched a variety of energy strategies, in order to ensure sustainable development. Hungary, as a member of the European Union has committed to raise the share of the counties renewable energy consumption to 14,65 %, by 2020. There is a lot room for improvement, considering that this share is only 7 % at present. Among the variety of renewable energy carriers emerges biogas, since almost every organic material can be converted to biomethane via anaerobic digestion. This is particularly useful when we must deal with organic waste produced by households, the industry or by the agriculture. These are often considered as

Strang O, Bagi Z, Ács N, Kovács E, Wirth R, Kovács KL (2013) Biogas production from cellulosic substrates. Acta Microbiol. Immunol. Hung., 60, 80-81. IF (2013): 0,787

Kakuk B, Strang O, Ács N, Kovács E, Rákhely G, Kovács KL, Bagi Z (2013) The impact of bacterial pretreatment on corn stover for biogas production. Bul. AGIR, 1:9-12.

## Patent application

Kovács E, Kovács KL, Maróti G, Wirth R, Rákhely G, Bagi Z, Ács N (2013) Production of biogas from protein rich resources. P1100510 Nr.Hungarian patent application, and PCT/HU2012/000092 Nr. International patent application, and European application, number 12805746.0

hazardous waste, which requires special handling, storage and disposal. Thus, handling these materials by the above mentioned method, we can save money on the elimination, as well as obtaining valuable energy at the same time.

The degradation of the organic material is catalysed by a highly complex microbial community, which can be divided into three distinct units. The first group includes the hydrolysing bacteria that are using their exoenzymes to break down the high molecular weight substrates. The resulting intermediates are used by the acetogenic bacteria, who build up the second section. The end metabolites they produce are volatile fatty acids. The methanogenic archaeabacteria, belonging to the third group are utilizing these compounds to generate the two main components of biogas, namely methane and carbon-dioxide.

The produced biogas can be diversely utilized. The easiest way is to burn the gas mixture in specialised gas engines, thereby converting it to electricity and heat. The sulphur removal from the biogas is essential in order to prevent the premature corrosion of the engine block.

Another problem is the unnecessary waste heat, which may harm the efficiency, if not used properly. A more advisable option to purify the biogas not only from sulphur, but from the unwanted carbon-dioxide as well, resulting pure biomethane. This energy carrier is more suitable for transportation, storage, or in case of relevant statutory provisions, the feed-in to the natural gas grid is also a possibility.

## Objectives

In order to raise the competitiveness of biogas compared with the fossil energy carriers, we need to increase the efficiency of the process. To achieve this, we need to get a more comprehensive knowledge about the biogas-producing community, what makes the use of molecular biological methods inevitable.

Before preparing the thesis I have aimed to apply several molecular biological methods (Real Time PCR and T-RFLP, or Terminal Restriction Fragment length Polymorphism) to examine a variety of biogas producing microbial communities, in particular their dominant

Bagi Z, Ács N, Kovács E, Kovács KL (2013) Anaerobic fermentation of distillery thin stillage. Acta Microbiol. Immunol. Hung., 60, 3. IF (2013): 0,787

Ács N, Bagi Z, Kovács E, Wirth R, Kovács KL (2013) Monitoring the biogas producing archaea community via molecular biological methods. Acta Microbiol. Immunol. Hung. 60:1. IF (2013): 0,787

**Cumulate IF: 4,565**

## Other publications

Kovács KL, Bagi Z, Ács N, Perei RK, Imre E, Telekes G, Bartha I (2009) Biotechnological methods to increase landfill gas production and degradation of organic waste. Proc. 3rd Int. Workshop “Hydro-Physico-Mechanics of Landfills”. Braunschweig, Germany, March 10-13 (2009)

Kovács KL, Kovács E, Ács N, Bagi Z (2009) Mikróbák a biogáz fermentorokban. Környezetvédelem. 17, 10-11.

Kovács KL, Kovács E, Ács N, Wirth R, Bagi Z (2010) Egy különösen hasznos megújuló energiahordozó: a biogáz. Energetika, 11, 5-8.

Bagi Z, Ács N, Kovács E, Kovács KL (2011) Improvement of biogas production via microbiology. Acta Microbiol. Immunol. Hung. 58:3-4. IF (2011): 0,551

Kovács E, Bagi Z, Ács N, Kovács KL (2011) Biogas production from protein rich substrates. Acta Microbiol. Immunol., 58, 54-55. IF (2011): 0,551

Kovács KL, Bagi Z, Kovács E, Maróti G, Szögi-Dorogházi E, Ács N, Wirth R, Tengölics R, Fülöp A, Rákhely G (2011) Industrial microbiology for the production of biohydrogen and biogas. Acta Microbiol. Immunol., 58, 171-172. IF (2011): 0,551

Bagi Z, Kovács E, Wirth R, Ács N, Böjti T, Strang O, Kakuk B, Kovács KL (2013) Microbiology and biotechnology of biogas production. In: Teparic, R., Frice, J., Mrsa, V (eds). Power of Microbes in industry and Environment. Zagreb: Croatian Microbiological Society ISBN: 978-953-7778-06-4. pp. 18.

Kovács E, Wirth R, Maróti G, Bagi Z, Ács N, Kovács KL (2013) Changes in microbial community metagenome upon adaptation to protein substrate. Acta Microbiol. Immunol. Hung., 60, 36-37. IF (2013): 0,787

members. The target genes of my investigation were on one hand unique, specific to the given strain (*celA*: large subunit of the celluleas gene, *ech*: extreme condensed hydrogenase gene), or on the other hand universal to the investigated group (*16S rRNA*: the gene of the ribosomal RNA small subunit both in case of eubacteria, and archaeabacteria, or *mcrA*: large subunit of the methyl-coenzyme M reductase gene in case of methanogenes). The prevalence of these genes was monitored in time, along to the modification of some key parameters, in order to obtain crucial information regarding the behaviour of the given biogas producing community.

## Methods

During my research I have analysed liquid samples withdrawn from 5 litre volumetric content continuous fermentors, fed by various biomass types (casein, pig blood, corn silage, liquid pig manure).The volume of the produced gas, and relevant key operating parameters (pH, redox potential, temperature) were

constantly monitored and registered by the specially developed devices. The gas composition was determined periodically by the help of a gas chromatograph during the whole fermentation. A high pressure liquid chromatograph (HPLC) was used in order to measure the concentration of the formed volatile fatty acids, and their quantitative change. The monitoring of the externally added hydrogen producer strains in the fermentors was carried out with Real-Time PCR method using specific primerpairs. T-RFLP method was implemented targeting the *mcrA* gene and the partial 16S *rRNA* gene to map the archaeal population. The eubacterial content of the samples was assessed by analysing a fragment of the *16S rRNA* gene. An online evaluation software, named T-REX was used to analyse the restriction patterns, whilst a clone library was prepared from every sample in order to identify the dominant peaks on the chromatogram. After careful screening and selection, a few clones carrying high importance were handpicked, and sequenced. The obtained sequence data was subjected to online similarity search (NCBI BLAST, RDP) to define their closest relatives among the known species.

## Conference abstracts


Kovács KL, Bagi Z, Ács N, Kovács E, Wirth R, Rákhely G (2011) Biohydrogen and Biogas Biotechnology. Proc. The Bioenergy Question: Reality or Wishful Thinking. Tulln, Austria, pp. 11-13.

Kovács KL, Bagi Z, Ács N, Kovács E, Wirth R, Maróti G, Rákhely G (2011) Engineering biogas microbial communities. Proc. 1st Int. Conf. on Biogas Microbiology. Leipzig, Germany. p. 46.

Ács N, Bagi Z, Kovács E, Wirth R, Rákhely G, Kovács KL (2011) Monitoring archaea and selected strains of bacteria in a biogas producing system via their unique genes. Proc. 1st Int. Conf. on Biogas Microbiology. Leipzig, Germany. p. 66.

Bagi Z, Ács N, Kovács E, Kovács KL (2011) Anaerobic fermentation of distillery thin stillage. Proc. 1st Int. Conf. on Biogas Microbiology. Leipzig, Germany. p. 68.

Ács N, Rákhely G, Bagi Z, Kovács E, Kovács KL (2011) The usage of real-timePCR to monitor specific eubacteria in biogas producing systems. Acta Microbiol. Immunol. Hung. 58:1-2. IF (2011): 0,551

## Publications closely related to the Thesis

Bagi Z, Ács N, Bálint B, Horváth L, Dobó K, Perei KR, Rákhely G, Kovács KL (2007) Biotechnological intensification of biogas production. Appl. Microbiol. Biotechnol. 76:473-482. IF (2007): 2,569

Herbel Zs, Rákhely G, Bagi Z, Ivanova G, Ács N, Kovács E, Kovács KL (2010) Exploitation of the extremely thermophilic *Caldicellulosiruptor saccharolyticus* in hydrogen and biogas production from biomasses. Env. Technol. 31(8-9):1017-1024. IF (2010): 1,007

Ács N, Kovacs E, Wirth R, Bagi Z, Strang O, Herbel Zs, Rákhely G, Kovács, KL (2013) Changes in the Archaea microbial community when the biogas fermenters are fed with protein-rich substrates. Bioresource Technology 131: 121-127. IF (2013): 4,750

Kovacs KL, Acs N, Kovacs E, Wirth R, Rakhely G, Strang O, Herbel Z, Bagi Z (2013) Improvement of biogas production by bioaugmentation. BioMed Research International, ArticleID482653, http://dx.doi.org/10.1155/2013/482653 IF (2013): 2,880

**Cumulate IF: 11,206**

## Results

1. I have successfully developed a method based on specific marker genes (*celA*, *ech*), to identify both hydrogen producing strains we routinely use. With the help of this method, I have proven that the previously identified decline in the intensification effect in some special circumstances is caused by the low rate of biomass input. As a consequence of the doubled food intake, the cell number of the inoculated strain proved to be stable, as well as the elevated biogas yield.

2. In my thesis I have presented the archaeal microbial composition of a fermentor operated at typical parameters, using T-RFLP method, targeting both the *mcrA* gene, as well as the 16S *rRNA* gene. Using the gathered sequence information I have identified the dominant species, or their closest relatives. The obtained data were compared with scientific literature, in order to interpret the results.

3. I have examined the methanogenic composition of several fermentors, adapted to two kinds of high-protein content biomass with the help of T-RFLP method, targeting the *mcrA* gene. I have identified the dominant species and their closet relatives in this case as well. I concluded that the adaptation affected the methanogenic population massively, which is an important discovery because they do not have a direct contact with the substrate, the chemical nature of the intermediates (volatile fatty acids and hydrogen) is constant.

4. I have determined the eubacterial composition - in particular the dominant species - of a continuously stirred fermentor fed with corn silage based on partial sequences of the 16S *rRNA* gene. The occurrence of the abundant species was corroborated by previous experiments of several research groups.

5. Subsequently, the fermentors were inoculated with the previously applied hydrogen producing strain while the same operating circumstances were kept. As a result of the integration to the biogas producing consortia, the gas yield elevated more than 20 % compared to the control fermentor. I have also defined the eubacterial composition of the inoculated fermentor that showed significant difference from the previously detected.