

Utilization of wastes derived from plants and animals for biogas production

Ph.D. Thesis

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INTRODUCTION

Today's society has to face several environmental problems, such as the increasing amount and management of organic wastes, the increased usage of fossil fuels, etc. Our resources are exhausted and their use leads to various environmental contaminations such as air, soil and water pollution. Due to the quickly growing population, the energy consumption of the modern world are increasing day by day and showing significant growth over the last few decades. While global energy demand was about 80.000 Terawatt hours (TWh) in 1978, by 2018 it had doubled to about 160.000 TWh. This energy demand is mostly covered with burning of fossil fuels, which results in the release of million-years-ago-fixed carbon-dioxide. The consequences of this fact that the atmosphere's carbon-dioxide content is rising which causes global warming and climate change. In 2017, 79.7 % and 20.3 % of the world energy production was covered with fossil fuels and renewable energie sources, respectively.

Nowadays, one of the biggest challenge for the researchers to find new, renewable energy sources and technologies to replace the fossil fuels and to cover our energy demand in a sustainable way. There are several solutions such as utilization of sun-, wind-, water-, geothermal-, nuclear energy or biomass conversion. Nevertheless, these energy sources also present problems which can significantly affect their accessibility. Due to the conversion of biomass into energy carrier, the amount of organic wastes could be reduced as well. Today, energy from biomass provides about 9.5 % of the total energy supply and 70 % of all energy from renewable sources (Reid et al., 2020). Biomass, with low moisture content, can be converted directly into heat by combustion. However, either liquid (bioethanol, biobutanol, biodiesel) or gaseous energy sources (biohydrogen, biogas) can also produced from it. These can be used in many ways, such as fuel, heating, electricity generation, etc. (Creutzig et al., 2015). From the point of view of my dissertation, the most important utilization method is the production of biogas, which is generated during the anaerobic digestion (AD) processes, in which a complex microbial community converts the organic materials into

biogas under anaerobic condition. These organic compounds can be directly produced for this purpose such as energy plants (eg. sugarcane, etc.), or might be organic wastes produced by intensive industrial- and/or agricultural activities. The quality and the quantity of the nascent biogas depends on the substrates used and the applied inoculum (Senés-Guerrero et al., 2019). Several kinds of by-products/wastes are suitable for biogas production e.g. sewage sludge, agriculture-, municipal waste, etc. (Anna Schnürer; Asa Jarvis, 2009; Kushkevych et al., 2018), therefore the „waste to biogas” approach is becoming more and more popular. It has dual benefits: handling the organic wastes and producing renewable energy carrier at the same time. These wastes might contain pathogenes, several hazardous chemicals and nutrients which can contaminate the groundwater and soils, as well. Besides, a significant amount of methane and carbon-dioxide are released from these untreated wastes under anaerobic condition increasing the risk of global warming and climate change. The proper handling of these wastes can reduce the greenhouse gas emission and environmental pollution. Production of renewable energy carrier from organic wastes corresponds to the the aims of circular economy: optimal utilization of raw materials and reduction of organic wastes. Furthermore, after the fermentation process, the remaining sludge is suitable as biofertilizer.

In my thesis, I examined the utilization of plant- and animal origin wastes originated from agricultural and food industry for biogas production.

APPLIED METHODS

Batch fermentation experiments were carried out in Wheaton serum bottles (120 mL and 500 mL) according to the VDI 4630 (Verein Deutscher Ingenieure) standards. The continuous fermentations were performed in 5 L Continuously Stirred Tank Reactors (CSTRs). In the case of CSTRs, the produced biogas was measured by gas-flow meter which recorded the gas mass and the methane content was determined by gas chromatography. The most important parameters of the fermentations, such as pH, ammonia, carbon and nitrogen content, Volatile Organic Acids/Total Inorganic Carbon (VOA/TIC), Total Solid (TS), organic Total Solid (oTS) were determined by pH meter, analytical kits coupled to photometric measurements, Elementar device, automatic titrator and oven, respectively. During the Anaerobic Digestion (AD) processes, in the step acidogenesis and acetogenesis volatile fatty acids (VFAs) were produced which were measured by High Performance Liquid Chromatography (HPLC).

The inoculum for the AD experiments was obtained from an industrial scale biogas plant (Zöldforrás Ltd., Sándorfalva, Hungary) and the applied substrates (Tomato plant waste and corn stover) derived from fields. The rumen contents were collected from abattoirs.

The DNA purification for metagenomic analysis was carried out with ZymoResearch Quick-DNA Fecal/Soil Microbe Kit according to the manufacturer's instruction. The quality and quantity of the purified DNA were determined by agarose gelelectrophoresis and Qubit 4.0 fluorometer, respectively. Both, amplicon (16S rRNA gene) and shotgun whole genome sequencing was performed on Illumina MiSeq sequencer. In the case of amplicon sequencing, DADA2, OneCodex, FactoMineR, phyloseq, DESeq2 and ggpubr softwares were used. For the analysis of shotgun whole genome sequencing reads, FastQC, MultiQC, Trimmomatic, DIAMOND, MEGAN, MetaSPAdes, MetaQUAST, MetaWRAP, CheckM, GTDB Tk, eggNOG Mapper and Circos programs were applied.

RESULTS OF THE THESIS

According to the physico-chemical parameters of TW, it is a promising substrate for biogas production. In batch fermentation experiments, TW was tested as mono- and co-substrate (with CS) for biogas production and their biomethane potentials (BMP) were compared to that of CS which was used as a control substrate.

In continuous fermentation tests, it was highlighted that the presence of TW apparently decreased the biogas production for both mono- and co-substrate (with CS) as compared to the control substrate CS. However, the most important fermentation parameters (pH, VOA/TIC, ammonium-ion cc.) did not differ from the normal values.

16S rRNA gene based metagenomic sequencing was carried out to follow the microbial compositions when the fermenters were fed with TW as mono- and co-substrate. Significant rearrangements were observed in the microbial community in the presence of TW. Moreover, the biodiversity was substantially dropped in the case of TW as compared to the control fermentation.

TW is known to contain antimicrobial and antifungal agents such as glycoalkaloids (tomatine and tomatidine) which can influence the microbial community thereby the biomethane formation. In batch fermentation experiments, the effect of these two glycoalkaloids on the biogas yield was tested at various concentrations (0.0025-2.5 µg/mL). Tomatidine had no effect on biomethane production, however tomatine decreased it at 0.025 µg/mL and above.

Summary, TW is a good substrate in batch fermentation or as a co-substrate with CS in a proper ratio in continuous fermentation. Nevertheless, further examinations are needed to maximize the biomethane formation from TW such as pre-treatment process(es), thermophilic system, dosage of applied TW, etc.

Sheep-, and cattle rumen contents (sRC and cRC) have an active hydrolizing and organic acid producing microbial community. Therefore, our primary aim was to use the microbial activity of the rumens for intensifying the biogas production. However, due to the intensive microbial activity, the usage for inoculation biogas reactors did not perform well since it lowered the pH in a short time. This observation did not coincide with the reports publishing the applicability of rumen content as inoculum. I hypothesized that the rumen contents rather served as substrates than inoculum in the biogas plants. Therefore, I set up a concept about the application of residual rumen contents as a substrate in biogas fermentations.

According to the results, rumen contents are suitable for organic acid and/or biogas production in two stage AD processes. In the first stage, the endogenous microbial community pre-digest the RC and converts it into methane but mainly into organic acids. In stage two, the pre-digested RC and the organic acids are converted into methane.

Both sRC's and cRC's microbiome needed 1-2 at longest 5 days to achieve the maximal organic acid production. In parallel, the pH drastically dropped in all cases similarly to the methane production. Acetic acid, propionic acid and iso-butyric acid were produced in the largest quantities in the case of both RCs.

Several metagenomic analyses were carried out on the RCs and on the samples which were collected on the 0., 2nd and 5th day of the first stage. The microbial compositions of rumen samples of sheeps and cattles were compared to each other (0. day). According to the analyses, the most important microbial groups are the same in both cases thereby the relative incidences of some microbial groups differed.

I examined the changes of the microbiome in the fermenters after 2 and 5 days during the first stage to elucidate which microbial groups emerge and decline in the presence of the accumulating organic acids. The results have proven that the Archaea domain remained unchanged during the examined period in the RCs. Therefore they were likely in inactive form. Bacteria domain changes were more prominent while the Eukaryotes have shown a

more drastic decline: some of them completely disappeared from the system by the 5th day of the incubation.

Binnig was carried out on all quality filtered reads pooled. As a result, 22 bins were identified at genus and/or species level. Due to the analysis, many Carbohydrate Active Enzyme (CAZy) genes were identified. Almost 70% of the identified genes belonged to the Bacteroidia and 18% of them belonged to the Clostridia families. Altogether, 840 Glycosyl Hydrolase (GH) genes were identified which were classified into 41 GH families. Overall, 56% of these identified GH genes belonged to 5 different GH families (GH3, GH5, GH9, GH31, and GH13).

Acetic acid was produced in the largest amount in every case, so genes belonging to the acetic acid production pathways (oxidative-, Wood-Ljungdhal- and Glycine synthase pathway) were searched in the identified bins. Genes of all three pathways genes were identified so the microorganisms genomically identified are probably important members of the organic acid production, as well.

To summarize this part of my work, the two stage procedure for utilization of RCs increased the BMP of both sRC and cRC with approximately 20 %.

PUBLICATIONS

Article the thesis based on:

1) **Árpád Szilágyi**; Attila Bodor; Norbert Tolvai; Kornél L. Kovács; László Bodai; Roland Wirth; Zoltán Bagi; Ágnes Szepesi; Viktória Markó; Balázs Kakuk; Naila Bounedjoum; Gábor Rákhely: **A comparison of biogas production from tomato bio-waste in mesophilic batch and continuous anaerobic digestion systems**, (2021), PlosOne, <https://doi.org/10.1371/journal.pone.0248654>

(D1; IF: 2.87)

Article closely related to the thesis, but wasn't used in the thesis:

2) Roland Wirth; Gyula Kádár; Balázs Kakuk; Gergely Maróti; Zoltán Bagi; **Árpád Szilágyi**; Gábor Rákhely; József Horváth; Kornél L. Kovács: **The planktonic core microbiome and core functions in the cattle rumen by next generation sequencing**, Frontiers in Microbiology, (2018), <https://doi.org/10.3389/fmicb.2018.02285>

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3) Krisztián Laczi; Ágnes Erdeiné Kis; **Árpád Szilágyi**; Naila Bounedjoum; Attila Bodor; György Erik Vincze; Tamás Kovács; Katalin Perei; Gábor Rákhely: **New Frontiers of Anaerobic Hydrocarbon Biodegradation in the Multi-Omics Era**, (2020), Frontiers in Microbiology, <https://doi.org/10.3389/fmicb.2020.590049>,

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(IF: -)

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1) Violetta Tripolszki; Viktória Markó; Norbert Tolvai; **Árpád Szilágyi**; Etelka Kovács; Zoltán Bagi; Kornél L. Kovács; Katalin Perei; Zoltán Pásztory; Gábor Rákhely: **Isolation of cellulose degrading fungi from wooden wastes**; II. Sustainable Raw Materials Conference Book – International Project Week and Scientific Conference (2019); pp. 191-194.

2) Attila Bodor, Tibor Sipos; György Erik Vincze; Péter Petrovszki; Gábor Feigl; Naila Bounedjoum; Krisztián Laczi; **Árpád Szilágyi**; Gábor Rákhely; Katalin Perei: **Alterations in Soil Fertility after Used Lubricating Oil Bioremediation**, (2018), pp. 266-269.

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1) **Árpád Szilágyi**; Ágnes Szepesi; Attila Bodor; Krisztián Laczi; Viktória Markó; Katalin Perei; Gábor Rákhely: **Tomato plant waste as a new substrate in biogas fermentation**, 7th International Conference on Sustainable Solid Waste Management, Heraklion, Crete, (2019)

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