Microbiological intensification of the biogas fermentation systems

Ph.D. Thesis

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Introduction

Mankind has to solve two energy related global problems in the near future, i.e., the environmental crisis that is also called as global warming due to an excessive use of fossil fuels and the foreseeable shortage of the non-renewable energy carriers. At present the world cover 80% of its energy consumption from not renewable energy sources (natural gas, fossil oil, coal). The main demand to increase consumption is due to the growing population and the industry. If we keep using fossil energy carriers at the same rate, the production will be not able to cover the consumption in 30-40 years. Researchers recognized that renewable energy sources are needed to supply the increasing global energy demand. A widely used renewable energy carrier is biomass, i.e., the organic material produced by biological systems using solar energy directly or indirectly. One of the most important means to convert biomass to other forms of energy is biogas production. Biogas is produced through conversion of biomass in anaerobic fermentation, the product can be used in several ways. Biogas can be burnt thereby producing heat, or it can be fed into a gas engine and electricity generator in order to convert it to heat and electricity, or -following purification it can be fed into the natural gas grid for further use, e.g., excellent fuel for cars. The fermentation residue is utilized as fertilizer, so that one can substitute artificial fertilizers on the field. Artificial fertilizers are produced in processes of huge energy requirement.

Biogas can be generated only under anaerobic conditions, although some facultative anaerobic strains are also found in the fermenters. During anaerobic decomposition of complex organic molecules the biochemical conversions are carried out by various microbe populations adapted to the different biomass types. The bacterial strains are organized into a microbiological food chain and the participants assume a distinct order. The survival of the individual strains depend on each other, they are working in a coordinated, concerted fashion, every specific strain has a set of unique molecules to decompose. The transformation of the complex organic molecules is successful only if the bacterial strains build up this special network in which every strain grows and produces its fermentation product that is utilized by the next bacterium in the degradation chain. Energy for the survival of each member of the consortium is produced

by degrading the complex organic molecules and their intermediers. A number of various bacterial strains are competing for the energy stored in the substrate molecules, the most viable ones will become a dominant strain. Simpler molecules are produced from the complex ones during the biodegradation. At the end of the process the produced volatile organic acids are consumed by the methanogens.

Biogas formation is thermodynamically possible when hydrogen is kept below a threshold concentration; thus, H_2 is barely detectable in biogas. At the same time, the biological activity of certain methanogens, i.e., the hydrogenotrophoic methanogens, requires a good supply of hydrogen to carry out the redox reaction. The relationship between the acetogens and methanogens is syntrophic, supported by a process called interspecies hydrogen transfer or interspecies electron flow.

The two main components of the biogas are methane (55-75%) and carbon-dioxide (28-48%). Biogas usually contains traces of other gases (1-2%), like hydrogen-sulfide, carbon-monoxide and nitrogen. The fossil fuel natural gas is composed of more than 90% methane. Technologies have been developed, which purify biogas to similar quality and therefore after compressing biogas can be used as transportation fuel. Biogas fermented from sewage sludge has the highest methane content (60-75%), biogas from manure and agricultural residue has lower methane content, domestic household waste in landfills produce biogas at the lowest rate.

Aims

The aim of my PhD thesis work was the enhancement of the efficacy of the biogas producing technologies exploiting the results of the several decades long study of the microbiological events leading to biogas production. These studies into the metabolic pathways of the biogas producing consortia revealed the hydrogen may be a rate limiting factor of the entire. I therefore studied the possibility to intensify the activity of the hydrogenotrophic methanogens by adding a suitably selected hydrogen producing strain, cultivated in pure culture, which would be able to intensify the efficacy of the biogas fermentation via supplying appropriate reducing agent in the form of hydrogen. In addition I intended to determine the metabolic pathway changes in the system under the altered conditions. I also examined whether the technology can be applied in laboratory batch and continuous fermentors and in scale-up field experiments. Field experiments were designed to test if the improved biogas production technology can be used at industrial renewable energy production scale.

Methods

Factors have been identified, which are important in the regulation of the activity of methanogens during anaerobic digestion. The experiments were carried out in batch fermentors with 0.5 liter capacity and automatically controlled semi continuous fermentors of 5 liter volume, these fermentors have been designed and constructed for this specific application. The changes in the composition of the biogas were followed by gas chromatography. The intermediers, produced during the anaerobic digestion process were measured using high pressure liquid chromatography (HPLC). The presence and relative concentration of the added hydrogen producing bacteria in the fermentors was followed using conventional and Real Time PCR (polymerase chain reaction) methods. The composition of the various biomass samples were determined in an automatic carbon and nitrogen measuring instrument and/or using an analytical workstation based on spectrophotometric assays. For the cultivation of the microbes conventional sterile microbiological techniques were used.

Results

1. It was demonstrated that H_2 plays an important role in anaerobic degradation of organic material. The regulatory roles of hydrogen levels and interspecies hydrogen transfer optimize the concerted action of the entire population.

2. I demonstrated that a significant increase in biogas productivity by the mixed methanogenic consortium can be triggered by adding appropriately selected hydrogen producing bacteria to the natural consortium, in either the mesophilic or thermophilic temperature range. Evidence links the effect to the presence and direct contact of living hydrogen producing bacteria with their methanogenic partners. The effect was not particularly substrate dependent. It occurred when various sources of biomass, and therefore various methanogenic consortia were used.

3. A set of experiments was designed to identify the parameter, which may be responsible for the increase in biogas formation. These led to the conclusion that the observed intensification effect took place only when the biogas consortium and the hydrogen producing bacteria were in close contact in the same space. H_2 administered from outside (either from a tank or from the head space of a growing hydrogen producing culture) did not affect the natural biogas formation process. Inactivated hydrogen producing strain (*Caldicellulosiruptor sacharolyticus*) and hydrogenase-minus hydrogen producing strain *(Escherichia coli*) were ineffective too.

4. It has been recognized that, to a certain degree, *in situ* generated hydrogen, provided by the syntrophic partner that carries out heterotrophic fermentation, helps to overcome a bottleneck of the complex process of methanogenesis.

5. I demonstrated the positive effect of the hydrogen producing bacteria in batch and continuous fermentation technology. In the laboratory scale semi-continuous fermentors the hydrogen producing bacteria failed to fit into the natural consortium for an extended period of time, the cells were diluted during the fermentation because of the inefficient

growth rate in the consortium. I demonstrated that there was a correlation between the C/N ratio of the input substrate material and the rate of the intensification. Biomass having higher C/N ratio is accompanied with more intensive intensification of biogas production. The intensification was more pronounced at mesophilic temperature than at thermophilic temperature.

6. Experimental results established the long-term benefit of the intensification process under “real-life” industrial scale-up field tests using a $5m^3$ fermentor. In this system the intensification effect was detectable for at least 4 month. With PCR reactions the hydrogen producing bacterial DNS was detectable in the fermentation residue after 4 month, which demonstrated that *C. saccharolyticus* could grow in the anaerobic biogas fermentor at an acceptable rate.

7. The *hypF* minus *E. coli* mutant strain provided direct evidence, linking the beneficial effect to the presence of an active hydrogen-producing enzyme. HypF is a pleiotropic accessory protein necessary for the biosynthesis of all hydrogenases in *E. coli*. The mutant strain, therefore, does not possess the ability to produce hydrogen, but it carried out anaerobic fermentation just as wild-type *E. coli* does.

8. For the use of the technology at industrial scale it is important to determine economically feasible media for the cultivation of the hydrogen producing bacteria. My work results show that the best cheap industrial medium for the *E. cloacae* is the melas, for the *C. saccharolyticus* is the soya powder.

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