

Methanogenic bioconversion of cellulose

The alternatives for coal or oil are renewable resources (food crops, hydrocarbon-rich plants, agricultural waste) and/or processes of their biotransformation. Two main pathways for energetic utilization of biomass are thermal or biochemical. The latter includes fermentation, dark fermentation and anaerobic digestion which yield biogas. Utilization of lignocellulosic biomass rather than food crops for energy generation is an approach corresponding to *sustainable development*, because (1) much greater quantity of energy can be obtained from a land unit and (2) there is no concurrence with food production.

Waste agricultural lignocellulosic feedstock abundance is estimated on over 4 billion tons annually. 35-50% of these resources comprise polysaccharides. Methanogenic utilization of cellulose requires either conversion to fermentable monosaccharides [1] or use of cellulolytic bacteria [2,3]. Average yield of biomethane from biomass anaerobic digestion is 0.3–0.4 m³/kg and depends on organic compounds presence, bacteria strain and loading, temperature (mesophilic 35°C, thermophilic 55°C) and retention time.

In the literature numerous approaches for lignocellulosic biomass fermentation have been described. The factors affecting the efficiency of the process are: (1) used microorganism species and (2) pretreatment method.

Initial experiments performed on bacterial consortium sampled from a municipal landfill resulted in substantial methane generation from waste pine dust during anaerobic fermentation in a 1-L bioreactor.

The main objectives of the project are (1) to determine fermentation procedures for high-yield bioconversion of lignocellulosic biomass to methane, (2) utilization of cellulose or simple sugars as carbon source and, in the latter case, finding the most effective pretreatment.

The experimental tasks covered by the project will be as follows:

1. Tests of biogas productivity for the selected bacterial species in model environment.
2. Chemical and enzymatic protocols for most efficient lignocellulosic biomass saccharification.
3. Evaluation of conversion of biomass to methane protocols.

Ad. 1.

The literature data indicate ca. 20 bacterial species enabling fermentation of lignocellulosics to biogas. At that stage of the project, fermentations of monosaccharide cocktails of pre-defined compositions or non-treated cellulose with selected species will be performed in 0.5-L bioreactors under mesophilic or thermophilic conditions. Environments of dark fermentation, photo fermentation under anaerobic conditions will be investigated. Preferably, methanogenic bacteria cultures existing in soil or animal gastrointestinal tract will be examined [4,5]. Produced gas will be

collected in gas-tight bags, and its volume be measured by the liquid replacement method. Biogas concentration will be determined by gas chromatography.

Ad. 2.

The main pretreatments developed for enhanced digestibility of phytomass are: chemical (ammonia, acids) and enzymatic. It is planned to investigate the most efficient biomass chemical pretreatment methods for maximized yield of fermentable monosaccharides. Additionally, since it is known from the literature that enzymatic pretreatment strongly affects the concentration of the inhibitors of microbial growth (i.e. fermentation stage), a series of cellulases of different origin under variable environment conditions will be used for the most effective saccharification of model cellulose or lignocellulosic raw materials. Efficacy of the treatments will be determined by means of HPLC.

Ad. 3

Evaluation of the labored approaches and efficiency of the developed protocols will be based on the investigations of biomethane productivity in the optimized systems involving most effective pretreatment for lignocellulosic material, most productive bacteria strain and most effective fermentation environment.

References

- [1] Danay Carrillo Nieves, Keikhosro Karimi, Ilona Sárvári Horváth, 2011. *Improvement of biogas production from oil palm empty fruit bunches (OPEFB)*, Ind. Crops Prod. 34, 1097–1101; Mirahmadi, K., Kabir, M.M., Jeihanipour, A., Karimi, K., Taherzadeh, M.J., 2010. *Alkaline pretreatment of spruce and birch to improve bioethanol and biogas production*. BioResources 5, 928–938
- [2] Maki M, Leung KT, Qin WS, 2009. *The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass*. Int. J. Biol. Sci., 5, 500–516
- [3] Azam Jeihanipour, Claes Niklasson, Mohammad J. Taherzadeh, 2011. *Enhancement of solubilization rate of cellulose in anaerobic digestion and its drawbacks*, Process Biochemistry 46, 1509–1514
- [4] Varel VH, Yen JT, Kreikemeier KK, 1995. *Addition of cellulolytic clostridia to the bovine rumen and pig intestinal tract*. Appl Environ Microbiol, 61, 1116-1119
- [5] Ohara H, Karita S, Kimura T, Sakka K, Ohmiya K., 2000. *Characterization of the cellulolytic complex (cellulosome) from Ruminococcus albus*. Biosci Biotechnol Biochem 64, 254-260