Genomic optimisation of hydrolysis in biogas production

Mike Larkin, Chris Allen, Leonid Kulakov, Joanna Grebosz
Queen's University Belfast, School of Biological Sciences
97 Lisburn Road, Belfast BT9 7BL , E-mail address: jgrebosz01@qub.ac.uk

Introduction

Biogas, which consists mainly of methane (60-70%) and carbon dioxide, is produced from the feedstock in the anaerobic digestion process. The process is carried out by several groups of microorganisms (Yadvika et al. 2004). The gas can be used as a renewable energy source (Berglund and Börjesson 2006). The biogas production process is composed of four stages, each carried out by different groups of microorganisms. The stages are: hydrolysis, acidogenesis (fermentation), acetogenesis and methanogenesis (Yadvika et al. 2004; Li et al. 2011).

One of the key factors influencing the baterial or archaeal groups can be hydrogen partial pressure. It is connected with the syntrophic relations between the microorganisms and with some microbial shifts (Schlüter et al. 2008). The structure and shifts in the uncultured microbial community can be investigated by the metagenomic tools (Wooley et al. 2010).

The purpose of the project is to investigate the possible syntrophy between fermentative, hydrogen producing bacteria such as the little characterised and uncultured OD1 and hydrogenotrophic methanogens and its meaning in the methane production yield in anaerobic digesters by using metagenomic tools.

Methods

A sample was taken from a mesophilic two-stage anaerobic digester (that uses slurry as a feedstock) located in Hillsborough (AFBI). The samples will be used to inoculate some laboratory-scale reactors with different substrates. The extraction of the DNA from the samples was attempted using PowerSoil DNA Isolation Kit (Mo Bio Laboratories). The possible approach for DNA extraction is the Griffiths et al. (2000) method. The DNA concentration was estimated using a fluorometer (Quantus, Promega). The technique qPCR or PCR followed by 454-pyrosequencing will be used to estimate the quantity of some microbial groups and to investigate the relationships between these groups. To investigate the OD1 in the samples, the general bacterial primers 341f and 805r or 63f and 1387r in PCR (followed by the 454-pyrosequencing) can be used.

Fig. 1. The mesophilic two-stage anaerobic digester located in Hillsborough (AFBI)

For OD1 quantification the qPCR with primers for the functional genes NiFe1aFor and NiFe1Rev can be conducted. To investigate the quantity of methanogens in the samples, qPCR can be used with 16S primers such as MCC495F, MCC832R, MBT857F, MBT1196R, MMB282F, MMB832R, MSL812F, MSL1159F, Msc380F, Msc828R, Mst702F and Mst862R or by using the primers complementary to some functional genes, such as *mcrA*, *acsB*, *fhs* etc.

Results

The concentration of extracted DNA was estimated at 7 ng/µl. After the electrophoresis (1% agarose gel; 100V/h) that had confirmed the presence of DNA, the PCR with 63f and 1387r 16S bacterial primers was conducted. The failure of the PCR might indicate the presence of some inhibitors of the PCR in the sample. The low DNA concentration can lead to the conclusion there's the need to optimize the DNA extraction method.

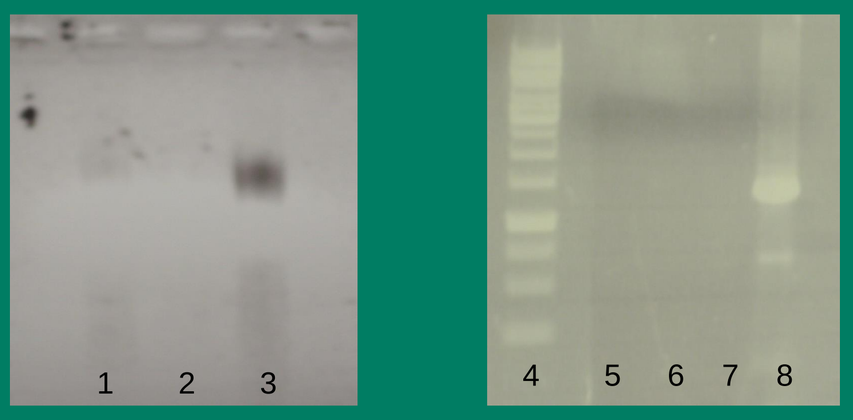

Fig. 2. The electrophoregrams obtained by using the extracted DNA (A) and the amplification products after PCR with 63f and 1387r primers (B). Designation: 1 and 2 – DNA isolated from the anaerobic digester's samples, 3 – DNA isolated from the soil sample for comparison purposes, 4 - 1kb molecular mass marker, 4, 5 and 6 – the anaerobic digester's samples amplicons, 7 – bacterial positive control

Hypothesis and conclusions

The candidate division OD1 is considered to have an influence on the methanogenic community due to the possibility to produce hydrogen (Wrighton et al. 2012). In the anaerobic reactors rich in OD1, the dominating *Archaea* are hydrogenotrophic methanogens that are suspected to give lower methane yield than acetoclastic methanogens (Ziganshin et al. 2011). Thus, OD1 might be a biomarker indicating low methane yields in the digesters that can be monitored by some robust metagenomic techniques (such as qPCR). OD1 investigation can bring the information about the most optimal hydrolysis conditions (for example by testing different feedstocks or different feedstock pre-treatment methods). Therefore, the most optimal conditions of hydrolysis step can be connected with low OD1 numbers and result in high methane yield.

The project will show the connection between specific microbial community shifts and biogas production efficiency in particular conditions using the more rapid molecular biology techniques.

References

Berglund M, Börjesson P (2006) Assessment of energy performance in the life-cycle of biogas production. Biomass Bioenergy 30:254-266

Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ (2000) Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and RNA-based microbial community composition. Appl Environ Microbioll 66:5488-5491

Li Y, Park SY, Zhu J (2011) Solid-state anaerobic digestion for methane production from organic waste. Renew Sust Energ Rev 15:821-826

SchlüterA, Bekel T, Diaz NN, Dondrup M, Eichenlaub R, Gartemann KH, Krahn I, Krause N, Krömeke H, Kruse O, Mussgnug JH, Neuweger H, Niehaus K, Pühler A, Runte KJ, Szczepanowski R, Tauch A, Tilker A, Viehöver P, Goesmann A (2008) The metagenome of biogas-producing microbial community of a production-scale biogas plant fermenter analysed by the 454-pyrosequencing technology. J Biotechnol 136:77-90

Wooley JC, Godzik A, Friedberg I (2010) A primer on metagenomics. PLOS Comput Biol 6:1-13

Wrighton KC, Thomas BC, Sharon I, Miller CS, Castelle CJ, VerBerkmoes NC, Wilkins MJ, Hettich RL, Lipton MS, Williams KH, Long PE, Banfield JF (2012) Fermentation, hydrogen and sulfur metabolism in multiple uncultivated bacterial phyla. Science 337:1661-1665

Yadvika, Santosh, Sreekrishnan TR, Kohli S, Rana V (2004) Enhancement of biogas production from solid substrates using different techniques – a review. Bioresour Technol 95:1-10

Ziganshin AM, Schmidt T, Scholwin F, Il'inskaya ON, Harms H, Kleinsteuber S (2011) Bacteria and archaea involved in anaerobic digestion of distillers grains with solubles. Appl Microbiol Biotechnol 89:2039-2052