Fluorescent *in situ* hybridization technique in anaerobic process studies

Tommy Wikström, tommy.wikstrom@ltu.se
Désirée Nordmark, desiree.nordmark@ltu.se
Markku Pelkonen, markku.pelkonen@ltu.se
Anders Lagerkvist

Waste science & Technology, Lulea university of technology, S-971 87 Luleå, Sweden

Introduction

FISH (Fluorescent *in situ* hybridization) is a technique that is used to identify and enumerate specific microbial groups. This technique can be used to determine whether specific genetic elements exist in a sample. This is useful for determining microbes that have a particular gene (rRNA) present (Amann, 2008). Fluorescent oligonucleotide probes (15-25 bases) are designed to attach to specific genetic regions of microbes that will differentiate them from other groups, by using different fluorescent dyes. Epifluorescent microscopy is then used to detect the presence or absence of individual microbial groups.

FISH can be applied to analyze microbial characteristics on general level or on more specific level, like different groups in landfill environment such as methane oxidizing bacteria in landfill cover and ammonia and nitrite oxidizing bacteria in harsh environment in landfill leachate treatment plant (Pelkonen, 2002). In this study microbiological communities in different anaerobic sludges were studied with FISH technique, aiming at to characterize biological phenomena in more detail together with other analysis methods.

Materials and methods

The sludges used in this study were from a slaughterhouse pig farm (referred to as sludge 1) and from a sewage treatment plant also containing municipal solid food waste as co-substrate (referred to as sludge 2). Both sludges were digested anaerobically in thermophilic digesters, with hydralic retention time over 15 days.

A general probe for *Archaea*, ARC915, was used to identify all methanogens, MSMX860 to *Methanosarcinales* and MB311 to *Methanobacteriales* (Crocetti et al. 2006). The probes were labeled at 5'-end with indocyanine dye (Cy3). Microscope used was Diaphot-300 epifluorescence microscope (Nikon, Japan) with 100 W mercury lamp, equipped with filter sets (Chroma Tech, USA), and used with 1000x magnification. Also metabolic tests were used by adding acetate or by producing conditions with hydrogen atmosphere in 110 ml serum bottles with sludge and incubated for weeks.

Results and discussion

The number of methanogens was in the same range. Microbial characterization showed only one methanogen type present in sludge 1 (Fig 1) and metabolic tests with acetate showed clearly lower methane production rate than in sludge 2. As hydrogen consumption was considerable in metabolic tests with sludge 1, results indicate that hydrogenotrophic activity was responsible on the methane production. Sludge 1 contained slaughterhouse waste feed

with high nitrogen content and ammonium-nitrogen in the liquid was as high as 2.4 g/l. When pH was high (around 8.4), these conditions combined with high temperature indicate strong ammonia concentration and inhibition to methane production. Absence of acetotrophic methanogens is indication on this, as they are more sensitive to ammonia inhibition. Methanogens in sludge 2 consisted of two dominating groups (Fig 2) with more diverse use of substrate. Ammonia inhibition was less probable (pH 7.4, NH_4-N < 1 g/l). This also gives more favorable conditions in possible transitions.

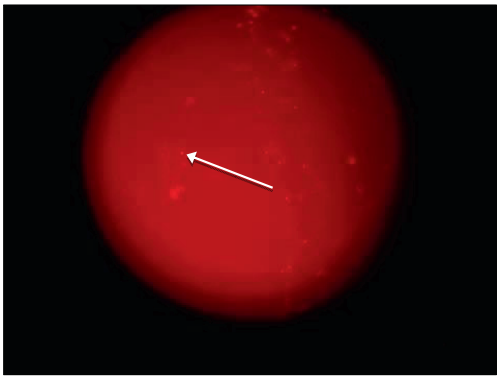

Figure 1. Sludge 1: hydrogenotrophic methanogens (probe ARC915).

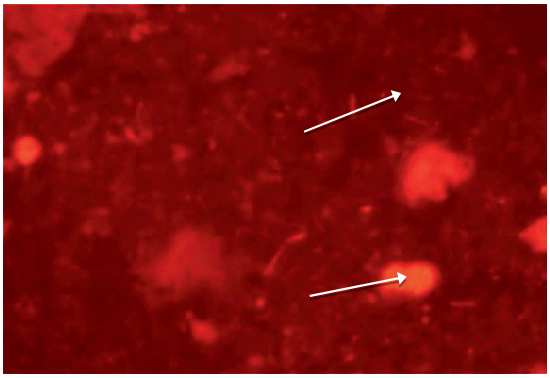

Figure 2. Sludge 2: diverse species of methanogens (*Methanosarcinales*, *Methanobacteriales*; probe ARC915).

Conclusions

Compared to the study of enrichment cultures, FISH gives faster results with less effort. In the cases studied it was fairly easy to identify a stressed anaerobic culture, in the one case, through the reduced variation of micro-organisms. This could be linked to high pH and high concentration of nitrogen resulting from a protein rich feedstock. The other reactor received a more varied substrate and displayed a good variation of the microbial community and hence a good biogas production.

References

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