Screening for Methane Productive Microbial Communities

Y. Isaieva¹, L. Khrokalo²

¹. Faculty of Biotechnology and Bioengineering, National Technical University of Ukraine “Kyiv Polytechnic Institute”, UKRAINE, Kyiv, Peremohy Ave. 37, E-mail: isaeva7@ukr.net

². Faculty of Chemical Technology, National Technical University of Ukraine “Kyiv Polytechnic Institute”, UKRAINE, Kyiv, Peremohy Ave. 37, E-mail: lkhrokalo@gmail.com

Abstract – The methane production of accumulative cultures of bacterial communities was investigated. The accumulative cultures were isolated from substrates: fermented residue from laboratory reactors, pig manure, poultry manure and sludge from the preliminary setting tank of the waste water treatment station. In the experiment culture method of bacterial communities growing on the liquid mineral medium with adding yeast extract and deoxidizing agent was used. The composition of metabolism gaseous products was analyzed on the chromatograph LHM-8MD. The most productive among accumulative cultures was the culture separated from poultry manure (content of methane was 33.08 vol. %). The samples of accumulative cultures before and after antibiotic processing were microscoped. Methanogenic bacteria of genera Methanosarcina and Methanothrix were identified. Basically, the storage cultures processed by antibiotic produced less of methane than ones before processing.

Keywords – bacterial anaerobic community, Zylina’s culture media, methanogenic bacteria, methane production, gas chromatography.

I. Introduction

Methane fermentation of organic waste and biomass on an industrial scale provides the agricultural industry with alternative renewable energy (biogas), high-quality organic fertilizer and helps to reduce human impact on the environment. Modern conceptions of methane fermentation processes are based on the research in biochemistry and microbiology of natural anaerobic biotic community (swamp, rumen, etc.) and medium of an industrial reactors (methane tanks). In this direction the studies of taxonomy, ecology, energetics and molecular biology are conducted. Advances in the study of the species composition of communities and their methane producing capacity and speed of fermentation make it possible to optimize this process on an industrial scale.

Ukraine needs to solve the problem with alternative energy. Thus, the research of methane digestion processes is important for development of new technological decision. The aim of this work was studying methane productive anaerobic community.

II. Materials and Methods

The culture method of anaerobic bacterial communities on the liquid mineral modified Zhylin’s medium with the addition of yeast extract (2 g/l) and deoxidizing agent – sodium sulfide (0.5 g/l) was used in the experiment. The methanol (10 ml/l) and sodium acetate (5 g/l) were used as the carbon source. The gas argon was passed through the culture medium to remove traces of oxygen. The solution of 0.2% resazurin sodium salt was used as the indicator of anaerobic conditions. The antibiotic ampicillin (0.12 g/l) was used for purification of the methanogenic bacterial cultures [1].

Cultivation was carried out in the thermostat at the temperature of 37 °C (mesophilic mode).

The composition of the gas phase was analyzed on the chromatograph LHM-8MD. The content of gaseous metabolic products of microbial communities (H₂, CO₂, N₂, CH₄) was calculated by the standard method of calculating areas of their peaks recorded by the tape recorder.

The microslides of cultures were stained by Gram and fuchsin. The microscopy was performed with the light microscope. The microslides were viewed under immersion at the magnification in 1500 times. The bacterial identification was provided by keys [1, 2].

The methane production of accumulative cultures of bacterial communities was investigated. The accumulative cultures were isolated from substrates: fermented residue from the laboratory reactors, pig manure, poultry manure and sludge from the preliminary setting tank of the waste water treatment station.

III. Results and Discussion

The accumulative culture derived from poultry manure on the medium with methanol was the most methane productive (33.08 vol. %). The culture derived from the fermented pig manure + wood on the medium with methanol was poorly productive (content of methane was 3.18 vol. %) (Fig. 1).

Among all the cultures processed with the antibiotic the culture isolated from sludge of the preliminary setting tank on the medium with sodium acetate had the highest yield of methane (32.28 vol. %) (Fig. 2). We can assume that this was due to inhibition of methanogenic bacteria with oxygen consumed by facultative anaerobes in the accumulative cultures of anaerobic bacterial communities.

The microscopy of antibiotic-processed cultures showed that the effect of the antibiotic was not effective enough, since gram-positive bacilli (probably putrefaction clostridia) were discovered (Fig. 3, a, b). The capsules of exopolysaccharides were found in foreign bacteria. The
Exopolysaccharides are released by the bacterial cell and surrounding them as unpainted rings. It is known that exopolysaccharides are a screening barrier to protect cells from the effects of antibiotics. Thus, the ineffectiveness of antibiotic in the experiment could be determined by the presence of capsules in bacteria-satellites.

Fig. 2. Dynamics of methane emission by storage cultures of anaerobic communities processed with the antibiotic: a) on the medium with sodium acetate; b) on the medium with methanol; 1 – fermented pig manure + wood; 2 – fermented pig manure; 3 – sludge; 4 – pig manure; 5 – poultry manure

Gram-negative bacilli, which are much smaller than *Methanosarcina*, may be members of the genus *Methanothrix* (Fig. 3, c).

Gram-positive and gram-negative cocci that are seen in the figures (Fig. 3, a, b, c) are similar in their morphological features to the bacteria genus *Methanosarcina*. They are irregular spherical cells, arranged singly or forming specific cell aggregates. Small units usually have views of packages of sarcinas ("pseudosarcinas"), but the plane of cell division is not perpendicular to one another. Gram coloration varies [2].

**Conclusion**

The most productive among accumulative cultures was the culture separated from poultry manure.

The culture processed with antibiotic had lower productivity of methane. Thus, the methanogenic communities are more productive than the accumulative cultures of pure methanogenic bacteria after processed by antibiotic.

Among methanogenic microorganisms the representatives of genus *Methanosarcina* and *Methanothrix* were identified.

**References**
