AVIATION IN THE XXI-ST CENTURY

INTERNATIONAL CIVIL AVIATION ORGANIZATION
NATIONAL ACADEMY OF SCIENCES OF UKRAINE
MINISTRY OF EDUCATION AND SCIENCE,
YOUTH AND SPORT OF UKRAINE
NATIONAL AVIATION UNIVERSITY

PROCEEDINGS

THE FIFTH WORLD CONGRESS "AVIATION IN THE XXI-st CENTURY"

"Safety in Aviation and Space Technologies"

Volume 2

September 25-27, 2012
Kyiv, Ukraine

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SELECTION OF METHANOGENIC BACTERIA FOR IMPROVING THE BIOGAS PRODUCTION

Overview of methane digestion and characteristic bacteria is provided. Selection of methane-producing bacteria pure cultures conducted on Zhilina's grows media with methanol and sodium acetate from storage cultures on a base of fermented residue from pig manure and wood, pig manure, poultry excrements, sewage sludge. Emissions of gases were analyzed on gas chromatograph. Pure cultures of the family Methanosarcinaceae were obtained on agar medium by the method of Hungate.

Biogas, the perspective renewable fuel has obtained from different wastes and biomass sources used in heat and electricity power engineering and transport (aviation) as well. The components of biogas are CH_4 (55-80 %), CO_2 (15-45 %), H_2 (1-4%), H_2S and mercaptans (about 3 %), NH_3 and N_2 (less than 1 %). Biogas is obtained by anaerobic digestion of organic compounds due to enzyme activity of specific bacteria community. The heat capacity of biogas is strongly depend on methane content and determine by substrate (feedstock) and parameters of fermentations such as temperature, pH, ammonium content *etc*. Other important aspect of fermentation activity is composition of bacteria community. Common anaerobic digestion consists of a series of bacterial events that convert organic compounds to methane, carbon dioxide, other primary compounds and new bacterial cells. These events are commonly considered as a three-stage process. The first stage of the process involves the hydrolysis of solids (particulate and colloidal wastes). The hydrolysis of these wastes results in the production of simplistic, soluble organic compounds (volatile acids and alcohols).The second stage of the process, acetogenesis, involves the conversion of the volatile acids and alcohols to substrates such as acetic acid (CH_3COOH), carbon dioxide and hydrogen gases. The third and final stage of the process, methanogenesis, is provided by methane-forming bacteria and lead to methane production.

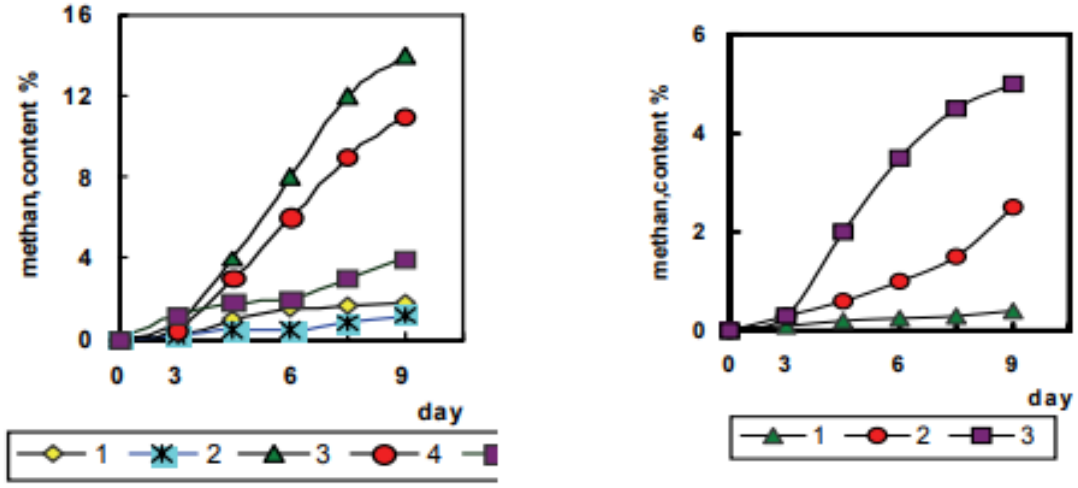
Methane-forming bacteria (other name methanogenic) are singular group of organisms which can produce methane (no other organism produces methane!). Methanogenic bacteria are morphologically various groups that have diameter sizes of individual cells 0.1–15 × 10^{-6} m of different shapes and growth patterns. There can be found as individual rods, curved rods, spirals, cocci or grouped as irregular clusters of cells, chains of cells or filaments, and sarcina or cuboid arrangements. Methanogenic bacteria are some of the oldest bacteria and are grouped in the domain *Archaea.* This domain is also included extremely halophilic bacteria, thermoacidophilic bacteria, and extremely thermophilic bacteria. However, the methane-forming bacteria are different from all other bacteria. They are oxygen-sensitive strict anaerobes, tolerate high concentrations of salt and could be found in habitats that are rich in destructive organic compounds in which oxygen is rapidly removed through microbial activity. Many ones occur as symbionts in animal digestive tracts. The rumen is a special organ in the digestive tract of cows, goats, sheep and some other herbivores, in which the destruction of cellulose and complex polysaccharides occurs. Ruminants cannot survive without such bacteria. The bacteria inhabit digestive tract obtain compounds and energy and ruminant animals get simply assimilated nutrients. Methane-forming bacteria also have unusually high sulfur content: approximately 2.5% of the total dry weight of the cell is sulfur. Some ones are capable of fixing molecular nitrogen. Methane-forming bacteria are classified according to their structure, substrate utilization, types of enzymes produced and temperature range of growth. Recently more than 60 species of methane-forming bacteria are identified. In microbial consortia methanogenic bacteria grow on limited number of substrates. *Methanobacterium formicium* (most

abundant bacteria in nature) grows on hydrogen and carbon dioxide and formate. But the majority of methane is produced by two genera of acetotrophic methanogens, *Methanosarcina* and *Methanothrix*. All methane-forming bacteria grow best in an environment with an ORP (oxidation-reduction potential) less than – 300 mV [1] .

The samples for cultivation of methanogenic bacteria involved in original experiment were such: fermented residue from pig manure and wood wastes; fermented residue from pig manure; pig manure; poultry excrements; sludge from station of waste water treatment. Storage bacteria culture have been cultivated in strict anaerobic conditions and temperature + 30 ^{0}C on Zhilina's liquid grows media with pH 6,0-8,0 (KH_2PO_4 – 1,5 g and NH_4Cl – 1,5 g dissolved in 500 ml of distilled water; $MgCl_2 \cdot 6H_2O$ – 0,15 g and $CaCl_2 \cdot 2H_2O$ – 0,3 g – in 500 ml; $NaHCO_3$ – 1,5 g and $Na_2S \cdot 9H_2O$ – 0,75 g – in 350 ml; yeast solution – 0,15 g in 150 ml of distilled water) sterilized in the autoclave. Storage cultures grew in 250 ml glass bottle filled in half-null by liquid nutrient media and blow out by argon gas using copper needle. Resazurin solution 0,1 % have been used as indicator of anaerobic conditions. Bottles were hermetic closed up by rubber plug kipped by wire yarn. Antibiotic amoxicillin is used for keeping the pure cultures of methane-forming bacteria 0,12 g/l. Selection of methanogenic bacteria have been made by grow of storage culture patterns on Zhilina's liquid media with add-on source of carbon feed. Methanol or sodium acetate was the carbon sources for growing of pure culture of methanogenic bacteria. Finally, the reinoculation of methanogenic bacteria has been made on solid agar medium a modified method by Hungate [2].

The purity of selection was controlled by microscopy and gas emissions. Gas compounds have been analyzed by gas chromatograph LHM-8MD. Microslides were colored by Gram method and viewed under light microscopy in lens magnification × 1875 and bacteria identification was provided by keys [3, 4].

Cultures that were isolated from different samples were grown for 9 days of the three models each replication according to standard procedure. Since accumulation of anaerobic microbial community biomass the patterns with high emission of carbon dioxide and low content of nitrogen were selected for pure culture of methane-producing bacteria growing. Pure cultures grew on liquid nutrient medium with additions of methanol (first experimental series) and sodium acetate (second series). Cultivation of pure cultures conducted in 9 days, gas emission was estimated on the 3th, 6th, 9th days of experiment (fig. 1).



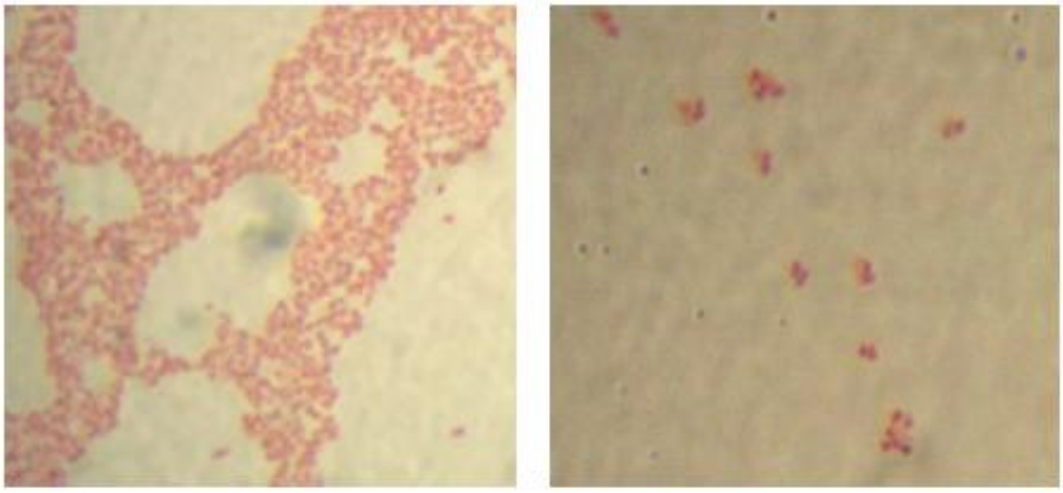
a)
1 - fermented pig manure & wood,
2- fermented pig manure, 3 - pig manure,
4 - poultry excrements, 5 - sludge)

b)
1 - pig manure, 2- poultry excrements,
3-sludge)

Fig. 1. Rates of methane emission of pure bacteria cultures on mediums:
with methanol (a), sodium acetate (b)

High yields of methane were observed in 9-day cultivation in the samples isolated from poultry excreta and sewage sludge on substrates - acetate and methanol, respectively. Obviously, these ecological niches are of great variety and population density of methane-producing bacteria.

Microscopy of cells of pure cultures showed a high number of coccus cells (a) and collected in bags irregular clusters (b), isolated from all samples growing on methanol and sodium acetate. Identification determined, that the isolated cultures may belong to the family *Methanosarcinaceae*.



(a) (b)

Fig. 2. Light microscopy of Gram-negative coccus cells (a), irregular clusters (b), increase × 1875

Conclusions

Selection of methane-producing bacteria pure cultures conducted on Zhilina's grows media with methanol and sodium acetate from storage cultures on a base of fermented residue from pig manure and wood, pig manure, poultry excrements, sewage sludge. Experiment results also demonstrated that activity of anaerobic digestion and methane emission is larger in patterns with bacteria communities than pure culture of methane-producing bacteria. The possibilities of further bacteria selection and construction the preparation of specific bacteria community for industrial application needs to be further investigated.

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