UDC 579.017.7
DOI: 10.17816/edgcc15809

SYSTEMS APPROACH TO THE STUDY OF MICROBIAL METHANOGENESIS IN WEST-SIBERIAN WETLANDS

O.R. Kotsyurbenko[1,2], M.V. Glagolev[1,2,3,4,5], A.F. Sabrekov[1,2,4,5], I.E. Terentieva[1,4]

[1]Yugra State University, Khanty-Mansyisk, Russia
[2]Institute of Water Problems, Russian Academy of Sciences, Moscow, Russia
[3]Lomonosov Moscow State University, Moscow, Russia
[4]Tomsk State University, Tomsk, Russia
[5]Institute of Forest Science, Russian Academy of Sciences, Uspenskoe (Moscow region), Russia

Corresponding author: kotsor@mail.ru

Citation: Kotsyurbenko O.R., Glagolev M.V., Sabrekov A.F., Terentieva I.E. 2020. Systems approach to the study of microbial methanogenesis in West-Siberian wetlands // Environmental Dynamics and Global Climate Change. Vol. 11. N.1. P. 54-68.

DOI: 10.17816/edgcc15809

Text of the article in English https://edgccjournal.org/EDGCC/article/view/15809

Abbreviations:
VFA – volatile fatty acids;
IPCC – Intergovernmental Panel on Climate Change.

This work is a lecture adapted to the format of a journal article on the course "Modern Topics in Biology", delivered by one of the authors in Yugra State University.

The modern stage of the development of science and biology, in particular, is characterized by a systematic approach to the evaluation of various phenomena. In the concept of hierarchical holism, which dominates the systems approach, various biological systems form a hierarchical structure in which an element of one system is an independent system of a lower level. In any individual system, the key points are the interaction of its components and the structure that determines the stability of the system. The microbial systems of wetlands in West Siberia play a crucial ecological role in the context of the problem of greenhouse gases and changes in climate and atmospheric composition. The greenhouse gas methane entering the atmosphere is formed by the methanogenic microbial community, which is a complex biological system containing microbial groups which are closely related to each other by trophic interactions. The result of the work and the efficiency of the methanogenic microbial community is also determined by various physicochemical parameters of the environment. The main microbial agents responsible for the production of CH_4 are methanogenic archaea, which are divided into three main trophic groups. The application of a systematic approach to the study of the methane cycle in wetlands of West Siberia allows us to comprehensively evaluate the vertical and horizontal system relationships, identify key elements and conduct a complex analysis of the problem under study.

Key words: System approach, methanogenesis, greenhouse gases, West Siberian wetlands, microbial communities, methanogens

BASIC PRINCIPLES OF SYSTEM APPROACH TO STUDY OF BIOLOGICAL SYSTEMS

The idea to consider the object under study as a whole system is mentioned in ancient philosophy and science, for example, in Anaxagoras's natur-philosophic theory of the indestructible elements stating that all things emerge due to the combination of the qualities of these elements (c. 500 BC – 428 BC) or in Aristotle's systemic worldview of space and biological expediency (384 BC – 322 BC). Later in the Middle Ages, the idea of a system organization of knowledge was suggested and then most developed in German classical philosophy and, in particular, in the ideas about the system interrelationship of animate and inanimate nature of I. Kant (1724–1804) and the necessity for subordination and coordination in the concept of I.G. Lambert (1728–1777).

In biology, the first ideas of system organization were associated with the views of Claudius Galen (ca. 130 – ca. 217), who postulated a different systemic function of arterial and venous blood in the human body. Much later, attempts to systematize nature as a whole and to study the influence of environmental factors on organisms were related to the works of C. Linnaeus (1707–1778), J.-B. Lamarck (1744–1829), C. Darwin (1809–1882) and I.V. Vernadsky (1863–1945).

In the early 1920s the Austrian biologist Ludwig von Bertalanffy (1901–1972) began to study living organisms as systems and summarized his findings in the book "Modern Theories of Development" (1929). It is Bertalanffy who is considered to be the developer of a system approach to the study of biological organisms, although he had several predecessors, in particular, the Russian scientist A. A. Bogdanov (1873–1928), who was developing the theory of organization.

In the 30s of the twentieth century, Bertalanffy created a general theory of systems – a scientific and methodological concept for the study of objects, including living organisms, which were considered as systems that constantly exchange matter and energy with the environment. In the framework of this theory, a system is a set of interacting elements, which, in turn, can be a system of a lower level. The structure of the system is a set of stable relationships between elements. The properties of an object as an integral system are determined by the special system-forming emergent properties of its structure, rather than by simple summation of the properties of its individual elements [Glagolev and Fastovets, 2012].

Further development of systems theory within the framework of biology led to the emergence of systems biology. Currently, this scientific direction has gained a powerful experimental base with the development of various omix technologies in biology and the accumulation of a huge amount of data that require processing and systematization.

The term "system approach" was being actively used in Russian scientific literature since the 1970s. In the English literature, “system analysis” and “systems theory” are more often used to denote this concept.

In general, the system approach is focused on studying the object as an integrated system, on identifying its elements and types of interactions between them and eventually on summarizing the results in a single theoretical concept. The structural similarity of laws established in various fields of science and practice allows identifying system-wide patterns that are the basis of a system approach.

SYSTEM APPROACH ON A FORMAL LEVEL

In general terms, the basic idea of system approach is to build a holistic picture of the object. If an ecosystem is considered as an object, then a system approach consists in (i) determining its constituent parts and environmental compartments interacting with it; (ii) establishing an ecosystem structure, i.e. the totality of internal links and relationships, as well as the relationships between the ecosystem and the environment and (iii) finding the functionality (the principle of functioning) of the ecosystem that determines the nature of the change in its components and the relationships between them under the influence of external objects [Fedorov and Gilmanov, 1980, 49, 52]. Back in the last century, there was a very clear pattern of applying the system approach (Table 1).

As the table 1 shows, the modern implementation of the system approach is, in fact, aimed at creating a mathematical model of the system. In this regard, the different stages of the system approach relate to the different scientific disciplines and should be performed by a team of relevant specialists, such as biologists, physicists, mathematicians and programmers. However, in this article, the discussion of the phenomenon of methanogenesis in wetland ecosystems is limited to the framework of biology, and, accordingly, to only the stages of “Conceptualization” and “Specification”. For a spacious approach to this problem, there is quite extensive information regarding the structure of mathematical models of methanogenesis [Kalyuzhny et al., 1988; James, 1993; Grant, 1998; Sabrekov and Glagolev, 2008].

SYSTEM APPROACH IN MICROBIOLOGY

In relation to microbial ecology and biogeochemistry, it is necessary to note the outstanding works of the Russian academician G.A. Zavarzin [1933–2011], who actively used the basic principles of a system approach to the study of microbial communities and their role in global ecology [Zavarzin, 2011]. Microorganisms associated in communities based on trophic interactions are complex biological systems involved in various biogeochemical cycles of elements on Earth.

The application of system approach to the study of biological systems is associated with the development of the concept of biological organization levels. For the most comprehensive analysis of the system, it is first necessary to determine its hierarchical level and its relationships with other systems. It is generally accepted to assign the whole organism to the organism level, its subsystems (for example, cells) to the suborganismal level, and the population-species organization to supraorganismal levels. However, when considering microbial systems, the researchers mostly deal with a single unicellular organism. In this case, a cell, for example, a bacterial cell as a unit of living matter, is considered at the organism level. Then systems of a higher and a lower (e.x. cellular structures) levels belong to supraorganismic and suborganismic levels, respectively. Finally, the interaction of various systems characterizing the biological organization levels and their relationship with the environment is so close that a comprehensive study of any systemic phenomenon requires determining its position in the hierarchical rank of subordinate systems and determining the main intersystem and intrasystem interactions.

The most accurate theoretical concept of this approach is hierarchical holism [Zavarzin, 1995a], which has been created in modern natural science and, as applied to biology, claims that biological phenomena or processes in nature are combined into systems, each of which can in turn be an element of another system of a higher rank and the precision in describing the studied object is dependent primarily on the correct identification of the system in which this object operates. An incorrect characterization of the system under study leads to false conclusions.

The variety of levels of the structural biological organization specifies the differentiation of biology, in which each such level is studied in more detail by the corresponding biological science. Additionally, a system approach promotes the integration of individual biological disciplines, offering a more general view of various phenomena that can only be understood by their coordinated investigation in various biological disciplines.

One of the challenging issues of global ecology at present is the problem of climate change caused by the emission of greenhouse gases into the atmosphere. Vast areas of West Siberian wetland ecosystems are the powerful sources of these gases. The application of a system approach to the study of this issue enables defining the appropriate hierarchy of interacting systems and their elements that play a key role in the biological processes under study (Fig. 1) and analyzing them by the use of a proper methodology.

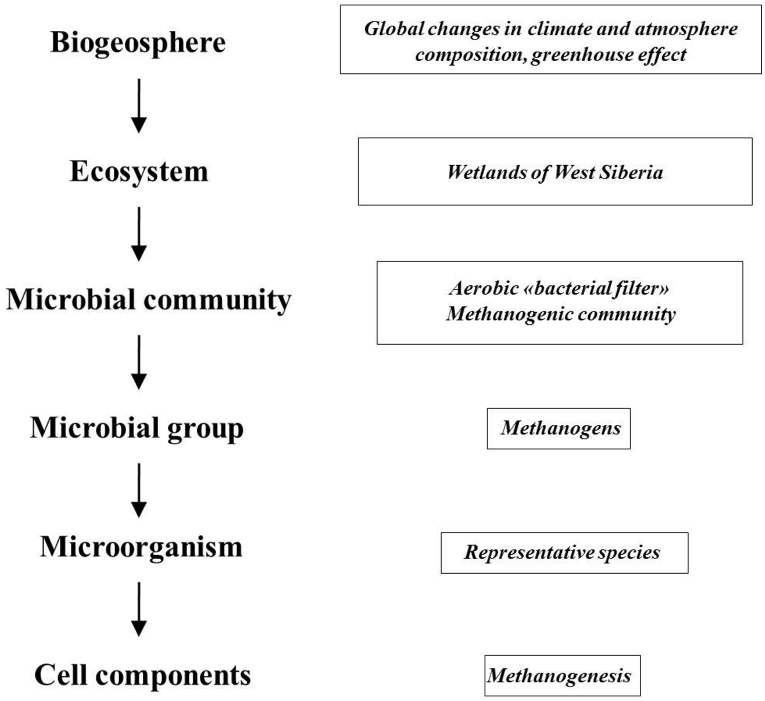

Fig. 1. Systems level hierarchy and key elements in the context of problem of methane emission into the atmosphere.

BIOSPHERE AND GLOBAL CLIMATE CHANGE

Changes in the composition of the atmosphere and climate is a planet-scale problem. These changes are caused by the permanent increase in atmospheric concentration of the main greenhouse gases – methane and CO2 [IPCC, 2013]. Along with an increase in the concentration of these gases, the rise of the average global temperature on Earth is observed at a rate of about 0.15°C per decade (Fig. 2). Such a temperature rise can subsequently have a strong impact on various regions of Earth. These impacts can be the melting of permafrost and polar glaciers, an increase in the frequency of extreme weather events and global changes in climatic zones.

Methane is the second most important greenhouse gas after CO2. Despite the fact that the concentration of methane in the atmosphere is much lower than that of carbon dioxide, the CH4 molecule has the global warming potential (GWP) that is 28 times stronger when calculated over the past 100 years, and 84 times stronger for a 20 years perspective than GWP of the CO2 molecule [IPCC, 2014, p. 87].

Another important feature of methane is its primarily biological origin. Totally, the microbial communities of various ecosystems generate 85-90% of methane passing to the atmosphere [Ehhalt and Schmidt, 1978]. Methane is released in the atmosphere unequally. Its basic amount was determined to be emitted by the ecosystems of northern latitudes, and more precisely by wetland ecosystems located in the boreal climate zone [Matthews and Fung 1987; Cicerone and Oremland 1988; IPCC 2013, p. 505–510].

Thus, the investigation of the role of methane as a greenhouse gas in the atmosphere at the level of the biosphere is related to identifying its most powerful sources. Numerous measurements

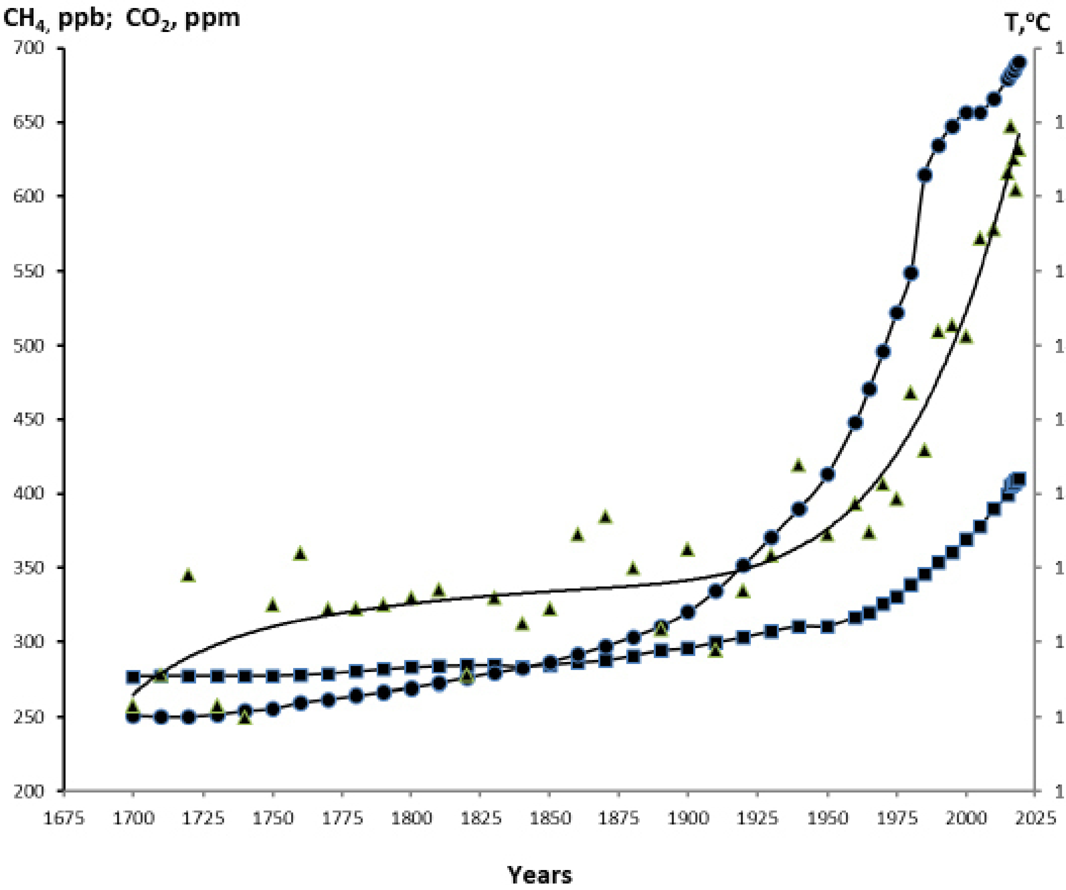

Fig. 2. The rise of temperature (▲) and atmospheric concentrations of greenhouse gases (CH_4 (●) and CO_2 (■)). Charts are plotted according to the data from: https://www.methanelevels.org/.

of methane emissions in various ecosystems are carried out to determine such sources and assess their potential [Adhya et al., 1994; Butterbach-Bahl et al., 2004; Kutzbach et al., 2004; Glagolev and Shnyrev, 2007]. The results of such measurements are processed and systematized in the framework of the Intergovernmental Panel on Climate Change (IPCC), which submits periodic reports. Such reports summarize the results of measurements of CH4 concentrations and its emission rates obtained by researchers using various methods [Glagolev and Suvorov, 2007; Haddaway et al., 2014; Anisimov and Kokorev, 2015; Kotsyurbenko and Glagolev, 2015, Lin et al. 2015; Terentieva et al., 2016; Li et al. 2019] and at different scales (local, regional, continental or global). Depending on the method used and the scale under consideration, stationary ground chambers, meteorological towers, airplanes, or satellite data can be used for measurements. Since it is impossible to make measurements at each geographical point, various types of mathematical models are used to reduce the uncertainties in estimations of the current methane emissions and predict their future changes, [Cao et al., 1995; Glagolev et al., 2012; Sabrekov et al., 2014; Kotsyurbenko et al., 2019].

WETLAND ECOSYSTEM AS A SOURCE OF METHANE

The main natural sources of methane emitted to the Earth’s atmosphere are the waterlogged soils of the boreal zone, which are located mainly in Russia, Canada, the USA and the Scandinavian countries [Glagolev et al. 2012; IPCC, 2013, p. 505–510; Haddaway et al., 2014; Webster et al. 2018]. Russia contains the world's largest territories of wetlands, including the West Siberian Lowland, the largest wetland in the world [Panikov, 1994]. Numerous field studies of these ecosystems have revealed that they are powerful sources of atmospheric methane [Panikov, 1995; Glagolev and Suvorov, 2007; Glagolev and Kleptsova, 2009; Sabrekov et al., 2014].

Wetlands of Western Siberia are characterized by great diversity and are divided into lowland, upland, and transitional ones according to their position in the relief; whereas their biogeocenoses are classified into eutrophic, oligotrophic and mesotrophic ones depending on the mineral nutrition type. Each of these systems is characterized by its vegetation and peat type [Masing, 1974; Liss et al., 2001, p. 84–85, 196–198]. Lowland fens are rich in minerals and, normally, contain diverse herbaceous vegetation and have slightly acidic, neutral, or slightly alkaline values of environmental pH. The upland bogs are mainly fed by precipitation and are therefore poor in mineral components and characterized by plants adapted to living under conditions of limited nutrition and the lowest pH values. The transitional wetlands have intermediate characteristics.

In the climatic conditions of Western Siberia, wetlands are defined as natural ecosystems being influenced by rather low average annual temperatures with their strong fluctuations on the surface in summer and winter seasons and with more stable temperatures in the deep layers [Whalen and Reeburgh, 2000].

The structure of the bog as a typical wetland includes various landscape elements such as hummocks, hollows, etc., which affect the water regime and oxygen balance and cause a high patchiness of greenhouse gas emissions from a single bog system [Sabrekov et al. 2014, 2016]. Depending on the spatial scale, various methods for estimating methane production and emissions are used. The accuracy of the assessment depends on the properly chosen method of measurements, which should take more carefully into account the specificity of a particular methane-generating soil system. In field measurements, chamber methods provide directly information on the specific gas flow from an area equal to the base chamber square, i.e. up to 1 m2. Estimates of flows by the chamber method from a large area can also be made, but the work input will increase drastically due to numerous chamber transpositions or the use of a great number of chambers. Besides, the measurements will be in some degree indirect due to the necessity for their extrapolation to surface areas on which the cameras were not placed. Micrometeorological methods give reliable estimates of greenhouse gas emissions, normally in the range of 105 ÷ 106 m^2 (for specific towers, on which the sensor is attached at heights of ~ 60 m, the footprint can increase up to 108 m^2), despite spatial and temporal heterogeneities in the system [Glagolev, 2010; Chen et al., 2010; Glazunov et al., 2016]. Moreover, the extremely important feature micrometeorological methods attributable to their physic basis is automatically averaging all heterogeneities in the system and generating an average flow data over the area. Receiving such data with the chamber method is very difficult because of the necessity to carry out numerous measurements on each type of microrelief (hummock, hollow, etc.) and take into account the proportion of each such landscape element to the total area of the wetland.

Altogether, various methods make it possible to compile a complete picture of methanogenesis and measure the rate of its emission into the atmosphere. Moreover, they allow measuring both detailed (taking into account heterogeneities of the spatial structure and specificity of physicochemical conditions at the measurement site), and averaged flow rates in the ecosystem under study. The latter is important for assessing the significance of a particular ecosystem as a source of atmospheric methane and increases the accuracy of forecasting greenhouse gas flows.

MICROBIAL METHANE CYCLE

Methane is formed in terrestrial natural ecosystems due to the activity of microorganisms. Its release into the atmosphere is the result of the incompleteness of the microbial cycle of methane, which is also called the Söhngen cycle [Zavarzin, 1995b]. The methane cycle is driven by the interaction of methanogenic and methanotrophic microbial communities [Zavarzin, 1995b; Kallistova et al., 2017], which are clearly spatially separated due to different attitudes towards oxygen. Microorganisms involved in the formation of methane are anaerobes. Methanogenesis ceases in the presence of O_2. In contrast, microbial oxidation of methane occurs with the participation of oxygen. In the water layer, the concentration of dissolved oxygen decreases and anaerobic conditions are created at a certain depth under which the methanogenic community can operate. Methane is formed as a result of anaerobic degradation of organic matter and then enters the upper layers, where, it is oxidized by aerobic methanotrophs in the presence of oxygen. Methanotrophic microorganisms are the main component of the so-called bacterial filter, a community of aerobic bacteria that trap out the products of activity of anaerobic microorganisms coming from the lower layers. If all the methane formed in the anaerobic zone is trapped by the bacterial filter, then the methane cycle is closed. However, in many types of wetland ecosystems, part of the methane passes through a bacterial filter and releases into the atmosphere, where it can then participate in various photochemical reactions and contribute to the greenhouse effect.

Thus, the microbial methane cycle is a complex system in which trophic interactions between its two main components (methanogenic and methanotrophic microbial communities) occur [Zavarzin, 1995c; Conrad, 2007]. The activity of these communities is regulated by various environmental factors such as temperature, pH, Eh, oxygen and mineral elements concentrations, availability of organic substrates, etc. Since these communities are spatially separated, their regulation by the above factors can occur to a large extent independently. Specifically, temperature conditions directly affecting the rate of metabolic processes can be substantially different on the surface and in the deep peat layers, and these differences can vary depending on the season. Accordingly, the result of the methane cycle operation should be also variable.

Due to the region specific features, the West Siberian waterlogged soil ecosystems are one of the most powerful natural sources of methane. In extensive wetlands area, the environmental conditions are favorable to anaerobic methane formation, and cold conditions impede the activity of bacteria in the aerobic filter that facilitates the emission of methane into the atmosphere.

Due to the spatial separation of two microbial communities, the process of delivery of the substrate (methane) from one community to another has a certain specificity. Methane enters the upper layers in three main ways: (i) by diffusion, (ii) with gas bubbles, or (iii) due to poorly studied transport mechanism associated with plants [Lansdown et al. 1992; Kutzbach et al. 2004].

Thus, the methane cycle is a complex system of interaction between microbial communities functioning in different spatial layers and is regulated by a large number of factors [Conrad, 1996; Limpens et al., 2008].

To study the operation of the methane cycle, researchers use mesocosms (large soil monoliths with an undisturbed structure) or microcosms (soil mixed with water or culture medium and placed in a container), which are incubated at a given temperature and analyze the dynamics of methane accumulation over time. The concentration of CH4 formed is determined chromatographically. The obtained data can also be used to construct appropriate models of ongoing processes. In the case of using mesocosms with minimal impact on the sample texture, the system generating methane is in a state as close to the in situ conditions as possible.

ANAEROBIC MICROBIAL COMMUNITY

The anaerobic microbial community producing methane is an excellent example of a complex biological system, where all its components are closely related to each other by trophic interactions. Complex organic substances decompose in such a community in stages – the products of one microbial group are substrates of another one. As a result, the total energy of degraded polymer compounds is shared between different microbial groups in the community (Fig. 3).

The feasibility of sequential metabolic reactions in the microbial community is dependent on their thermodynamics. Gibbs energy value of a certain reaction must be negative enough in order to allow the microbial group gaining energy to support life.

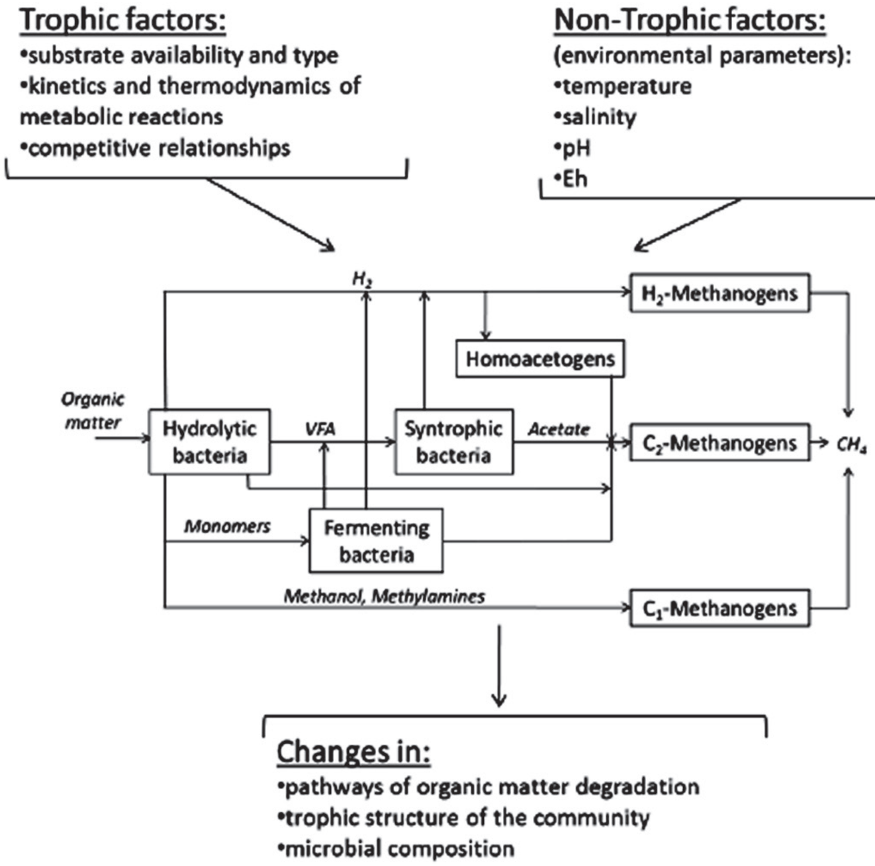

Рис. 3. Methanogenic microbial community as a system, main regulating factors and sustainability strategies.

Anaerobic processes have energy efficiency much lower compared to that of aerobic processes; therefore, anaerobic organisms are forced to form close trophic cooperative relationships (McInerney and Beauty, 1988; Schink, 1997; McInerney et al., 2010).

Depending on the type of organic matter entering the anaerobic zone, the trophic structure of the methanogenic community can vary greatly. If the main primary component is plant debris, then the complete trophic chain is formed. This is the most general situation happened in natural ecosystems.

Cellulose (the main component of plant debris) is broken down by exoenzymes of hydrolytic microorganisms with the release of glucose molecules, which are then consumed by the same hydrolytics, as well as a large group of microorganisms that perform various types of fermentation. The main products of fermentation of glucose and other saccharides as well as compounds of a different biochemical nature, for example, amino acids, which can also be present in the system and decomposed parallel to the main metabolic pathway, are volatile fatty acids (VFAs), as well as hydrogen and carbon dioxide.

Decomposition of VFA is a result of syntrophic relationships. They are the closest microbial interactions in the anaerobic community due to thermodynamics of such processes that become energy-yielding for microorganisms only if the products of these metabolic reactions (acetate and hydrogen) are actively removed from the system as a result of consumption by another microbial group [Schink, 1997; Jakson and McInerney, 2002], usually situated at the very end of the trophic chain. Such a terminal group can comprise microorganisms using simple compounds for their metabolism and performing various types of anaerobic respiration. They also use various inorganic electron acceptors contributing differently to the thermodynamic efficiency of corresponding reactions that decreases in the following order:

$$NO_3^- > Mn^{4+} > Fe^{3+} > SO_4^{2-} > CO_3^{2-}$$

Since in natural wetland systems all electron acceptors, except carbonates, are usually limited, anaerobes that perform the carbonate type of respiration are the main terminal group, and their metabolic products are the end products of the community [Kotsyurbenko, 2005]. In the vast majority of cases, such microorganisms are methanogens belonging to Archaea. However, possible competition between methanogens and other terminal groups, for example, iron-reducing bacteria for common substrates should be also taken into account [Küsel et al., 2008].

The methanogenic microbial system is regulated by various factors, which can be divided into two main groups – trophic and non-trophic (Fig. 3). Trophic microbial interactions are the structural basis for the functioning of the methanogenic community. When balanced functioning, the community as a system has a closed trophic structure and methane is the final product of its work. This is facilitated by stable environmental conditions and regular intake of necessary nutrients into the system. Under these conditions, trophic interactions are formed in the community in such a way that the concentration of intermediate metabolites is at a stable low level. If a linear increase in methane concentration is observed in the system, then the methanogenic system is balanced. Such a state, for example, can be achieved in laboratory incubation experiments in which methanogenic degradation of organic matter occurs under controlled conditions.

In nature, this state can be achieved transiently. Seasonal changes in environmental conditions, and particularly temperature as a non-trophic factor, affect the stability of the methanogenic community, which tends to adapt to the changes through a rearrangement in its trophic structure. As a result, the main pathways of degradation of organic compounds can also change, which leads to redirection of organic matter flows in the microbial community.

The main changes in the trophic structure of the methanogenic community are related to changes in metabolic pathways driven by the same microbial group to produce, for example, more reduced compounds, or to the replacement of key microbial groups in the trophic chain. The former changes are most characteristic of microbial groups located at the beginning of the trophic chain and capable of fermenting complex organic compounds with the formation of several products. For example, if the consumption of hydrogen, the main intermediate compound in the microbial system involved in various regulatory interactions, is impeded, then the fermentation shifts from acetate as the most oxidized product to ethanol or more reduced forms of VFA in order to compensate for the excess of reducing equivalents in system. The latter change relates more to the terminal microorganisms using simple compounds and synthesizing one or two end products.

In general, the strategy of the methanogenic microbial community is aimed at maintaining its basic system structure under changing environmental conditions. To determine the metabolic potential of a single microbial group, which is a part of the methanogenic community, the method of introducing elective substrates and the study of the dynamics of the products accumulated at their decomposition is used. The possibility of varying the conditions in incubation experiments allows obtaining data for Ⓢodeling microbial methanogenic processes.

To determine the rate of a particular methanogenesis pathway, specific inhibitors are used that block either general methanogenesis or its certain metabolic pathways [Conrad, Klose, 2000: Kotsyurbenko et al., 2004, 2007], as well as radioactive substrates of methanogens are introduced into the system, which are converted to radioactive methane during the activity of microorganisms that can be further detected by special radioactivity counters [Conrad, Klose, 1999].

SPECIAL ASPECTS OF THE EXISTENCE OF MICROBIAL COMMUNITIES AS A BIOLOGICAL SYSTEM

The basic principle of hierarchical holism is the subordination of various systems. Microbial communities are key systems in environmental microbiology. Elements of such systems are microorganisms as units of living matter. They are trophically connected and actively interacting with the environment. The main systemic properties of the microbial community were examined in detail in the works of G.A. Zavarzin [2015].

The formation of the microbial community occurs according to the systemic principle of expediency. The inclusion of an organism in its composition does not depend on how high the potential this organism has in relation to a certain metabolic process, but depends on how beneficial is its inclusion for the community in relation to increasing the environmental potential of the community as a whole and increasing its sustainability as a system.

In the meanwhile, microorganisms included in the community enter into cooperative mutually beneficial relations. This is an indication of the fact that the microbial community is formed on the basis of non-Darwinian laws and the main driving force in its formation is cooperation rather, than competition. The latter only plays a regulatory role [Zavarzin, 1995a].

The proper functioning of a community depends on the coordinated work of all its elements, and its structural stability is determined by Le Chatelier's principle, formulated initially for physical and chemical systems: if a system in equilibrium is subjected to any influence deviating it from an equilibrium state, then the appropriative processes in the system occur that counteract this deviation. In this regard, the community as a system resists the inclusion of an alien element in its structure that can reduce its stability. The artificial introduction of an organism into the natural system will lead to its elimination in case of a conflict with the systemic principle of expediency for the microbial community.

The stability of the microbial community as a system is determined by its ability to maintain the basic structure under the influence of various environmental factors. The stability of such a biological system depends on keeping its functional significance for the external ecosystem – a system of a higher order. The methanogenic microbial community has various mechanisms of adaptation to changing environmental conditions. As already mentioned, the system reacts to the impacts of various environmental factors by restructuring its trophic structure and changing the composition of key microorganisms (Fig. 3).

The more diverse the metabolic pathways and microbial groups performing them, the higher is the ability of the community to adapt. An important systemic feature of the microbial community is the preponderance of the functional constituent over the taxonomic one. For example, it was found that in different types of wetlands (eutrophic, mesotrophic and oligotrophic) methanogenic microbial communities are functionally similar, but can be different to a great extent taxonomically [Hunger et al., 2015]. The key microbial groups characteristic of the trophic structure of each of these communities can be represented by phylogenetically different microorganisms [Stoeva et al., 2014]. In other words, the diversity of metabolic pathways and taxonomic groups in the community is an important mechanism for maintaining the function.

The more extreme the external conditions, the less microbial diversity, and the more difficult is for the microbial community to maintain the functionality when the external conditions change and, hence, the lower is its adaptive potential. Nevertheless, under quite stable conditions, a microbial system consisting of extremophilic microorganisms is formed. Its sustainability is primarily determined by specific conditions of the ecological niche, in which unique non-competitive adaptive survival mechanisms of extremophiles and their proper functionalities are in demand.

Thus, the functionality is the most important characteristic of the microbial community and determines the feasibility of its existence in a particular ecosystem. Specialization increases the potential of an organism to perform a function, while versatility increases its adaptive capabilities. Obviously, nature chooses one or another strategy depending on ecosystem requirements. If the adaptation capabilities of the community are not enough to withstand external impacting factors, in other words, if the impact on the system is too strong, then Le Chatelier's law is no longer fulfilled, and the community is to be destroyed or changes its structure, which means a transition to a new stationary state – a new biological system.

METHANOGENS AS A KEY MICROBIAL GROUP AND THEIR COMPETITORS

Methanogenic archaea are situated at the end of the trophic chain of the anaerobic microbial community, which forms methane as the final product of complex organic matter decomposition. In the absence of electron acceptors other than CO_2, methanogens are the only terminal microbial group. This situation is especially characteristic of bog ecosystems depleted in mineral components.

In the trophic chain of the anaerobic microbial community, methanogens are represented by three groups: hydrogen-consuming (with CO_2 as a carbon source and electron acceptor), acetate-consuming (acetoclastic) and using simple single-carbon (C_{1-}) compounds (methylotrophic). In freshwater terrestrial ecosystems, including wetlands, the main trophic groups of methanogenic archaea are acetoclastic and H_{2-} consuming methanogens. The percentage ratio of these two pathways of methanogenesis in the ecosystem is an important characteristic of the microbial community that degrades organic matter. Usually, the ratio of acetoclastic and hydrogen-dependent methanogenesis is 2/1 in a balanced system at moderate temperatures, neutral pH, and the absence of electron acceptors other than CO_2. This is due to the fact that the decomposition of organic matter in natural terrestrial ecosystems normally occurs with the formation of approximately 1 mole of acetate and 2 moles of hydrogen, when methanogens are the terminal group. Then, the above ratio (2/1) of these methanogenesis pathways is composed based on the stoichiometry of the reactions of methane formation from acetate and hydrogen and CO_2,

$$CH_3COOH = CH_4 + CO_2 \qquad (1)$$

$$4H_2+CO_2 = CH_4 + H_2O \qquad (2)$$

A shift in this ratio can be due to a change in set of products of organic matter degradation. The formation of more hydrogen equivalents or consumption of acetate as a result of another metabolic process shifts the ratio toward H_2-methanogenesis, whereas an increase in the competition of methanogens with other microbial groups for hydrogen is favorable to acetoclastic methanogenesis.

In freshwater terrestrial systems, the main competitors of methanogens for hydrogen are homoacetogenic bacteria, which also perform the autotrophic consumption of hydrogen and carbon dioxide, but with the formation of acetate instead of methane:

$$4H_2 + 2CO_2 = CH_3COOH + 2H_2O \qquad (3)$$

Homoacetogens have a higher growth rate compared to methanogens at low temperatures, typical of most terrestrial ecosystems, including bog systems, and are able to outcompete them for hydrogen if it is available in excess [Kotsyurbenko et al., 2001]. However, usually, under natural conditions, methanogens intercept H_2, reducing and keeping its concentration inaccessible to homoacetogens due to a much higher affinity for this substrate. As a result of this competition, the consumption of hydrogen can take place via the homoacetogenic pathway A–C–B with subsequent consumption of acetate by methanogens and a corresponding increase in the contribution of acetoclastic methanogenesis to the total production of CH_4 rather, than via the methanogenic pathway A – A (Fig. 4a).

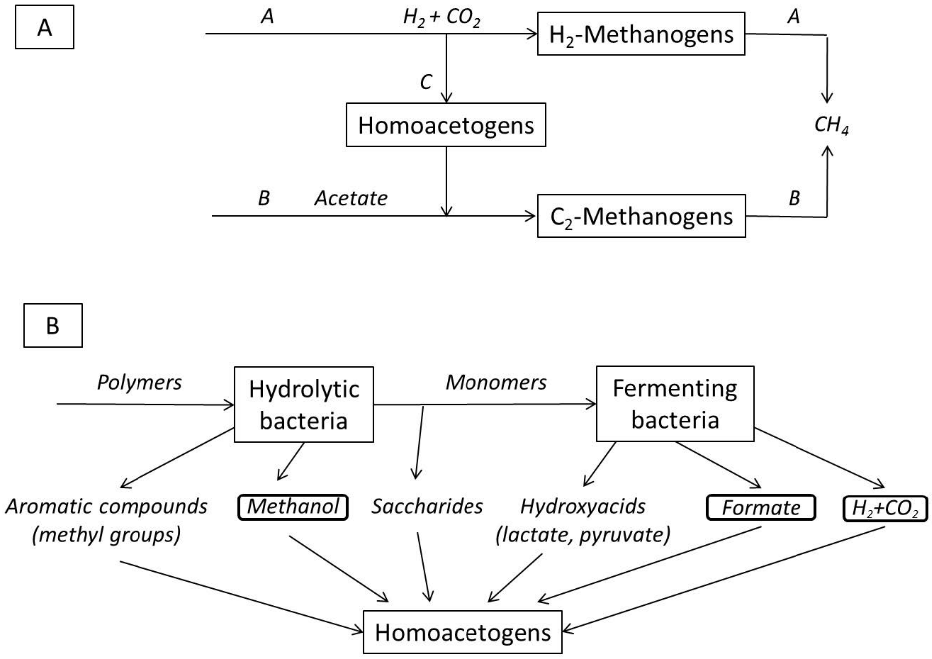

Рис. 4. Trophic relationships between homoacetogenic bacteria and methanogenic archaea in the anaerobic microbial community. **A** – The main matter flows passing through the terminal microbial groups of the methanogenic community, **B** – The availability of substrates for homoacetogenic bacteria in the anaerobic microbial community (compounds in the frame are competitive substrates of homoacetogens and methanogens).

However, unlike methanogens, the substrate spectrum of homoacetogenic bacteria is quite diverse [Drake et al., 1997, 2008]. Therefore, they can maintain their number in the community at a sufficient level, since they can switch their metabolism to other substrates (Fig. 4b).

Thus, a combination of factors, both trophic and non-trophic, determines the result of competition between these two microbial groups for hydrogen. The role of homoacetogenic bacteria can increase in eutrophic systems at low temperatures, when sufficiently intense processes of degradation of organic matter occur [Kotsyurbenko, 2005]. In stable oligotrophic ecosystems, methanogens are the main terminal group, and the role of hydrogen-dependent methanogenesis can increase.

The most important non-trophic factors affecting the function of microorganisms are temperature and pH. Since the vast majority of wetland systems, including the entire ecosystem of Western Siberia, are subjected to low temperatures, the discovery of psychrophilic or psychrotolerant methanogens and the study of their physiology were topical issues in the context of formation of methane and its emission into the atmosphere. Later, G. A. Zavarzin introduced the term: “psychoactive” [Zavarzin, 1995b], which more precisely indicates the ecological role of this physiological group. Indeed, many microorganisms in cold terrestrial ecosystems have sufficiently high temperature growth optima, which can be much higher than the temperature maxima in their habitats. At the same time, they quite actively (conditionally actively to be exact, since all processes at low temperatures occur at a much lower rate than those at elevated temperatures) participate in degradation processes, maintaining a balance in the community. Interestingly, it is psychroactive microorganisms that often dominate in permanently cold ecosystems.

The pH values in wetland systems range from close to neutral (6.5–7.0) in eutrophic fens to fairly acidic (3.5–4.0) in oligotrophic bogs. For a long time, all attempts to isolate acidophilic methanogens failed. However, the progress was achieved when researchers have made a strong emphasis on the composition of cultivation medium. They have used the medium with mineral components corresponding to their composition in natural habitats rather, than that described in the traditional handbook for methanogens [Bräuer et al., 2006, 2011]. It turned out that acidophilic methanogens from bogs are especially sensitive to high ionic strength, which is attributable to elevated concentrations of mineral salts.

Thus, at the microbial group system level, the main problems to be solved are identifying the key microbial agent responsible for the formation of methane in wetland ecosystems and determining its potential in competitive relationships with other microbial groups and the main factors regulating its activity.

BIOCHEMISTRY OF METANOGENESIS

Methanogenesis as a metabolic process is one of the subsystems that function in the cells of methanogenic archaea. A feature of this metabolic pathway is a unique enzyme system, which is found only in methanogens [Thauer, 1998]. Thanks to specific enzymes that perform this reaction chain, methanogens are able to gain energy for their life during the synthesis of methane from various simple compounds.

Microbial methanogenesis is considered to be one of the oldest, if not the oldest, metabolic processes performed by terrestrial organisms. Among three possible pathways of methane synthesis (hydrogen-dependent, acetoclastic and methylotrophic), the hydrogen-dependent pathway is defined as the most ancient one [Battistuzzi et al., 2004; Borrel et al., 2016; Martin and Sousa, 2016]. According to the latest estimations, the so-called LUCA (Last Universal Common Ancestor) [Markov and Naimark, 2014], quite likely, had a protosystem of metabolic reactions based on energy production through the generation of CH_4 [Weiss et al., 2016].

In addition to their living in ancient terrestrial ecosystems, methanogens are considered as possible model organisms inhabiting extraterrestrial ecosystems in the framework of the astrobiology concept of searching for life elsewhere [Taubner 2015, 2018]. These microorganisms, capable of using such simple compounds as hydrogen and carbon dioxide, as sources of carbon and energy with the formation of methane, can exist in almost any ecosystems poor in organics, where the above gases are available in sufficient quantity. In this respect, the microbial community of the deep aquafer ecosystem is noteworthy, where the trophic chain begins just with the aforementioned gases, and where lithotrophic methanogenic archaea and homoacetogenic bacteria were found to be highly predominant microbial groups [Kotelnikova and Pedersen, 1997; Chapelle et al., 2002]. Methane is, in particular, suggested as an important biomarker of possible microbial activity on other cosmic bodies [Seager et al., 2016].

The uniqueness of methanogenesis enzymes was the basis for the development of a method of specific markers of methanogenic archaea for their qualitative and quantitative identification in the studied microbial community. This method is based on the specific sequence of a gene encoding one of the key enzymes of methanogenesis, methyl coenzyme M reductase (MCR) and allows detecting the presence of various methanogenic archaea in ecosystems [Friedrich, 2005]. This method of identifying methanogens by functional gene is also widely used to study the diversity of methanogens in wetlands. It has been established that in wetlands located in various regions, including Western Siberia, methanogens of all trophic groups are present, and their diversity strongly depends on the type of the wetland system [Kotsyurbenko et al., 2004, 2007; Yavitt et al., 2006, 2012; Andersen et al., 2013]. The main predominant families of methanogens are *Methanomicrobiaceae, Methanobacteriaceae, Methancoccaceae* and, relatively recently discovered acid-tolerant methanogens of the *Methanoregulaceae* family, which perform the hydrogen-dependent methanogenesis pathway, as well as acetoclastic representatives of the *Methanosaetaceae* and the *Methanosarcinoaceae* [Basiliko et al., 2003; Kotsyurbenko et al., 2004; Galand et al., 2005; Narrowe et al., 2017] (Fig. 5).

CONCLUSION

Consistency and integrity are the most important features of living objects. Biological systems are one of the most complex systems with regard to organization structure. A complex system consists of a large number of independent variables and their relations, that leads to an increase in its emergent properties.

Using the principle of systemicity for the successful study of a scientific problem involves, in particular, identifying the main system within which the task will be solved, determining its key elements and, the relationships between them and the main regulatory factors, as well as establishing the position of the system under study relative to other systems (hierarchy level).

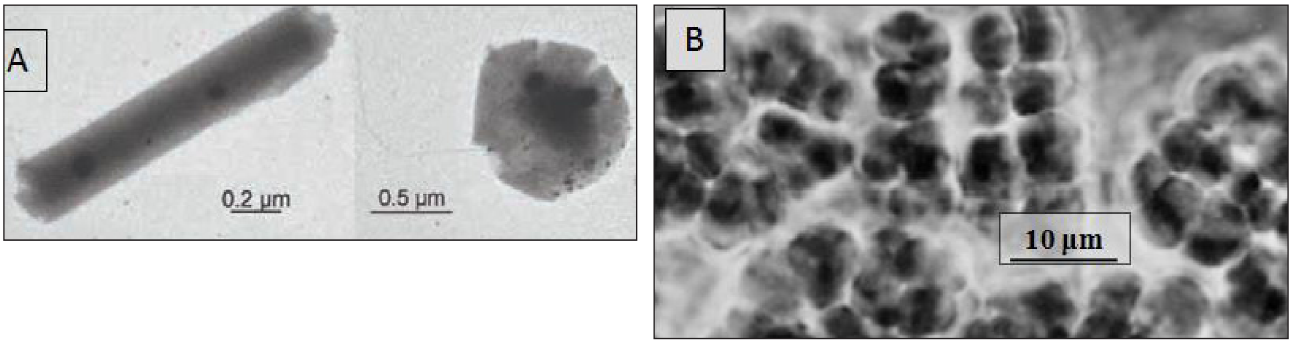

Fig. 5. Methanogens isolated from cold freshwater ecosystems. **A** – Methanoregula boonei strain 6A8 with rod-shaped (a) and coccoid (b) morphology (Photos taken from Genome Portal: https://genome.jgi.doe.gov/portal/metbo/metbo.home.html. Original publication: Врдuer et al., 2011); **B** – Methanosarcina lacustris. (Photos provided by the author of the original publication: Simankova et al., 2011).

The impact of greenhouse gases on climate is a complex problem. To investigate it comprehensively, the system approach is needed. To understand the mechanisms of formation and emission of methane, one of the main and active greenhouse gases of biological origin, it is necessary to study the interaction of systems of various levels in which this gas plays a key role. The structure and relationship of different systems should be determined both on a horizontal and a vertical level.

The vast territories of wetlands of Western Siberia are key ecosystems in the context of the aforementioned issue as sources of large amounts of methane releasing into the atmosphere. Methanogenic microbial communities operating in these regions produce methane under conditions of low temperature and pH, as a result of complex trophic interactions. The main microbial group in these interactions is methanogenic archaea situated at the end of the trophic chain of the anaerobic microbial community and possessing a unique enzyme system for energy generation. Their methanogenic activity is regulated by various factors that should be taken into account for the predictive modeling of methanogenesis under conditions of environmental changes.

The system analysis applied to the problem of methane greenhouse gas formation requires the involvement of the methodology and scientific potential of various biological disciplines such as ecology, biogeochemistry, microbiology, molecular biology, etc. As a result of the comprehensive approach to the objectives to be achieved, a wide range of scientific issues from the functioning of modern biogeochemical cycles to the problem of the origin of life is considered.

ACKNOWLEDGMENTS

The work was funded in the framework of the RFBR project 18-45-860015 p_a and state program AAAA-A15-115122810146-4

REFERENCES

1. Анисимов О.А., Кокорев В.А. 2015. Сравнительный анализ наземных, морских и спутниковых измерений метана в нижней атмосфере российской части Арктики в условиях изменения климата // Исследование Земли из космоса. Т. 2. С. 1–14. [Anisimov O.A., Kokorev V.A. 2015. Comparative analysis of land, sea and satellite measurements of methane in the lower atmosphere of the Russian part of the Arctic under climate change// Earth exploration from space. V 2. P. 1–14. (In Russian)]. doi: 10.7868/S0205961415020037
2. Глаголев М.В. 2010. К методу «обратной задачи» для определения поверхностной плотности потока газа из почвы // Динамика окружающей среды и глобальные изменения климата. Т.1. № 1. С. 17-36. [Glagolev M.V. 2010. On the "inverse problem" method for determining the surface density of gas flow from the soil // Environmental Dynamics and Global Climate Change. V. 1. No.1. P. 17-36. (In Russian)].
3. Глаголев М.В., Клепцова И.Е. 2009. Эмиссия метана в лесотундре: к созданию «стандартной модели» (Аа2) для Западной 613 Сибири // Вестник Томского государственного педагогического университета. Т. 3. С. 77-81. [Glagolev M.V., Kleptsova I.E. 2009. Methane emission in the forest-tundra: towards the creation of a "standard model" (Aa2) for Western Siberia // Bulletin of Tomsk State Pedagogical University. V 3. P. 77-81. (In Russian)].
4. Глаголев М.В., Суворов Г.Г. 2007. Эмиссия метана болотными почвами Средней Тайги Западной Сибири (на примере Ханты-Мансийского Автономного Округа) // Доклады по экологическому почвоведению. Т. 2. С. 90-162. [Glagolev M.V., Suvorov G.G. 2007. Methane emission by marsh soils of the Middle Taiga of Western Siberia (on the example of the Khanty-Mansiysk Autonomous Okrug) // Reports on ecological soil science. V. 2. Iss. 6. P. 90-162. (In Russian)].
5. Глаголев М.В., Фастовец И.А. 2012. Апология редукционизма (редукционизм – как мировоззренческая основа 619 математического моделирования) // Динамика окружающей среды и глобальные изменения климата. Т. 3. С. 1-24. [Glagolev M.V., Fastovets I.A. 2012. Apology of reductionism (reductionism as the worldview of mathematical modeling) // Environmental dynamics and global climate change. V. 3. № 2 (6). P. 1-24. (In Russian)].
6. Глаголев М.В., Шнырев Н.А. 2007. Динамика летне-осенней эмиссии СН4 естественными болотами (на примере юга Томской области) // Вестник Московского университета. Серия 17: Почвоведение. Т. 1. С. 8-14. [Glagolev M.V., Shnyrev N.A. 2007. Dynamics of summer-autumn CH_4 emission by natural bogs (by the example of the south of the Tomsk Region) // Moscow University Herald. Series 17: Soil Science. V. 1. P. 8-14. (In Russian)]. doi: 10.3103/s0147687407010024
7. Заварзин Г.А. 1995а. Анти-рынок в природе. Природа. Т. 3. С. 46-60. [Zavarzin G.A. 1995a. Anti-rynok v prirode // Priroda. V. 3. P. 46-60. (In Russia)].
8. Заварзин Г.А. 1995б. Микробный цикл метана в холодных условиях. Природа. 6:3-14. [Zavarzin G.A. 1995b. Mikrobnyy tsikl metana v kholodnykh usloviyakh // Priroda. V. 6. P. 3-14. (In Russian)].
9. Заварзин Г.А. 2015. Избранные труды. МАКС Пресс, Москва: 512 с. [Zavarzin G.A. 2015. Selected Works. M.: MAX Press. 512 p. (In Russian)].
10. Заварзин Г.А. 2011. Какосфера. Философия и публицистика. Ruthenica, Москва: 460с. [Zavarzin G.A. 2011. Kakosfera. Filosofiya i Publitsistika. M.: Ruthenica. 460 p. (In Russian)].
11. Каллистова А.Ю., Меркель А.Ю., Тарновецкий И.Ю., Пименов Н.В. 2017. Образование и окисление метана прокариотами // Микробиология. Т. 86. С. 661-683. [Kallistova A.Yu., Merkel A.Yu., Tarnovetsky I.Yu., Pimenov N.V. 2017. The formation and oxidation of methane by prokaryotes // Microbiology. V. 86. № 6. P.661-683. (In Russian)]. doi: 10.7868/S002636561706009X
12. Калюжный С.В., Пузанков А.Г., Варфоломеев С.Д. 1988. Биогаз: проблемы и решения // Биотехнология (Итоги науки и 627 техники ВИНИТИ АН СССР). Москва, Т. 21. С. 26-32 [Kalyuzhny S.V., Puzankov A.G., Varfolomeev S.D. 1988. Biogaz: problemy i resheniya // Biotekhnologiya (Itogi Nauki i 627 Tekhniki VINITI AN SSSR), Moscow, V. 21. P. 26-32. (In Russian)].
13. Лисс О.Л., Абрамова Л.И., Аветов Н.А., Березина Н.А., Инишева Л.И., Курнишкова Т.В., Слука З.А., Толпышева Т.Ю., Шведчикова Н.К. 2001. Болотные системы Западной Сибири и их природоохранное значение. Тула: Гриф и К. 584 с. [Liss O.L., Abramova L.I., Avetov N.A., Berezina N.A., Inisheva L.I., Kurnishkova T.V., Sluka Z.A., Tolpysheva T.Yu., Shvedchikova N.K. 2001. Bolotnyye Sistemy Zapadnoy Sibiri i Ikh Prirodookhrannoye Znacheniye. Tula: Grif and K. 584 P. (In Russian)].
14. Мазинг В.В. 1974. Актуальные проблемы классификации и терминологии в болотоведении // Типы болот СССР и принципы их классификации / Под ред. Т.Г. Абрамовой, М.С. Боч и Е.А. Галкиной. Л: Наука. С. 6-12. [Masing V.V. 1974. Aktual'nye problemy klassifikatsii i terminologii v bolotovedenii // Tipy Bolot SSSR i Printsipy Ikh Klassifikatsii / Abramova T.G, Botch M.S., Galkina E.A. (eds.). Leningrad: Nauka. P. 6-12. (In Russian)].
15. Марков А., Наймарк Е. 2014. Эволюция. Классические идеи в свете новых открытий. М: АСТ: CORPUS. 57 с. [Markov A., Naimark E. 2014. Evolyutsiya. Klassicheskie Idei v Svete Novykh Otkrytiy. M.: AST: CORPUS. P. 57. (In Russian)].
16. Сабреков А.Ф., Глаголев М.В. 2008. К математическому моделированию микробного сообщества цикла метана // Динамика окружающей среды и глобальные изменения климата. Т. 1. С. 84-97. [Sabrekov A.F., Glagolev M.V. 2008. On the mathematical modeling of the microbial community of the methane cycle // Environmental Dynamics and Global Climate Change. V 1. P. 84-97. (In Russian)].
17. Федоров В.Д., Гильманов Т.Г. 1980. Экология. Москва: Изд-во МГУ. 464 с. [Fedorov V.D., Gilmanov T.G. 1980. Ecologiya. M.: Publishing House of Moscow State University. 464 p. (In Russian)].
18. Adhya T.K., Rath A.K., Gupta P.K., Rao V.R., Das S.N., Parida K.M., Parashar D.C., Sethunathan N. 1994. Methane emission

from flooded rice fields under irrigated conditions // Biol. Fertil. Soils. V. 18. P. 245-248.

19. Andersen R., Chapman S.J., Artz R.R.E. 2013. Microbial communities in natural and disturbed peatlands: a review // Soil Biol. Biochem. V. 57. P. 979–994.
20. Aselmann I., Crutzen P.J. 1989. Global distribution of natural freshwater wetlands and rice paddies, their net primary productivity, seasonality and possible methane emissions // J.Atmos.Chem. V. 8. P. 307–358.
21. Basiliko N., Yavitt J.B, Dees P.M, Merkel S.M. 2003. Methane biogeochemistry and methanogen communities in two northern peatland ecosystems, New York State // Geomicrobiol. J. V. 20. P. 563–577.
22. Battistuzzi F.U., Feijao A., Hedges S.B. 2004. A genomic timescale of prokaryote evolution: insights into the origin of methanogenesis, phototrophy, and the colonization of land // BMC Evol. Biol. V. 4. P. 44. doi: 10.1186/1471-2148-4-44
23. Borrel G., Adam P.S., Gribaldo S. 2016. Methanogenesis and the Wood–Ljungdahl Pathway: An Ancient, Versatile, and Fragile Association Genome // Biol. Evol. V. 8. No. 6. P. 1706–1711. doi:10.1093/gbe/evw114
24. Brдuer S.L., Cadillo-Quiroz H., Ward R.J., Yavitt J.B., Zinder S.H. 2011. Methanoregula boonei gen. nov., sp. nov., an acidiphilic methanogen isolated from an acidic peat bog // Int. J. Syst. Evol. Microbiol. V. 61. P. 45–52. doi: 10.1099/ijs.0.021782-0
25. Brдuer S.L., Cadillo-Quiroz H., Yashiro E., Yavitt J.B., Zinder S.H. 2006. Isolation of a novel acidiphilic methanogen from an acidic peat bog // Nature. V. 442. P. 192–194.
26. Butterbach-Bahl K., Kock M., Willibald G., Hewett B., Buhagiar S., Papen H., Kiese R. 2004. Temporal variations of fluxes of NO, NO_2, N_2O, CO_2, and CH_4 in a tropical rain forest ecosystem // Global Biogeochem. Cycles. V. 18. GB3012.
27. Cao M., Dent J.B., Heal O.W. 1995. Modeling methane emissions from rice paddies // Global Biogeochem. Cycles. V. 9. P. 183–195.
28. Chapelle F.H., O'Neill K., Bradley P.M., Methe B.B.A., Ciufo S.A., Knobel L.L., Lovley D.R. 2002. A hydrogen-based subsurface microbial community dominated by methanogens // Nature. V. 415. P. 312-315.
29. Chen B., Ge Q., Fu D., Yu G., Sun X., Wang S., Wang H. 2010. A data-model fusion approach for upscaling gross ecosystem productivity to the landscape scale based on remote sensing and flux footprint modelling // Biogeosciences. V. 7. Iss. 9. P. 2943–2958.
30. Cicerone R.J., Oremland R.S. 1988. Biogeochemical aspects of atmospheric methane // Global Biogeochem. Cycles. V. 2. P. 299–327.
31. Conrad R. 1996. Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, NO) // Microbiol. Rev. V. 60. P. 609-640.
32. Conrad R. 2007. Microbial ecology of methanogens and methanotrophs // Advan. Agron. V. 96. P. 1–63. doi: 10.1016/s0065-2113(07)96005-8.
33. Conrad R., Klose M. 1999. Anaerobic conversion of carbon dioxide to methane, acetate and propionate on washed rice roots // FEMS Microbiol. Ecol. V. 30. № 30. P. 147–155.
34. Conrad R., Klose M. 2000. Selective inhibition of reactions involved in methanogenesis and fatty acid production on rice roots // FEMS Microbiol. Ecol. V. 34. № 1. P.27–34.
35. Drake H.L., Daniel S.L., Кьsel K., Matthies C., Kuhner C., Braus-Stromeyer S. 1997. Acetogenic bacteria: what are the in situ consequences of their diverse metabolic versatilities? // BioFactors. V. 6. P. 13–24.
36. Drake H.L., Göβner A.S., Daniel S.L. 2008. Old Acetogens, New Light // Ann. N.Y. Acad. Sci. 1125: 100–128, doi: 10.1196/annals.1419.016
37. Ehhalt D.H., Schmidt U. 1978. Sources and sinks of atmospheric methane // Pageoph. V. 116. P. 452–464.
38. Friedrich M.W. 2005. Methyl-Coenzyme M reductase genes: unique functional markers for methanogenic and anaerobic methane-oxidizing archaea // Methods Enzymol. V. 397. P. 428–442. doi: 10.1016/s0076-6879(05)97026-2
39. Galand P.E., Fritze H., Conrad R., Yrjдlд K. 2005. Pathways for methanogenesis and diversity of methanogenic archaea in three boreal peatland ecosystems // Appl. Environ. Microbiol. V. 71. P. 2195–2198.
40. Glagolev M.V., Sabrekov A.F., Kleptsova I.E., Filippov I.V., Lapshina E.D., Machida T., Maksyutov S.S. 2012. Methane emission from bogs in the subtaiga of Western Siberia: the development of standard model // Eurasian Soil Sci. V.45. P. 947–957. doi: 10.1134/S106422931210002X
41. Glazunov A., Rannik Ь., Stepanenko V., Lykosov V., Auvinen M., Vesala T., Mammarella I. 2016. Large-eddy simulation and stochastic modelling of Lagrangian particles for footprint determination in the stable boundary layer // Geosci. Model Dev. V. 9. P. 2925-2949.
42. Grant R.F. 1998. Simulation of methanogenesis in the mathematical model ecosys // Soil Biol. Biochem. V. 30. P. 883-896.
43. Haddaway N.R., Burden A., Evans C.D., Healey J.R., Jones D.L., Dalrymple S.E, Pullin A.S. 2014. Evaluating effects of land management on greenhouse gas fluxes and carbon balances in boreotemperate lowland peatland systems // Environ. Evid. V. 3:5. doi: 10.1186/2047-2382-3-5
44. Hunger S., Göβner A.S., Drake H.L. 2015. Anaerobic trophic interactions of contrasting methane-emitting mire soils: processes versus taxa // FEMS Microbiol. Ecol. V. 91. № 5. doi: 10.1093/femsec/fiv045
45. IPCC. 2013. Carbon and other biogeochemical cycles. Chapter 6 //: Climate change. The physical science basis. Global methane budget. Cambridge University Press. Cambridge. United Kingdom and New York. NY. USA. P. 505–510.
46. IPCC. 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 p. https://www.ipcc.ch/site/assets/uploads/2018/02/SYR_AR5_FINAL_full.pdf
47. Jackson B.E, McInerney M.J. 2002. Anaerobic microbial metabolism can proceed close to thermodynamic limits // Nature. V. 415. P. 454–456.
48. James R.T. 1993. Sensitivity analysis of a simulation model of methane flux from the Florida Everglades // Ecol. Model. V. 68. P. 119-146.

49. Kotelnikova S., Pedersen K. 1997. Evidence for methanogenic Archaea and homoacetogenic Bacteria in deep granitic rock aquifers // FEMS Microbiol. Rev. V. 20. P. 339-349.
50. Kotsyurbenko O.R. 2005. Trophic interactions in the methanogenic microbial community of low-temperature terrestrial ecosystems. Mini-Review // FEMS Microbiol. Ecol. V. 53. P. 3-13.
51. Kotsyurbenko O.R., Chin K.-J., Glagolev M.V., Stubner S., Simankova M.V., Nozhevnikova A.N., Conrad R. 2004. Acetoclastic and hydrogenotrophic methane production and methanogenic populations in an acidic West-Siberian peat bog // Environ. Microbiol. V. 6. № 11. P. 1159-1173.
52. Kotsyurbenko O.R., Glagolev M.V. 2015. Protocols for measuring methanogenesis. // Hydrocarbon and lipid microbiology protocols (Springer Protocols Handbooks) / McGenity T.J., Timmis K.N., Nogales B. (Eds.) Springer-Verlag. Berlin Heidelberg. P. 227-243. doi: 10.1007/8623_2015_89
53. Kotsyurbenko O.R., Glagolev M.V., Nozhevnikova A.N., Conrad R. 2001. Competition between homoacetogenic bacteria and methanogenic archaea for hydrogen at low temperature //FEMS Microbiol. Ecol. V. 38. P. 153-159.
54. Kotsyurbenko O.R., Glagolev, M.V., Merkel, A.Y., Sabrekov, A.F., Terentieva, I.E. 2019. Methanogenesis in soils, wetlands and peat // Handbook of hydrocarbon and lipid microbiology Series. Biogenesis of hydrocarbons / A.J.M. Stams and Diana Z. Sousa (Eds.) Springer-Verlag. Berlin Heidelberg. doi: 10.1007/978-3-319-53114-4_9-1
55. Kotsyurbenko O.R., Friedrich M.W., Simankova M.V., Nozhevnikova A.N., Golyshin P., Timmis K., Conrad R. 2007. Shift from acetoclastic to H2-dependent methanogenesis in a West Siberian peat bog at low pH // Appl. Environ. Microbiol. V. 73. No. 7. P. 2344-2348.
56. Küsel K., Blöthe M., Schulz D., Reiche M., Drake H.L. 2008. Microbial reduction of iron and porewater biogeochemistry in acidic peatlands // Biogeosciences. V. 5. P. 1537–1549.
57. Kutzbach L., Wagner D., Pfeiffer E.M. 2004. Effect of microrelief and vegetation on methane emission from wet polygonal tundra, Lena Delta, Northern Siberia // Biogeochemistry. V. 69. P. 341–362. doi: 10.1023/B:BIOG.0000031053.81520.db
58. Lansdown J.M, Quay P.D, King S.L 1992. CH4 production via CO2 reduction in a temperate bog: a source of 13C-depleted CH4 // Geochim. Cosmochim. Acta V. 56. P. 3493–3503.
59. Li T., Li H., Zhang Q., Ma Z., Yu L., Lu Y., Niu Z., Sun W., Liu J. 2019. Prediction of CH4 emissions from potential natural wetlands on the Tibetan Plateau during the 21st century // Sci. Total Environ. V. 657 P. 498–508.
60. Limpens J., Berendse F., Blodau C., Canadell J.G., Freeman C., Holden J., Roulet N., Rydin H., Schaepman-Strub G. 2008. Peatlands and the carbon cycle: from local processes to global implications – a synthesis // Biogeosciences. V. 5. P. 1475–1491.
61. Lin Y., Liu D., Ding W., Kang H., Freeman C., Yuan J., Xiang J. 2015. Substrate sources regulate spatial variation of metabolically active methanogens from two contrasting freshwater wetlands // Appl. Microbiol. Biotechnol. V. 99. P. 10779-10791. doi: 10.1007/s00253-015-6912-7
62. Martin W.F., Sousa F.L. 2016. Early Microbial Evolution: The Age of Anaerobes // Cold Spring Harb. Perspect. Biol. 8:a018127. doi: 10.1101/cshperspect.a018127
63. Matthews E., Fung I. 1987. Methane emission from natural wetlands: global distribution, area and environmental characteristics of sources // Global Biogeochem. Cycles. V. 1. P. 61–86.
64. McInerney M., Hoehler T., Gunsalus R.P., Schink B. 2010. Introduction to microbial hydrocarbon production: bioenergetics // Handbook of Hydrocarbon and Lipid Microbiology / Timmis K.N. (Ed.). Springer, Berlin, Heidelberg.
65. McInerney M.J., Beaty P.S. 1988. Anaerobic community structure from a nonequilibrium thermodynamic perspective // Can. J. Microbiol. V. 34. P. 487-493.
66. Narrowe A.B., Angle J.C., Daly R.A, Stefanik K.C., Wrighton K.C., Miller C.S. 2017. High-resolution sequencing reveals unexplored archaeal diversity in freshwater wetland soils // Environ. Microbiol. V. 19. P. 2192–2209.
67. Panikov N.S. 1994. CH4 and CO2 emission from northern wetlands of Russia: Source strength and controlling mechanisms // Proceedings of the International Symposium on Global Cycles of Atmospheric Greenhouse Gases. Sendai: Tohoku University. P. 100–112.
68. Panikov N.S., Sizova M.V., Zelenev V.V., Machov G.A., Naumov A.V., Gadzhiev I.M. 1995. Methane and carbon dioxide emission from several Vasyugan wetlands: spatial and temporal flux variations // Ecol. Chem. V. 4. No. 1. P. 13-23.
69. Sabrekov A.F., Runkle B.R.K., Glagolev M.V., Kleptsova I.E., Maksyutov S.S. 2014. Seasonal variability as a source of uncertainty in the West Siberian regional CH4 flux upscaling // Environ. Res. Lett. V. 9. 045008.
70. Sabrekov A.F., Filippov I.V., Terentieva I.E., Glagolev M.V., Il'yasov D.V., Smolentsev B.A., Maksyutov S.S. 2016. The Spatial Variability of Methane Emission from Subtaiga and Forest–Steppe Grass–Moss Fens of Western Siberia // Biology Bulletin. V. 43.No. 2. P. 62–168.
71. Schink B. 1997. Energetics of syntrophic cooperation in methanogenic degradation // Microbiol. Mol. Biol. Rev. V. 61. No. 2. P. 262–280.
72. Seager S., Bains W., Petkowski J.J. 2016. Toward a list of molecules as potential biosignature gases for the search for life on exoplanets and applications to terrestrial biochemistry. Astrobiology. V. 16. № 6. doi: 10.1089/ast.2015.1404
73. Simankova M.V., Parshina S.N., Tourova N.P., Kolganova T.V., Zehnder A.J.B., Njzhevnikova A.N. 2001. Methanosarcina lacustris sp. nov., a New psychrotolerant methanogenic archaeon from anoxic lake sediments // System. Appl. Microbiol. V. 24. P. 362–367.
74. Stoeva M.K., Aris-Brosou S., Chйtelat J., Hintelmann H., Pelletier P, Poulain A.J. (2014) Microbial community structure in lake and wetland sediments from a high arctic polar desert revealed by targeted transcriptomics // PLoS ONE. V. 9. № 3. e89531.
75. Taubner R.-S., Pappenreiter P., Zwicker J., Smrzka D., Pruckner C., Kolar P., Bernacchi S., Seifert A.H., Krajete A.,

Bach W., Peckmann J., Paulik C., Firneis M.G., Schleper C., Rittmann S.K.-M.R. 2018. Biological methane production under putative Enceladus-like conditions // Nature Com. V. 9. P. 748.

76. Taubner R.-S., Schleper C., Firneis M.G., Simon Rittmann S. K.-M. R. 2015. Assessing the ecophysiology of methanogens in the context of recent astrobiological and planetological studies // Life (Basel). V. 5. N. 4. P. 1652–1686.
77. Terentieva I.E., Glagolev M.V., Lapshina E.D., Sabrekov A.F., Maksyutov S.S. 2016. Mapping of West Siberian taiga wetland complexes using Landsat imagery: implications for methane emissions // Biogeosciences. V. 13. No. 16. P. 4615–4626.
78. Thauer R.K. 1998. Biochemistry of methanogenesis: a tribute to Marjory Stephenson // Microbiology. V. 144. P. 2377-2406.
79. Webster K.L., Bhatti J.S., Thompson D.K., Nelson S.A., Shaw C.H., Bona K.A., Hayne S.L., Kurz W.A. 2018. Spatially-integrated estimates of net ecosystem exchange and methane fluxes from Canadian peatlands // Carbon Balance Manag. V. 13. P. 16.
80. Weiss M.C., Sousa F.L., Mrnjavac N., Neukirchen S., Roettger M., Nelson-Sathi S., Martin W.F. 2016. The physiology and habitat of the last universal common ancestor // Nature microbiology. V. 1. doi: 10.1038/NMICROBIOL.2016.116
81. Whalen S.C., Reeburgh W.S. 2000. Methane oxidation, production, and emission at contrasting sites in a boreal bog // Geomicrobiol. J. V. 17. P. 237–251.
82. Yavitt J.B, Basiliko N., Turetsky M.R., Hay A.G. 2006. Methanogenesis and methanogen diversity in three peatland types of the discontinuous permafrost zone, boreal western continental Canada // Geomicrobiol J. V. 23. P. 641–651.
83. Yavitt J.B., Yashiro E., Cadillo-Quiroz H., Zinder S.H. 2012. Methanogen diversity and community composition in peatlands of the central to northern Appalachian Mountain region // North Am. Biogeochem. V. 109. P. 117–131.
84. Zavarzin G.A. 1995b. Soengen psychrophilic cycle // Ecol. Chem. V.4. P. 3-12.
85. Zhu X., Zhuang Q., Qin Z., Song L., Glagolev M. 2013. Estimating wetland methane emissions from the northern high latitudes from 1990 to 2009 using artificial neural networks // Global Biogeochem. Cycles. V. 27. No. 2. P. 592-604..