

METHANOGENIC MICROBIAL COMMUNITY FROM THE PEAT BOG «CHISTOE» (WEST SIBERIA): PRELIMINARY DATA AND PERSPECTIVES

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Introduction

West-Siberian Wetlands are considered to be the most potent terrestrial source of atmospheric methane on Earth. Methane emission from wetlands is controlled by a complex set of abiotic and biotic factors like temperature, soluble carbon source availability, Eh, pH, water availability, properties of plants covering the site, soil texture, microbial community composition, etc., linking the physical-chemical and biological characteristics of environments [Glagolev, 1998; Wagner *et al.*, 1999; Merilä *et al.*, 2006]. Thus, the methane dynamics in situ is strongly connected to the special characteristics of a particular site. In our previous study of the methanogenic community of tundra soil and the Bakchar bog (Southern Taiga of West Siberia), some specific features of the communities functioning have been revealed, the main finding of which was that temperature and pH act as factors regulating trophic microbial interactions and influences methanogenic pathways [Kotsyurbenko *et al.*, 1996, 2004, 2007]. It is as yet unclear whether the conclusions made for Bakchar bog are also true for other wetland ecosystems that differ in environmental conditions. Differences in environmental conditions lead to differences in the rates of degradation of organic matter, altering methanogenic community compositions inhabiting these ecosystems, as well as turning on/off corresponding pathways of methanogenesis.

The track of the expedition in 2007 went along the whole West Siberian wetland from the South to the North and to cover areas of different climatic zones including taiga and tundra regions as well as permafrost.

The bog “Chistoe” is located in Khanty-Mansijsk administrative area and belongs to a belt of ombrotrophic sphagnum bogs, the most extensive type of environments in West Siberia.

The aim of this work is to explore the methanogenic potential of the above mentioned individual ecosystem in the context of the comparative studies of different types of wetlands through the analysis of methanogenic community, its trophic microbial structure and main factors regulating its activity.

Results and discussion

The samples were transported to the laboratory under conditions similar to those detaining in situ, and then used for the laboratory experiments. The pH was 4.3-4.4. The potential of the microbial community to produce methane has been estimated in the incubation experiments in the temperature interval from 4 to 25°C (Fig.1). The Q_{10} has been calculated according to the rates of

methanogenesis and is 2.5. The increase of the methanogenesis rate with temperature increasing indicates the existence of psychroactive methanogenic community operating at low temperature, but having its optimum at moderate temperature.

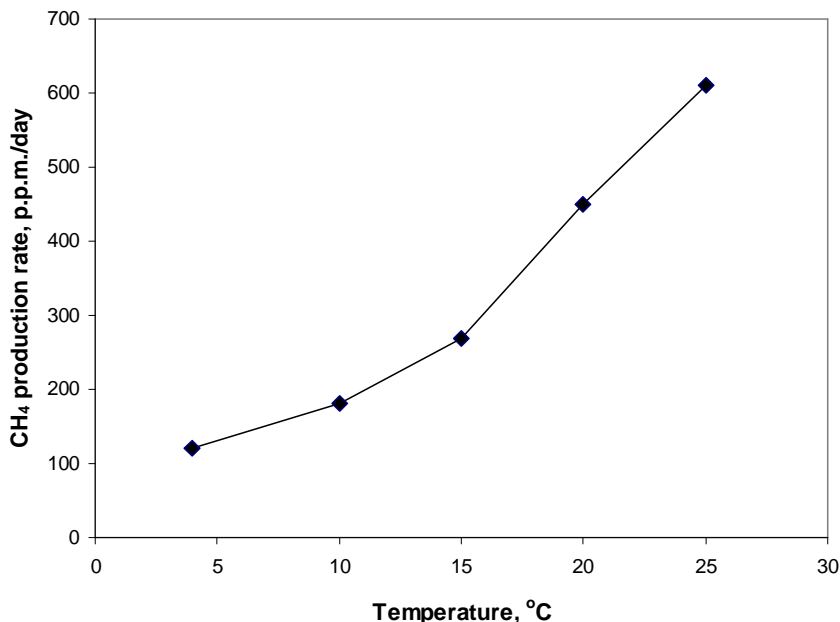


Fig.1. The rates of methanogenesis in the samples from the peat bog “Chistoe” at different temperatures.

The anaerobic community represents a biological system that is balanced by the coordinated interactions of the constituent microbial groups. So, the study of biological production of methane should involve not only field measurements and isolation of the key microorganisms, but also microbial interactions at the community level. The functional groups are hydrolytic, fermentative, syntrophic, homoacetogenic and methanogenic microorganisms. To stimulate a response of the functional microbial groups, characteristic substrates were introduced into the microbial system. The dynamics of substrate decomposition and product formation were monitored over time during the incubation at 15°C. The substrates used in the experiments were glucose that is indicative of the activity of fermenting bacteria, acetate (acetoclastic methanogens), formate and H₂+CO₂ (hydrogenotrophic methanogens), as well as methanol and trimethylamine (C1-methanogens) [Kotsyurbenko, 2005]. Glucose stimulated

the development of both fermenting microorganisms and methanogens. The most favourable methanogenic substrate appeared to be formate and H_2+CO_2 probably indicating the H_2 -dependent methanogenesis as the predominant pathway in the bog "Chistoe".

Application of culture-independent molecular techniques based on 16S rRNA gene sequences (creating clone libraries and SSCP [Schwieger and Tebbe, 1998]) reveals predominant microbial clusters among Bacteria and Archaea. The former are presented by *Alphaproteobacteria* as well as by *Acidobacteriaceae* and *Oxalobacteriaceae*, whereas the latter includes *Methanomicrobiales* and *Methanosarcinales* with representative clones from *Methanosarcinaceae* and *Methanosaetaceae*. The methanogenic archaea from the aforementioned orders are belonging to two nutritional categories: hydrogenotrophs and acetoclastic methanogens. This is the evidence for the operation of two main methanogenic (H_2 -dependent and acetoclastic) pathways at the degradation of organic matter in the studied ecosystem.

Outlook

The additional study of the predominant methanogenic pathways will be done by using radioactive tracers ($[2-^{14}C]$ acetate and $NaH^{14}CO_3$) [Kotsyurbenko *et al.*, 2004] for the immediate methanogenic precursors that can be incorporated into CH_4 . The contribution of different pathways to total methanogenesis will be then calculated.

SSCP molecular approach will be applied to investigate the microbial successions in the system activated by adding different substrates characteristic for the key microbial groups.

The samples supplemented with different substrates and incubated for kinetic experiments will be further used to obtain active enrichments and pure cultures of the key microorganisms.

To quantify bacterial and archaeal components of the community as well as acetoclastic methanogens, FISH [Amann *et al.*, 1995] and qPCR [Yu *et al.*, 2006] techniques will be used with probes and primers specific for Bacteria, Archaea and for the order *Methanosarcinales*.

The data collected will provide insights into correlation between trophic structure, microbial composition and functioning of the methanogenic community. The results will be compared to those obtained for other West Siberian wetlands to better understanding peculiarities of microbial ecology of methanogenic communities inhabiting such ecosystems.

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МЕТАНОГЕННОЕ СООБЩЕСТВО МИКРООРГАНИЗМОВ ИЗ ОЛИГОТРОФНОГО БОЛОТА «ЧИСТОЕ» (ЗАПАДНАЯ СИБИРЬ): ДАННЫЕ ПРЕДВАРИТЕЛЬНОГО ИЗУЧЕНИЯ И ПЕРСПЕКТИВЫ

Коцюрбенко О.Р., Да Сильва А.П., Глаголев М.В.

Образцы торфа из болота «Чистое», расположенного рядом с пос. Шапша (Ханты-Мансийский АО) были отобраны в 2007 с глубины 15-30 см. При инкубировании данных образцов образование метана происходило во всем выбранном температурном интервале от 4 до 25°C. Скорость метаногенеза возрастала при повышении температуры с коэффициентом $Q_{10} = 2.5$. Предварительная молекулярно-биологическая характеристика состава анаэробного микробного сообщества из вышеуказанных образцов, проведенная методами 16S-РНК и T-RFLP показала присутствие метаногенов из семейств Methanomicrobiaceae Methanosarcinaceae и Methanosaetaceae. Введение в систему различных селективных субстратов: глюкозы, ацетата, метанола, триметиламина, формиата и H_2/CO_2 показало наличие активной бродильной микрофлоры, а также водород- и ацетат-использующих метаногенов. Таким образом, наши данные указывают на функционирование сбалансированного психроактивного метаногенного сообщества в исследуемых образцах. Разложение органического вещества происходит как по водородному, так и по ацетокластическому пути. Для более детального изучения анаэробного сообщества планируется применение радиоизотопных исследований, а также ряда молекулярно- биологических методов, таких как FISH и qPCR для количественной оценки основных микробных групп и SSCP для изучения микробных сукцессий при варьировании различных параметров (температуры и субстратов, используемых для активации определенных микробных трофических групп).