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Microbial mat of the thermal springs Kuchiger Republic of Buryatia: species composition, biochemical properties and electrogenic activity in biofuel cell

Denis Aleksandrovich Yuriev¹, Svetlana Viktorovna Zaitseva², Galina Olegovna Zhdanova¹, Mikhail Yuriyevich Tolstoy³, Darima Dondokovna Barkhutova², Olga Feodorovna Vyatchina¹, Elena Yuryevna Konovalova¹ and Devard Iosifovich Stom¹,³,⁴,⁵

¹ Irkutsk State University, Irkutsk, 1, K. Marx st., Russia;
² Institute of General and Experimental Biology SB RAS, Ulan-Ude, 6, Sakhyanovoy str., Russia;
³ Irkutsk National Research Technical University, Irkutsk, 83, Lermontova st., Russia;
⁴ Baikal museum of the Irkutsk Scientific Center of the SB RAS, Listvyanka, 1A, Academicheskay st., Russia
⁵ stomd@mail.ru

Abstract. Electrogenic, molecular and some other properties of a microbial mat isolated from the Kuchiger hot spring (Kurumkansky District, Republic of Buryatia) were studied. Molecular analysis showed that representatives of Proteobacteria (85.5 % of the number of classified bacterial sequences) prevailed in the microbial mat of the Kuchiger springs, among which sulfur bacteria of the genus Thiothrix were the most numerous. In the microbial mat there were bacteria from the families Rhodocyclaceae, Comamonadaceae and Flavobacteriaceae. Phylum Bacteroidetes, Cyanobacteria/Chloroplast, Fusobacteria, Fibrobacteres, Acidobacteria, Chlorobi, Spirochaetes, Verrucomicrobia, Firmicutes, Deinococcus-Thermus, Chloroflexi and Actinobacteria are also noted in the composition of the microbial mat. Under the experimental conditions using Kuchiger-mat 16 as bioagents, glucose and peptone as substrates, the power of BFC was 240 and 221 mW / m², respectively. When replacing the substrate with sodium acetate, the efficiency of the BFC was reduced by a factor of 10 (20 mW / m²). The prospects of using a microbial mat "Kuchiger-16" as an electron in BFC when utilizing alkaline waste water components to generate electricity are discussed.

1. Introduction
Thermal springs of the Transbaikal are characterized by different temperature conditions (from 30 to 74 °C) and low salinity (up to 1 g/l) [1]. Some of them contain reduced sulfur compounds [2]. The pH value in them is shifted to the alkaline region. Kuchiger also belongs to this kind of reservoirs. It found a large number of microbial mats. The latter are consortia of microorganisms of the most diverse systematic groups. This circumstance determines the versatile metabolism of microbial mats [3, 4, 5, 6, 7]. At the same time, mats isolated from Transbaikalian springs grow at elevated temperatures, alkaline pH values and high mineralization of waters containing sulphides. These features open great prospects for the use of such consortia as bioagents in biofuel cell (BFC). BFCs are able to convert the chemical energy of sewage components into electricity, biofuels and biofuels while simultaneously...
eliminating pollutants [8]. Currently, the development and research in the field of BFC are on the way of modifying electrodes, creating enzyme and organelle systems, with the search for stable microbiocenoses as bioagents capable of operating under extreme conditions [9, 10, 11, 12, 13]. Based on the above, the purpose of this work was to study the electrogenic activity of a microbial mat isolated from the Kuchiger thermal spring.

2. Materials and equipment

The microbial mat (Kuchiger-16) was isolated in July 2016 from the Kuchiger hot spring (Kurumkansky District, Republic of Buryatia, 54°52’934”N, 111°00'050''E, 36 °C, pH – 9.8). Mat Kuchiger-16 is presented in the form of benthic microbial fouling. It was brought together in the lower current of a spring.

The primary production of the microbial mat was calculated based on the concentration of chlorophyll a [14]. Molecular genetic analysis of the microbial mat was performed by pyrosequencing the 16S RNA gene in the Genomics Center (Novosibirsk, Russia).

The electrogenic activity was evaluated in biofuel cell (BFC) [15]. The electrodes were made from carbon cloth "Ural" T22R (OJSC "Svetlogorskkhimvolokno", Republic of Belarus). The design of the BFC consisted of chambers made of organic glass. Between them is a proton exchange membrane CMI (Membraness International, USA) (thickness 0.45 ± 0.025 mm, exchange capacity 1.6 ± 0.1 meq/g, electrical resistivity <30 Ohm/cm²). The anode chamber is hermetically sealed. In its lower part there is a stub. It allows using a syringe to repeatedly take samples for chemical and biological analysis, as well as to make the necessary components during the operation of the BFC. At the same time there is no violation of sterility and anaerobic conditions of BFC. The current and voltage were recorded in two ways. We used either a DT32 DIGITAL multimeter or an automatic data recording system based on the microprocessor board "Arduino Mega 2560".

The sodium carbonate/bicarbonate buffer 0.05 M pH – 9.6-9.8 (Na₂CO₃ – 1.59 g, NaHCO₃ – 2.93 g, distilled H₂O – 1000 ml) was used as growth medium [16]. CH₃COONa (0.25 g/l) was used as the substrate. The prepared medium were poured into 5 ml tubes and sterilized at 1 atm.

The microbial mat was added in an amount of about 0.05 g/l. In microaerophilic variants of the experiments, after applying the mat, 1 ml of sterile vaseline oil was layered on top. Cultivation was carried out in accordance with the temperature regime of the microbial mat selection spring. After 4 days of incubation, presence (clouding of the medium, formation of the film) or lack of growth in test tubes was noted (table 1).

The experiments were performed in 5 biological replicas with 3 parallel variants. Statistical processing of the experimental data was carried out using the Excel software package. The tables show the average values for the sample and their standard deviations. The findings are made with the probability of an error-free forecast P ≥ 0.95.

3. Results and discussions

Based on the results of pyrosequencing of the 16S RNA gene, representatives of Proteobacteria (85.5 % of the number of classified bacterial sequences) prevailed in the microbial mat of the Kuchiger spring. Of these, sulfur bacteria of the genus Thiothrix were the most numerous (66.1 % of the number of classified bacterial sequences). In the microbial mat there were bacteria from the families Rhodocyclaceae and Comamonadaceae. Phylum Bacteroidetes contained 1,038 sequences. They belong mainly to the family Flavobacteriaceae (6.8 %) and unclassified at the genus level. Phylums Cyanobacteria/Chloroplast, Fusobacteria, Fibrobacteres, Acidobacteria, Chlorobi, Spirochaetes, Verrucomicrobia, Firmicutes, Deinococcus-Thermus, Chloroflexi and Actinobacteria are also noted in the composition of the microbial mat. But they were represented by a small number of sequences (no more than 0.5 % of the total number of classified sequences).

In the microbial mat of the Kuchiger spring, the chlorophyll α concentration was 139 mg/m². Low values of primary production can be fully explained by the prevalence of sulfur bacteria of the genus Thiothrix in it.
When studying the ability of a microbial mat to generate an electric current, the primary task was to establish its mineral specificity. To select optimal conditions for cultivation of the Kuchiger-16 mat, experiments were performed with various combinations of orthophosphoric acid and nitrogen salts. The obtained materials were summarized in table 1.

### Table 1. Nature of growth of a microbial mat isolated from the Kuchiger thermal spring on media with different content of orthophosphoric acid and nitrogen salts.

| Nitrogen salts | Conditions for cultivation | Salts of orthophosphoric acid |
|----------------|---------------------------|-----------------------------|
|                |                           | Na₂HPO₄ | NaH₂PO₄ | K₂HPO₄ | KH₂PO₄ |
| NaNO₃          | Aerobic                   | ++      | ++      | ++      | ++      |
|                | Microaerophilic           | ++      | ±       | ++      | +       |
| NH₄Cl          | Aerobic                   | ++      | ++      | +       | ++      |
|                | Microaerophilic           | ±       | +       | –       | +       |
| NH₄NO₃         | Aerobic                   | ++      | ++      | ++      | ++      |
|                | Microaerophilic           | +       | +       | ++      | ±       |
| (NH₄)₂SO₄      | Aerobic                   | ++      | +       | ++      | ±       |
|                | Microaerophilic           | ±       | ±       | +       | +       |
| CH₃N₂O         | Aerobic                   | –       | ±       | +       | –       |
|                | Microaerophilic           | ±       | +       | +       | +       |

Note: «++» – is good growth; «+» – average growth; «± » – poor growth; «–» – there wasn't growth

The analysis showed that the Kuchiger-16 microbial mat under microaerophilic conditions is cultured fairly well on practically all tested versions of mixtures of phosphorus and nitrogen salts. In subsequent experiments, sodium carbonate / bicarbonate buffer with NaNO₃ (0.25 g/l) and Na₂HPO₄ (0.25 g/l) was used to prepare the working medium.

On the 5th day of incubation, the mat Kuchiger-16, with glucose (0.25 %) as a substrate, gave a voltage increase of up to 289 mV. The current strength accordingly reached 3727 μI (figure 1, 2). During the experiments the pH of the anolyte varied from 9.6 to 7.8. By replacing NaNO₃ with urea (0.25 g/l) in the variant with glucose (0.25 %), the EMF value was raised to 247 mV, the current strength was up to 1770 μI. The pH varied from 9.6 to 8.5. Using peptone (0.25 %) as a substrate, the potential reached values of 315 mV and the current strength was 3180 μI. The hydrogen index remained practically unchanged (9.4 at the beginning and 9.2 at the end of the experiment). The buffer system containing the mat Kuchiger-16 and sodium acetate did not exceed 137 mV and 728 μI. The pH didn’t change. Consequently, sodium acetate, under the conditions of our experiments, turned out to be a poorly suitable substrate for use in BFC combined with the Kuchiger-16.
Figure 1. Dynamics of the voltage generated in the BFC using a microbial mat Kuchiger-16 (medium: sodium carbonate/bicarbonate buffer 0.05 M + Na$_2$HPO$_4$ (0.25 g/l) + NaNO$_3$ (0.25 g/l) + substrates: 1) glucose (0.25 %); 2) glucose (0.25 %) with urea (0.25 g/l), but without NaNO$_3$; 3) peptone (0.25 %); 4) sodium acetate (0.25 %)); T – 36±1 °C, time – 5 days.

Figure 2. Dynamics of the electric current generated in the BFC using a microbial mat Kuchiger-16 (medium: sodium carbonate/bicarbonate buffer 0.05 M + Na$_2$HPO$_4$ (0.25 g/l) + NaNO$_3$ (0.25 g/l) + substrates: 1) glucose (0.25 %); 2) glucose (0.25 %) with urea (0.25 g/l), but without NaNO$_3$; 3) peptone (0.25 %); 4) sodium acetate (0.25 %)); T – 36±1 °C, time – 5 days.
The maximum capacity of BFC obtained with microbial mats was calculated in the work. When used as a substrate for glucose and peptone, the microbial mat of Kuchiger-16 showed a very close electrical power normalized to a unit of the geometrical surface of the electrode – 240 and 221 mW/m² respectively. Replacement of the springs of nitrogen from mineral to organic (urea) resulted in a decrease in electric power by a factor of 2. With urea, it was – 110 mW/m² (figure 3).

Figure 3. The maximum power of the Kuchiger-16 microbial mat is normalized to the unit of the geometrical surface of the electrode, when used as substrates: glucose (0.25%); glucose (0.25 %) with urea (0.25 g/l); peptone (0.25 %); sodium acetate (0.25 %).

Thus, the electrogenic activity of a microbial mat isolated from the Kuchiger thermal spring is shown. The materials obtained allow us to talk about the prospects of using a microbial mat as an electron in BFC when utilizing alkaline waste water components to generate electricity.

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