# Bioelectrochemical processes of oxidation of dicarboxylic amino acids by strain *Micrococcus luteus* 1-l in a biofuel cell

# Bioelectrochemical processes of oxidation of dicarboxylic amino acids by strain *Micrococcus luteus* 1-I in a biofuel cell

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**Abstract**. A facultative anaerobic strain was isolated and studied from the activated sludge of the treatment facilities of a petrochemical enterprise. Its morphological and cultural, physiological and biochemical, tinctorial, molecular genetic characteristics have been investigated. Based on the data obtained, strain 1-I was assigned to the species *Micrococcus luteus*. The electrogenic activity of this bacterium in BFC was shown using dicarboxylic amino acids - glutamic and aspartic. The open-circuit voltage indices in the BFC with *M. luteus* 1-I increased in 6 days to 511.5 mV with the addition of aspartic acid, and 419 ± 38.5 mV with the addition of glutamic acid. In this case, the short-circuit current increased to 3.17 ± 0.12 and 1.6 ± 0.14 mA, respectively. The specific power of BFCs based on *M. luteus* 1-I was the highest with the addition of aspartic acid (40-50 mW / $m^2$ at a current density of 0.15 to 0.4 A / $m^2$). The indicated indicator in a similar BFC with glutamic acid was 26-32 mW / $m^2$ (at a current density of 0.08 to 0.28 A / $m^2$). The oxidation of these compounds by the studied bacterial strain was also confirmed by the methods of cyclic voltammetry.

## 1. Introduction

One of the main directions of biofuel cell technology (BFC) is the generation of electric current using raw materials with negative cost (various wastes, pollutants, waste water). Microorganisms are the most important link in these processes. In this case, the decisive factor in the functioning of the BFC is the withdrawal of electrons from the contaminant and the transfer to the acceptor (electrode). Given the wide range of chemical structures of pollutants, a continuous search and study of new strains of electrogenic microorganisms is underway [1-8]. The purpose of this communication was to study the *Micrococcus luteus* 1-I strain isolated from activated sludge of a petrochemical plant. The task of this work also included the study of the electrochemical parameters of BFCs functioning on the basis of this microorganism. Dicarboxylic amino acids - glutamic and aspartic – were used as substrates for microorganisms in BFC. They play an important, integrating role in nitrogen metabolism of proteins: for mutual transformation into each other, all nonessential amino acids in organisms are first converted into glutamic or aspartic ones.

## 2. Materials and methods

Strain *M. luteus* 1-I was isolated from activated sludge of JSC Angarsk Petrochemical Plant "Rosneft". The tinctorial, cultural, and physiological-biochemical properties of the isolated strain were studied according to the methods generally accepted in microbiology [9]. Identification was carried out by MALDI-TOF mass spectrometry and 16S rRNA gene analysis at the Institute of Biochemistry and Physiology of Microorganisms. G.K. Scriabin RAS.

In this work, we used a BFC, the design of which is described in [10]. Carbon fabric "Ural T-22P A" (JSC SvetlogorskKhimvolokno, Republic of Belarus) served as electrodes in the BFC. The size of the electrodes was 1 × 120 × 30 mm. The anolyte and catholyte in the BFC was our modified model wastewater. Organic carbon sources - sodium acetate and peptone - were removed from its composition (GOST P 50595 - 1993). Thus, the medium consisted of the following salts (mg / l): sodium carbonate - 50.0; monosubstituted potassium phosphate - 25.0; disubstituted ammonium phosphate - 25.0; calcium chloride - 7.5; magnesium sulfate - 5.0. Amino acids (aspartic and glutamic) at a concentration of 0.5 g / L were introduced into the anode chamber. The values of the current and voltage in the BFC were evaluated using a digital multimeter tester "DT-266". The measurements of these indicators were carried out in the "no-load" mode (open-circuit voltage, short-circuit current strength). Also, measurements of these indicators were carried out when the BFC was operated on an external load - resistance in the range from 10 to 10,000 ohms. Calculated power, specific power and current density [11]. Voltammetric studies were carried out in accordance with [10; 12]. Working electrodes for cyclic voltammetry measurements were made of glassy carbon (“GC-2000”) cut into 2 × 38 × 6 mm plates. The reference electrode was Ag | AgCl | NaCl 3M (BAS RE1, Japan). The platinum grid served as a counter electrode. Polarization curves were recorded using an IPC Pro potentiostat (Russia) and IPS 2000 software. The potential sweep rate in all cases was 100 mV / s. Electron microscopy of the strain under study was carried out using a Philips SEM525-M SEM at the Electron Microscopy PC, which is part of the Ultramicroanalysis Center for Collective Use of the Limnological Institute of the Siberian Branch of the Russian Academy of Sciences. All experiments were conducted in at least five independent tests with three parallel measurements in each. The experimental data were statistically processed using the Statistica and Windows Excel programs. The significance of differences in the results was determined using the Student's t-test ($p \geq 0.95$).

## 3. Results

### *3.1 Characteristics of the strain Micrococcus luteus 1-I*

The strain isolated from the activated sludge of a petrochemical plant was gram-positive cocci. The cell size was 0.93-1.3 μm (figure 1a). The strain grew well both on synthetic medium No. 1 with hexadecane, and on protein media (fish-peptone and mesopatamia agar). When growing on these media, the strain formed shiny round colonies, yellow in color, with a convex profile, and a homogeneous structure. The edge of the colonies was flat. The surface is smooth. The consistency is oily (figure 1b). In a liquid medium (mesopatamia broth), the growth is near-bottom, loose or flaky.

The isolated strain gave a negative growth reaction on a medium with carbohydrates such as glucose, lactose, maltose, sucrose, mannitol, sorbitol. It possessed lecithinase, amylolytic, lipolytic, catalase activity. The investigated strain carried out proteolysis of gelatin. The determinations showed that the isolate was a facultative anaerobic. The strain reduced nitrate to nitrite and was capable of denitrification. The isolate was characterized by weak growth on Ashby nitrogen-free medium. This made it possible to classify it as an oligonitrophil.

The ability of the isolated bacteria to use organic acids, amino acids, and aliphatic hydrocarbons (hexadecane) as the only source of carbon and energy was studied. *M. luteus* 1-I grew on media with hexadecane, salicylic, cinnamic and α-anthracene carboxylic acids. Scant growth was observed on a medium containing alanine, mandelic and oxalic acids. In the variant with ascorbic acid and with glycine, there was no growth.

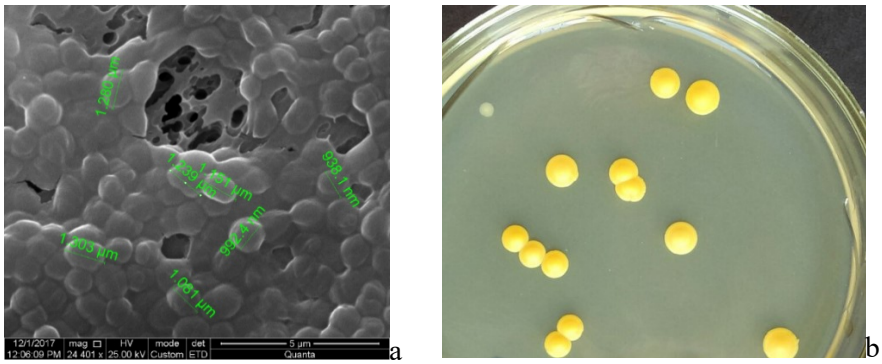


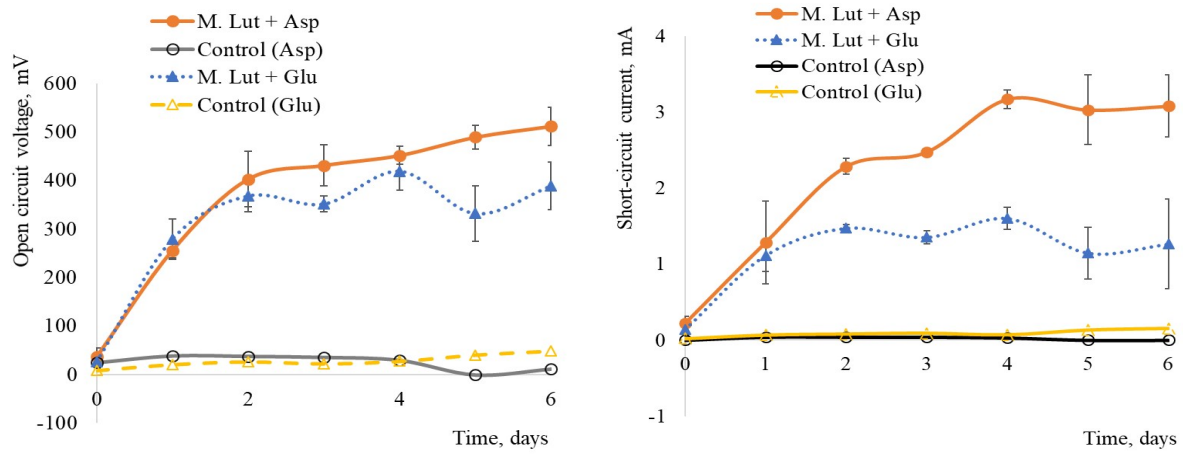
**Figure 1.** Cells of M. luteus strain 1 (SEM, Philips SEM525-M) (a) and colonies on fish-peptone agar (b).

The investigated strain turned out to be rather halotolerant. It showed good growth at up to 6.5% sodium chloride. The culture showed poor growth even in the presence of 0.05% potassium cyanide. *M. luteus* 1-I showed antibiotic resistance to amoxicillin, furadonin. The sensitivity of this strain to antibiotics such as ampicillin, vancomycin, gentamicin, ticarcillin / clavulenic acid, ciprofloxacin, cefuroxime, cefaclor was revealed.

Based on the studied morphological, cultural, physiological and biochemical characters, as well as the results of 16S rRNA gene sequencing and MALDI / TOF mass spectrometry, strain 1-I was assigned to the species *Micrococcus luteus*.

*3.2 Electrochemical characteristics of BFC based on M. luteus 1-I and dicarboxylic amino acids*

The open-circuit voltage in BFC with *M. luteus* after 6 days of exposure increased to 511.5 mV with the addition of aspartic acid, and 419 ± 38.5 mV with the addition of glutamic acid. At the same time, the most intensive increase in electrical indicators was observed in the first 2 days. Further increase in electrical parameters was insignificant (figure 2a). In parallel, the short-circuit current was measured in the investigated BFCs. In BFC with aspartic acid, this indicator was characterized by an increase during the first 4 days. It reached its maximum on 4 days – 3.17 ± 0.12 mA. In BFC with glutamic acid, the current increased less intensively. The highest values of this indicator (1.6 ± 0.14 mA) were also recorded on the 4th day of the experiment (figure 2b).




a b

**Figure 2.** Dynamics of open circuit voltage (a), short-circuit current (b) generated in BFC by *M. luteus* 1-I strain when using aspartic (0.5 g / l) and glutamic (0.5 g / l) acids.

The specific power of the investigated BFCs was measured when operating on an external load with a resistance in the range of 0.01 - 100 kΩ. The highest values of electrical parameters were obtained using resistance from 0.1 to 1.0 kΩ (figure 3).

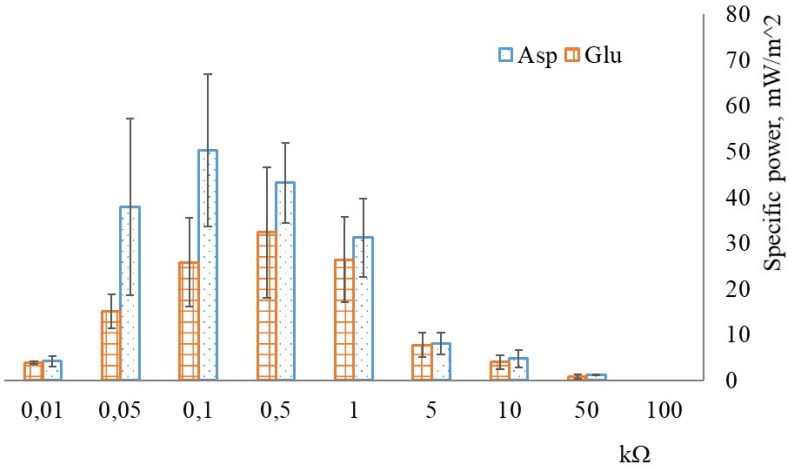



**Figure 3.** Specific power indicators of BFC based on *M. luteus* 1-I strain using aspartic (0.5 g / l) and glutamic (0.5 g / l) acids, measured at resistance in the range from 0.01 to 100 kΩ.

The maximum power developed by the BFC is achieved when the internal and external resistance is equal [13; 14]. The obtained power values of the investigated BFCs based on *the M. luteus* 1-I strain allow us to assume that their internal resistance is in the range of 0.1 - 1.0 kΩ.

In figure 4 shows the current-voltage characteristics of BFCs with *M. luteus* 1-I and with the studied amino acids on days 1 (a) and 4 (b) of the experiment.

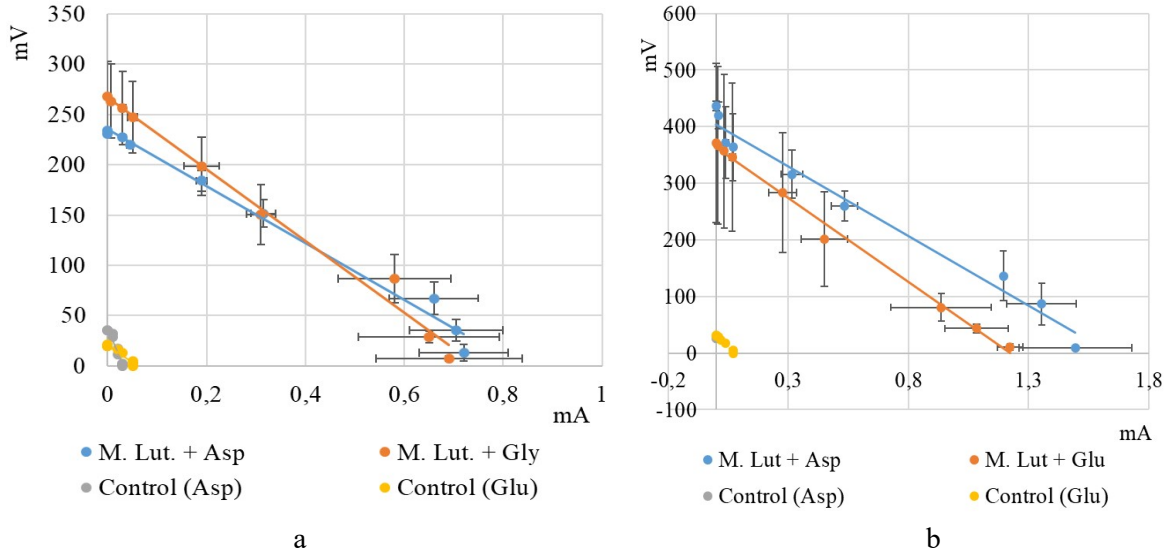



**Figure 4.** Current-voltage characteristics of BFC based on *M. luteus* 1-I strain when using aspartic (0.5 g / l) and glutamic (0.5 g / l) acids on the first (a) and fourth (b) days of the experiment.

The highest values of the specific power of BFCs with aspartic acid and *M. luteus* 1-I bacteria (40-50 mW / $m^2$) were recorded at a current density of 0.15 to 0.4 A / $m^2$. The indicators of similar BFC

with glutamic acid were somewhat lower. In this case, the specific power increased to 26-32 mW / $m^2$ at a current density of 0.08 to 0.28 A / $m^2$ (figure 5).

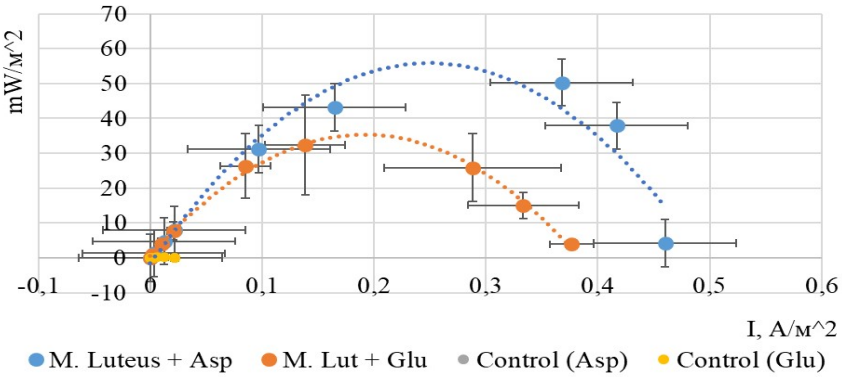



**Figure 5.** The ratio of the specific power (mW / $m^2$) to the specific strength (A / $m^2$) of the BFC current based on the *M. luteus* 1-I strain when using aspartic (0.5 g / l) and glutamic (0.5 g / l) acids on 4 days of exposure (4th day).

The oxidation of aspartic and glutamic acids by the studied bacterial strain *M. luteus* 1-I was also confirmed by voltammetric studies. Thus, on the cyclic voltammogram in the presence of *M. luteus*, an increase in the anodic current values was observed in comparison with the control. This confirms the oxidation of dicarboxylic amino acids by the studied bacteria (figure 6).

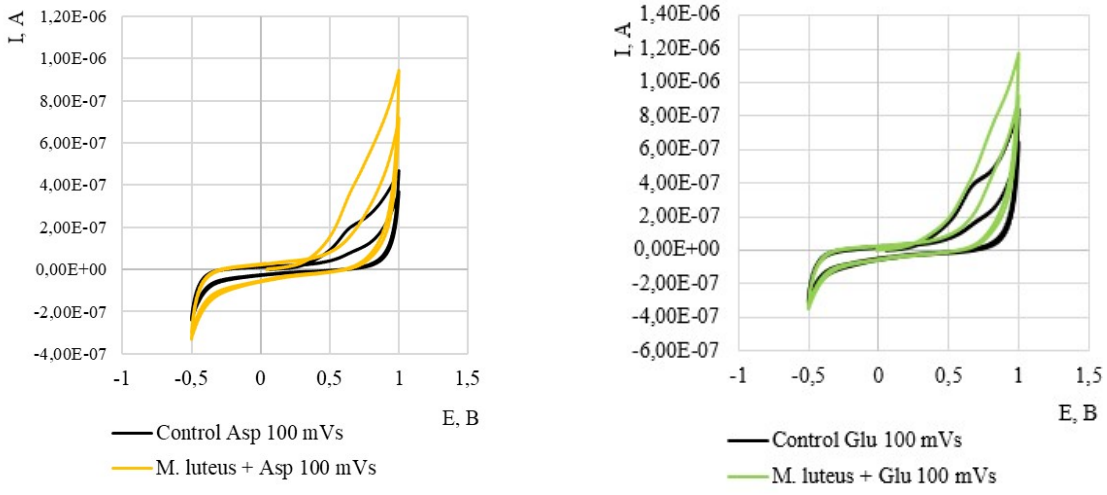



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**Figure 6.** Cyclic voltammograms measured in solutions of dicarboxylic amino acids in the presence of *M. luteus* 1-I strain (potential sweep rate 100 mV/s)

## 4. Conclusion

Thus, a facultative anaerobic strain was isolated from the activated sludge of the treatment facilities of a petrochemical enterprise. It was identified as *M. luteus* 1-I. The isolate had lecithinase, amylolytic, proteolytic, lipolytic, catalase activity. The strain reduced nitrate to nitrite and was capable of denitrification. It was characterized by weak growth on Ashby's nitrogen-free medium (this made it possible to classify it as an oligonitrophil). *M. luteus* 1-I grew on media with cetane, salicylic, cinnamic and α-anthracene carboxylic acids. Lean growth was observed on a medium with alanine,

mandelic and oxalic acids. The investigated strain turned out to be rather halotolerant. It showed good growth up to 6.5% NaCl. The culture showed poor growth even in the presence of 0.05% potassium cyanide.

The *M. luteus* 1-I strain generated an electric current in the BFC using dicarboxylic amino acids – glutamic and aspartic. The oxidation of these compounds by the studied bacterial strain was also confirmed by the methods of cyclic voltammetry. Earlier, Choi et al. [15] showed electrogenic activity in BFC of another *M. luteus* strain (KCCM 40166, IFO 3066). Thus, the experimental data obtained in our work, on the one hand, are consistent with the data on the ability of *M. luteus* strains to generate an electric current. On the other hand, the electrogenic activity of the strain isolated by us was demonstrated on dicarboxylic amino acids. In the work of Choi et al. [15] carbohydrates were used as substrates.

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