# Express Method for Redox Potential and pH Measuring in Microbial Cultures

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***Abstract:*** *The new modification of potentiometric pH and Eh measuring was developed. The essence of the method is to measure pH and Eh in compact hermetic redox cell. This modification provides reliable and fast pH and Eh measuring with minimum expense of culture liquid (1 $cm^3$ for each measuring). The application of the developed modification in microbiology was shown on the example of bacterial culture Kocuria carniphila isolated from Negev desert soil (Israel). Dynamics of the Eh changing in the growing culture allows establishing the influence of the carbon and energy sources concentration on the microbial growth, the rate of oxygen consumption and character of microbial interaction with toxic compounds. Copiotriphic conditions (800 mg/l C) were shown to be optimal for K. carniphila. It was shown that K. carniphila was resistant to 200 ppm of $Cu^{2+}$, nevertheless it did not extract $Cu^{2+}$ out of liquid medium.*

***Keywords:*** *Redox potential, Method modification, Kocuria carniphila, Carbon concentration, Copper.*

## Introduction

Energy metabolism of all organisms, as well as microorganisms, is based on redox reactions. In these reactions, multi-enzyme systems provide coupled reaction of catabolism – the oxidation of electron donors and reduction of electron acceptors for energy obtaining. It is known that redox potential is one of the main indicators of direction and efficiency of the redox reactions. Redox potential (Eh, mV) allows to predict the final result of the microbial interaction with chemical elements in ecosystems, to determine the effectiveness of growth and metabolic processes in vitro, to create the theoretical background for the regulation of microbial metabolism. Regulation of microbial metabolism plays an important role in the biotechnological processes, for example, optimizing the microbial synthesis of energy (hydrogen, methane), increasing the efficiency of industrial wastewater treatment from toxic metals, organic xenobiotics, etc.

Value of Eh enables to evaluate both direction of metabolic processes and the efficiency of microbial growth. For example, lowering the redox potential to -100 ... -200 mV during cultivation of aerobic microorganisms in liquid medium clearly indicates the lack of oxygen. So, it is necessary to provide better aeration or to reduce the concentration of carbon and energy sources. Addition of toxic compounds to the culture liquid inhibits metabolic processes of microorganisms and, therefore, the Eh increases sharply. Dynamics of Eh changing mapped to growth of biomass enable us to classify the microorganisms as aerobic or facultative and obligate anaerobic [3]. Decrease of Eh value and biomass growth of aerobic

and facultative anaerobic microorganisms occurs simultaneously. In contrast, no obligate anaerobic microorganisms grow up until Eh decreases to -200 ... -400 mV [3].

The investigation of redox potential in biological systems was started in the end of the XIX$^{th}$ century. The history of Eh investigation of living objects was exhaustively described by Jacob [4]. Ehrlich first used aniline dyes to characterize the redox state in animal tissues in 1886. Potter first realized the electrochemical measurement of the redox potential in the growing culture of microorganisms in 1910. Gillespie suggested using the term "redox potential" for bacterial cultures in 1920. Since then the measurement of the redox potential has been widely used in biology.

Measurement of redox potential in growing cultures of microorganisms is widespread in present researches. There are two methods for measuring the redox potential – colorimetric and potentiometric.

## Colorimetric method for measuring the redox potential

The method is based on the ability of redox-indicators (organic dyes – aromatic derivatives) to change color or become colorless (leukoform) at specific redox potential values, which are specific for each indicator. The most common redox-indicators in biochemistry and microbiology are methylene blue, and sodium resazurin and benzylvialogen [3]. The most complete information on the usage of redox indicators in microbiology is presented in the classical works of Jacob [4], Meynell [5], Duda [3]. However, in these studies only values of standard redox potential of redox-indicators reduction are presented. By definition, standard redox potentials values are the values at equal concentrations of oxidized and reduced forms. Such mode of expression the redox potential has been generally accepted, however, this makes impossible the practical use of redox-indicators in microbiology to determine the redox potential. Indeed, it is practically impossible to visually determine the redox potential value by decreasing the color intensity of the culture liquid. Therefore, earlier we created a scale of redox indicators, which allows to measure redox-potential with the step 20-30 mV, in the range from -30 to -420 mV [9].

Obviously, both colorimetric and potentiometric (electrode) methods for measuring the redox potential have advantages and disadvantages. Advantages of colorimetric measurement of redox potential are:

1. Possibility of simultaneous Eh measurement in the tens and even hundreds of variants of the experiment (tubes, flasks).
2. Possibility to measure the redox potential visually without depressurizing of cultivators and without any other additional operations.
3. Simplicity and affordability of the method.

The disadvantages include the following:

1. Many redox-indicators are toxic to microorganisms (for instance, methylene blue, brilliant green, benzylvialogen).
2. Some redox-indicators change color and intensity of color due to both Eh and pH value changing. For example, sodium resazurin decolorizes in neutral conditions at Eh = -100 mV. However, sodium resazurin becomes light yellow (almost colorless) in acidic conditions at pH in the range from 3 to 4. It is known that strong acidification of the medium occurs during the microbial fermentation of sugars. Hence, in these conditions discoloration of sodium resazurin can be assessed by mistake as a reducing Eh value to -100 mV and below.

3. Some dyes can play the role of high-potential redox buffers in the visually-detectable concentrations, and thus have a negative impact on the growth of microorganisms.

## Potentiometric method for the redox potential determining

The method is based on redox-potential measurement on the potentiometer with a pair of electrodes. One electrode is measuring; the second is the reference electrode. Platinum polished measuring electrode, such as EPV-1 and silver chloride flow reference electrode EVL-1MZ are the most common electrodes used in microbiology. Besides the redox potential value, the pH value is also measured with the respective electrode, such as ECL-63-07 (coupled with a silver chloride reference electrode).

The advantages of potentiometric measuring of the redox potential are:

1. Ability to measure Eh accurately (± 10 mV) and continuously;
2. Ability to measure accurately redox-potential of many objects by a set of calibrated electrodes (for measuring pH, Eh, and the reference electrode).

Two modifications of potentiometric measuring of the redox potential in growing microbial cultures are known. In the first modification, the culture liquid is sterilely taken out of cultivator and transferred to non-sterile system of Eh measurement. In the second modification, sterile electrodes are fitted directly in the cultivator over the entire experiment.

In the first modification, reference and measuring electrodes are inserted in a tall, narrow cylinder. The electrodes must be at the bottom of the cylinder. Next, at least 30-50 ml of culture liquid is taken out of the cultivator with the syringe and brought in cylinder with the electrodes to the final height of the culture liquid column 8-10 cm. High layer of the culture liquid is necessary to minimize diffusion of air oxygen to the lower layer, wherein measured redox potential is directly hold and, respectively, the distortions in the measurement of the redox potential. To increase the accuracy of measurements, it is advisable to continuously blow the cylinder with argon both before bringing in the culture liquid and during the measurement [4].

Disadvantages of this modification are following:

1. Potential contact of culture liquid with atmospheric oxygen leads to high probability of inaccurate Eh measuring.
2. Large volumes of culture liquid (30-50 ml) are required to measure the pH and Eh. Therefore, the amount of pH and Eh measurements for a single experiment is limited.
3. Blowing cylinder with argon complicates the measurement procedure of Eh.

In the second modification, pH and Eh are continuously measured throughout the experiment by electrodes hermetically fitted in a cultivator. The electrodes may be fitted in a cover of a cultivator, or in rubber plugs of specially made glass measuring cell [2].

This modification has several disadvantages that obstruct its use.

1. Measuring electrodes have to be sterilized because during the whole experiment the electrodes are inserted in the culture liquid. Both measuring and reference electrodes can not be sterilized thermally. Platinum electrode cracks during heating in the flame because platinum and glass have different thermal expansion coefficients. Flow reference electrode, which contains saturated solution of KCl inside the cylinder, comes into disrepair during sterilization in flame. Thus, KCl solution boils during the

electrode heating and KCl crystals form inside the electrode. Furthermore, the crystals clog asbestine wick on the tip of the electrode.
2. Chemical sterilization of electrodes is complex and unreliable. The 33% solution of $H_2O_2$ or less concentrated solution of $H_2O_2$ with the addition of sulfuric acid is usually used for electrodes sterilizing. Sterilization of the reference electrode is almost impossible task. Even when the entire outer surface of the electrode is sterilized effectively, it is not possible to sterilize internal fragments (KCl solution and asbestine wick saturated with AgCl).
3. The necessity of costly production of cultivators with complex design.
4. Potentiometric method is impossible to use in the experiments with a large number of variants (replicates). For example, for ten variants of the experiment it is necessary to use ten sets of measuring electrodes and the same amount of expensive cultivators.
5. The measurements are inaccurate during prolonged cultivation. Surface of electrode is being covered by a dense film of microorganisms in 18-24 hours of cultivation. It is obvious that the values of the redox potentials of the biofilm and the cells suspended in the medium differ greatly.
6. Mineralization of culture liquid increases when a saturated solution of KCl diffuses out the flow reference electrode to the culture liquid. This inhibits the growth of microorganisms.

So, it is necessary to develop new method modification of the redox potential measuring, which is devoid of the disadvantages of both colorimetric and potentiometric methods.

Accordingly, the aim of the work is to develop an express method for measuring the redox potential, and to evaluate the possibility of applying this method to microbiology. The influence of extreme factors on the growth of microorganisms and interaction of microorganisms with extreme factors with the help of the express-method was investigated. As objects we used the dominant culture of the Negev desert soil (Israel) *Kocuria carniphila* and toxic metal Cu(II).

## Materials and methods

### *pH-buffers preparing*

Standard buffers-fixanals were used to control pH:
potassium tetraoksalat (pH = 1.68), the mixture of sodium hydrogen phosphate and potassium dihydrogen phosphate (pH = 6.86) and sodium tetraborate (pH = 9.18).

### *Eh-buffers preparing*

Redox buffers were used to control the measurement of Eh:
mixture of potassium ferricyanide and potassium ferrocyanide (Eh = +480 mV, pH = 4.5), ferric citrate (Eh = -200 mV, pH = 7.0), titanium(III) citrate (Eh = -480 mV, pH = 7.0).

The composition of the redox buffer is as follows:

- 13.5 g/l of $K_3Fe(CN)_6$ (potassium ferricyanide) and 3.8 g/l of $K_4Fe(CN)_6$ (potassium ferrocyanide);
- 10 g/l $Fe^{2+}$, 20 g/l $Na_3C_6H_5O_7$, 50 ml of distilled water;
- 5 ml of a 15% solution of $TiCl_3$, 3.57 g of $Na_3C_6H_5O_7 \times 5.5\ H_2O$ (trisodium citrate), 3.2 g of $NaHCO_3$, 50 ml of distilled water.

### *Preparing of mixture of potassium ferricyanide and potassium ferrocyanide*

Potassium ferricyanide (13.5 g/l) and potassium ferrocyanide (3.8 g/l) were put into 1 l volumetric flask and dissolved in 300 ml of distilled water. Then the volume of the solution was made up to 1 l with the distilled water.

### *Preparing of ferric citrate*

Trisodium citrate ($C_6H_5O_7Na_3 \cdot 5, 5H_2O$) 5.0 g were dissolved in a beaker in 50 ml of distilled water. The solution was transferred into a 100 ml flask with a screw thread on the neck. The flask was blown with argon to remove atmospheric oxygen. The needle for argon supply was introduced to the bottom of the flask; the needle was fixed in the neck of the flask with a cotton-gauze plug. The flask with the solution was blown with argon for 20 minutes at a flow rate of argon of 0.5 l/min to remove dissolved oxygen completely. Thereafter, the 2.5 g of $FeSO_4 \cdot 7H_2O$ were added into the flask. Then the flask was closed with an elastic rubber plug and fixed on the neck of a flask with aluminum screwthread clamp. The excess pressure was created. The elastic plug was pierced therethrough with a thin needle, and argon was supplied to the flask to the final pressure of 0.3 atm.

### *Preparing of a titanium(III) citrate*

Two solutions ("A" and "B") were used to prepare solution of titanium(III) citrate.

### *Preparing of solution "A"*

First the 20 ml flask was blown with argon for 5 minutes at a flow rate of argon of 0.5 l/min to remove air oxygen from the flask. Argon was supplied in a flask by the needle that was fixed by a cotton-gauze plug near the inner surface of the flask neck. Then 6 ml of 15% $TiCl_3$ solution (qualification "*Purum*") were added into the flask during the continuous argon blowing. The flask was closed with an elastic rubber plug, and at the same time the needle was removed. Then, a plug was fixed onto the neck of the sealed flask with the aluminum screwthread clamp. After this, a thin needle was injected through the elastic rubber plug and argon was fed into the flask to the final overpressure of 0.5 atm. That was necessary to avoid the rarefaction, when titanium chloride would be taken out of the sealed flask by the syringe.

### *Preparing of solution "B"*

Trisodium citrate (3.57 g) and $NaHCO_3$ (3.2 g) were dissolved in a beaker of 50 ml of distilled water. Then alkali citrate solution was transferred to a 100 ml flask. After that the solution was being blown by argon for 10 minutes at a flow rate of argon of 0.5 l/min. Argon was supplied to the flask by a needle. Next, the flask was closed with a rubber plug, and it was fixed on the neck of the flask with an aluminum screwthread clamp.

The solution "A" was transferred to the flask with solution "B" in the following way. 1 ml of $TiCl_3$ was taken with 5 ml plastic syringe[1] out of the flask with the solution "A". The syringe was washed with $TiCl_3$ solution and then this solution was discarded. This operation was done to remove completely traces of air oxygen from the syringe as well as possible oxidizers on its internal surfaces. Then, 5 ml of $TiCl_3$ were transferred with the same syringe from the flask with solution "A" to the flask with solution "B". Hydrochloric acid, which was in excess in solution of titanium chloride, reacted with $NaHCO_3$. Due to this reaction of neutralization, carbon dioxide gas was generated, and the pressure in the flask

[1] If syringe with metal parts is used, surface coating (e.g., metallic Ni) dissolute in a strongly acidic solution of $TiCl_3$. Cation $Ni^{2+}$ strongly inhibits the growth of microorganisms when titanium(III) citrate is added to the culture medium.

increased to 1.0-1.2 atm. In one hour, pH and Eh values of titanium citrate solution came to an equilibrium state (pH = 7.0-7.2 and Eh = -480 ... -520 mV).

## *pH and Eh measuring*

Redox potential was determined using a potentiometer pH meter-milivoltmeter "pH-121" (or "EV-74") with electrodes: a platinum measuring electrode EPV-1, a glass ESL-63-07 (pH measurement) and a flow silver chloride reference electrode EVL-1MZ.

## *Cultivation of bacterial strain Kocuria carniphila*

*K. carniphila* was cultivated in copiotrophic (800 mg/dm$^3$ of total C) and oligotrophic (80 mg/dm$^3$ C) conditions. For cultivating *K. carniphila* in copiotrophic conditions, nutrient broth was used (HiMedia Laboratories Pvt. Ltd.). The concentration of the organic compounds in the nutrient broth was 800 mg/dm$^3$ C. For cultivation of *K. carniphila* in oligotrophic conditions, the poor broth was prepared. Poor broth is nutrient broth diluted with sterile distilled water by 10 times. Thus, the concentration of organic compounds in poor broth was ten times lower than in nutrient broth, and amounted 80 mg/dm$^3$ C.

Both nutrient and poor broth (100 ml each) were put in 150 ml flasks. Then, suspended in physiological solution *K. carniphila* was added to the broth. The final optical density of the broth was 0.05 units. Flasks were sealed with elastic rubber plugs and has their necks fixed with aluminum clamps. Microorganisms were cultivated at 28°C for 72 hours without shaking.

Culture *K. carniphila* was cultivated in the presence of 200 pmm of $Cu^{2+}$, as well as in both copiotrophic and oligotrophic conditions. For this reason, nutrient and poor broth (100 ml each) were put in 150 ml flasks. Then 1 ml of copper citrate solution (20.000 ppm of $Cu^{2+}$) was added into each flask. After that, *K. carniphila* biomass was added to the flask until the final optical density of the broth was 0.05 units. Flasks were sealed with elastic rubber plugs and had their necks fixed with aluminum clamps. Microorganisms were cultivated at 28°C for 72 hours without shaking.

## *Biomass growth*

Biomass growth in the culture liquid was determined by the change in the optical density at the PEC at $\lambda$ = 540 nm, the length of the optical step 0.5 cm.

## *Preparing of solution of copper(II) citrate*

Standard copper citrate solution contained 20.000 ppm of $Cu^{2+}$. 5.34 g of $CuCl_2 \cdot 2H_2O$ were dissolved in 50 ml of distilled water in a beaker. In the result of copper chloride dissolution, light blue transparent solution formed. Thereafter, 20 g of disodium citrate ($Na_2C_6H_6O_7 \cdot 1½H_2O$) were added to the solution of $CuCl_2$. After that the light blue solution of $CuCl_2$ became dark blue, which evidenced the copper(II) citrate formation. The solution was transferred from the beaker to a 100 ml volumetric flask, and the volume of the solution was brought to the mark with distilled water. Before using copper, the citrate solution was sterilized in a boiling water bath for 10 minutes.

## *Preparing of liquid copper-containing medium*

Nutrient broth and poor broth (100 ml each) were put in 150 ml flasks. Then 1 ml of sterile copper citrate (20 g/l of $Cu^{2+}$) was added to the broth. Thus copper-containing medium with 200 ppm of $Cu^{2+}$ was obtained.

### *Concentration of $Cu^{2+}$ determination in culture liquid*

Concentration of $Cu^{2+}$ was determined by the colorimetric method. The method is based on the colored complex formation of $Cu^{2+}$ with organic metal indicator *p*-aminoresorcin (PAR). PAR (0.5 ml of 0.5% aqueous solution) was added to the sample solution (3 ml). Then absorbance was measured at $\lambda$ = 490 nm (optical step 0.5 cm). Before determining the copper concentration, microorganisms were removed from the analytical sample by centrifugation at 10.000 g for 15 minutes.

## Results and discussion

### *Express method of redox potential and pH measuring*

The essence of the modified method of redox potential measuring is in using a sealed measuring redox-cell of small volume (0.5 ml) for the potentiometric measurement of Eh and pH. Cell design is extremely simple. Cell consists of a translucent tube (elastic silicone rubber), where measuring and reference electrodes are hermetically fixed (Fig. 1).

Process of equipment preparing and measurement is in the following (Fig. 1). In the tripod clamps (1), a flow reference electrode is rigidly fixed in the upright position (2). A silicone rubber tube (3) (outer diameter 11 mm, inner diameter 8 mm, respectively, wall thickness 1.5 mm, length 40 mm) is hermetically attached to the lower end of the electrode. The silicone tube has to be rigidly fixed on the reference electrode, so that the bottom end of the electrode is inserted no less than 1.0-1.5 cm into the tube.

Then a platinum electrode (4) (to measure Eh) is hermetically inserted into the free end of the tube. The tube's diameter is picked up in such a way to provide rigid fixation of the electrodes inside the tube (Fig. 1).

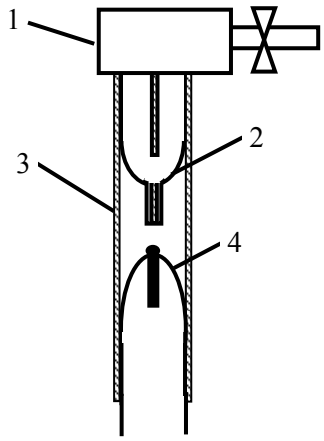


Fig. 1 Scheme of a measuring redox cell
1 – claw of tripod; 2 – silver chloride reference electrode;
3 – silicone tube; 4 – platinum measuring electrode.

Inner surface of the tube was wetted with distilled water to facilitate "screwing" the electrodes into the tube. The surfaces of the electrodes in a fixed state were spaced 2-3 mm from each other. In this case, the culture liquid volume required to fill the measuring cell is minimal (about 0.5 $cm^3$). General view of the redox cell is shown on Fig. 2.

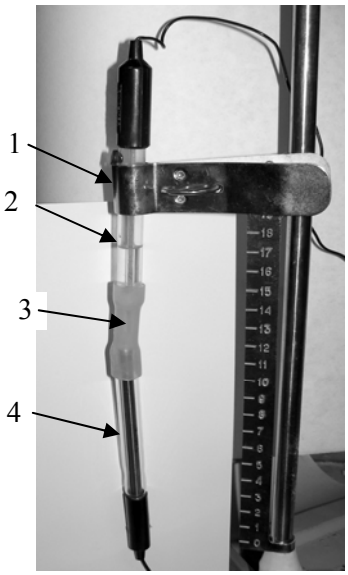


Fig. 2 Measuring redox cell
1 – claw of tripod; 2 – silver chloride reference electrode;
3 – silicone tube; 4 – platinum measuring electrode.

Order of pH and Eh measuring is shown on Fig. 3. First, the rubber plug of the sealed flask with culture liquid is punctured with a syringe and 1 $cm^3$ of the culture liquid is taken. Next, bottom part of the silicone tube is punctured directly at the surface of the platinum electrode by a syringe with culture liquid, and 0.5 $cm^3$ of the culture liquid is injected into the measuring cell (Fig. 3a). Due to this operation, air and potential oxidizers are removed from the surface of the electrode and the cell. The upper part of the silicone tube near the reference electrode is punctured by the needle of a syringe before introducing the culture liquid into the cell. This operation is to ensure free output of air from the measuring redox cell (Fig. 3b). A second portion of the culture liquid (0.5 $cm^3$) is injected into the cell directly for measuring the redox potential (Fig. 3c). Culture liquid has to fill the redox cell completely. Excess of culture liquid gets into the syringe, inserted into the upper bottom of the silicone tube.

Numerous injections of the culture liquid into the redox-cell cause the disturbance of the silicone tube. So, it is necessary to leave the needle of the syringe punctured in the bottom of the tube to avoid the disturbance of the tube during the experiment. In this case, the needle serves as a catheter for the culture liquid in the further measurements.

Measurement of pH is performed in the same order. An electrode for pH measuring is inserted instead of a platinum electrode in the lower part of the tube. Then, all above described operations are repeated.

The reliability of the developed method modification was verified with standard pH and redox buffers. The developed modification was shown not to distort the pH and Eh values of the buffers. Standard buffers-fixanals were used to control pH: potassium tetraoksalat (pH = 1.68), the mixture of sodium hydrogen phosphate and potassium dihydrogen phosphate (pH = 6.86) and sodium tetraborate (pH = 9.18). The values of pH of the buffers during measuring in the redox cell were the following: potassium tetraoksalat – 1.57±0.01, a mixture of sodium hydrogen phosphate and potassium dihydrogen phosphate – 6.99±0.01, sodium tetraborate – 9.30±0.01.

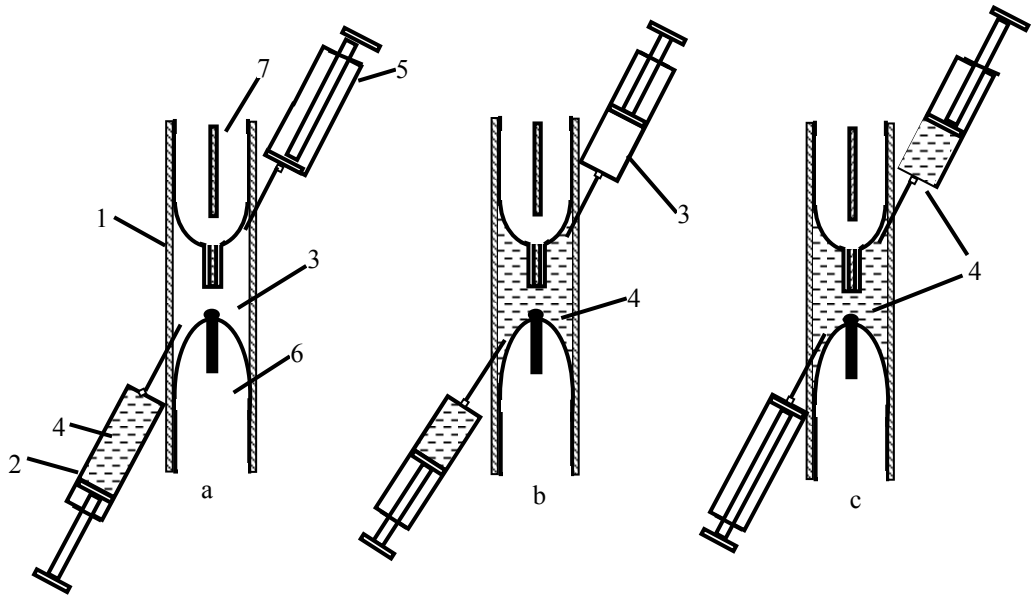


Fig. 3 Process of redox potential of culture liquid measuring the Eh in the redox cell
a – injection of the silicone tube by a syringe with a culture liquid and by an empty syringe to remove air from the redox cell; b – injection of culture liquid to the redox cell and removing the air from the redox cell; c – rinsing the redox cell by culture liquid and injection a sample in the redox cell to measure Eh; 1 – silicone tube; 2 – syringe with a culture liquid; 3 – air; 4 – culture liquid; 5 – empty syringe to remove air from redox cell; 6 – platinum measuring electrode; 7 – silver chloride reference electrode.

Redox buffers were used to control the measurement of Eh:
mixture of potassium ferricyanide and potassium ferrocyanide (Eh = +480 mV, pH = 4.5), ferric citrate (Eh = -200 mV, pH = 7.0), titanium(III) citrate (Eh = -480 mV, pH = 7.0). The following Eh values during measuring in the redox cell were obtained: +468±0.01 mV – mixture of potassium ferricyanide and potassium ferrocyanide, -202±0.01 mV – ferric citrate, -440±0.01 mV – titanium(III) citrate. So, experimentally obtained values of Eh do not differ from the standard values redox buffers.

Therefore, measuring of Eh in redox cell is valid. Moreover, the values obtained in the redox cell correspond to the values that were obtained with commonly accepted, but more laborious methods. Thus, the Eh values obtained during measuring in a glass cylinder with continuous argon blowing were the following: +467±0.01 mV – mixture of potassium ferricyanide and ferrocyanide, -214±0.01 mV – ferric citrate and -440 ±0.01 mV – titanium(III) citrate.

To ensure the validity of each set of measurements, the following operations should be provided. The electrodes should be tested with pH and redox buffers before the measuring. The tube of redox-cell has to be hermetically sealed on the electrodes. In the hermetic redox-cell, the tube does not slide off the electrodes, the culture liquid does not leak through the fissure between the electrode and the tube, and the Eh of low potential redox buffer does not rise up during measuring. For the additional check of redox-cell tightness, the culture liquid with sodium resazurin can be injected into the cell before the each set of measurements. If the liquid becomes pink, the redox-cell is not hermetic. Though the needle punctured in the bottom of the tube serves as a catheter, it is necessary to use a new silicone tube (without needle punctures) for each new set of measurements.

The advantages of our developed method modification are:

1. Culture liquid has no contact with oxygen of air during pH and Eh measuring.
2. Minimum amount of culture liquid is needed for pH and Eh measuring – 1 $cm^3$ (0.5 $cm^3$ for each measuring).
3. Measurement of pH and Eh is simple, fast and reliable.

## *Application of express method of Eh and pH measuring in microbiology*

Dynamics of the Eh changing in the growing culture enables to establish the influence of the carbon and energy sources concentration on the microbial growth, the rate of oxygen consumption and character of microbial interaction with toxic metals, such as copper(II) citrate.

Here is an example of the practical use of our method modification of measuring the redox potential in the microbiology. We isolated the dominant culture *K. carniphila* out of maximum dilution of Negev Desert soil (Israel). Concentration of organic compounds in the nutrient medium is one of the key parameters affecting the growth of the microorganisms. Obviously, it was necessary to establish the organic compounds concentration in the sample of desert soil for determining the physiological parameters of growth of *K. carniphila*.

We determined by the method of permanganate redox titration [11] that the concentration of organic compounds in the soil of the Negev Desert is 80 $mg/dm^3$ C. So, the dominant microbial strains of this ecosystem can be assumed as oligotrophic. However, higher concentrations of organic compounds can be optimal for some microorganisms abundant in poor oligotrophic conditions. Therefore, we investigated the growth of the dominant strain of the Negev desert soil *K. carniphila* in both oligotrophic (80 $mg/dm^3$ C) and copiotrophic (800 $mg/dm^3$ C) conditions.

On the $19^{th}$ hour of cultivation, the redox potential value was positive (+22 mV) and on the $60^{th}$ hour it even went up to +80 mV in oligotrophic conditions (Fig. 4, curve 2). In copiotrophic conditions (Fig. 4, curve 2), the redox potential decreased to negative values (-76 mV) on the $19^{th}$ hour of cultivation. And the minimum value of the redox potential (-190 mV) was registered on the $60^{th}$ hour of cultivation. Falling of the redox potential to negative values in copiotrophic conditions indicates intensive oxygen consumption.

Rate of $O_2$ consumption exceeds the rate of its diffusion into the medium. That is why the anaerobic conditions are created in the medium, which is indicated by a negative value of the redox potential (Eh = -76 ... -190 mV at pH 6.6 - 6.7). Therefore, *K. carniphila*, which exists in "oligotrophic" conditions in the soil of the Negev desert, can be assumed as copiotroph.

Larger yield of biomass in copiotrophic conditions compared to oligotrophic ones confirms *K. carniphila* to be copiotroph as well (Fig. 5). Thus, the optical density on the $19^{th}$ hour of cultivation in copiotrophic conditions was by 1.8 times higher than one in the oligotrophic conditions (0.54 and 0.31 units, respectively).

Presence of dominant copiotrophic bacteria *K. carniphila* in the poor oligotrophic soils of Negev desert requires further discussion. In the extreme conditions of desert, high mortality of animals (insects, reptiles, rodents, etc) is common. Specific name of bacteria "*carniphila*": "*caro, carnis*" – "*meat, flesh*", "*phila (philê)*" – "*to love*" indicates the biological significance of this species in the carbon cycle of the Negev desert ecosystem. Supposably, this culture provides the destruction of polymeric protein compounds of dead animals. This may explain

the distribution of the copiotrophic species of microorganisms in oligotrophic conditions of the desert.

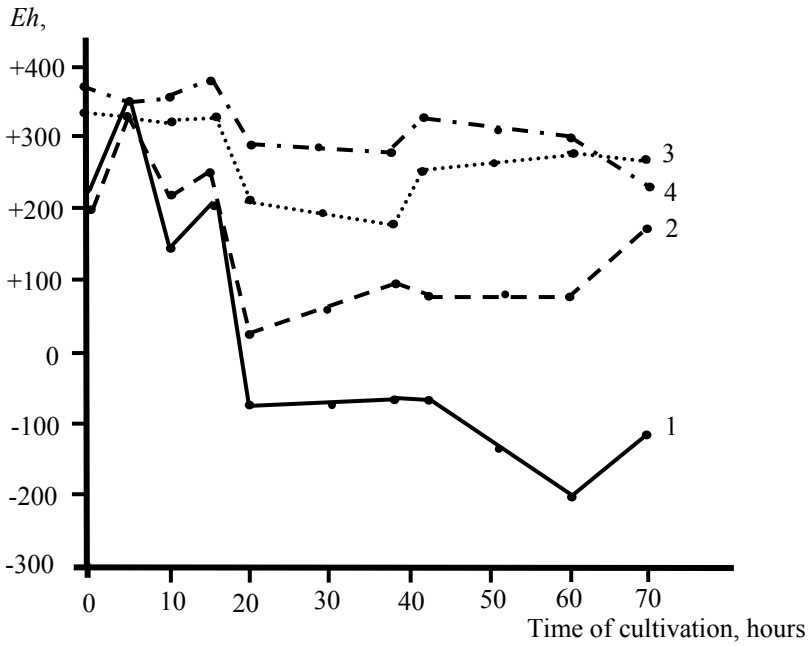


Fig. 4 Dynamics of redox potential changing in the growing culture *Kocuria carniphila*

1 – copiotrophic conditions; 2 – oligotrophic conditions; 3 – copiotrophic conditions in the presence of 200 ppm of $Cu^{2+}$; 4 – oligotrophic conditions in the presence of 200 ppm of $Cu^{2+}$.

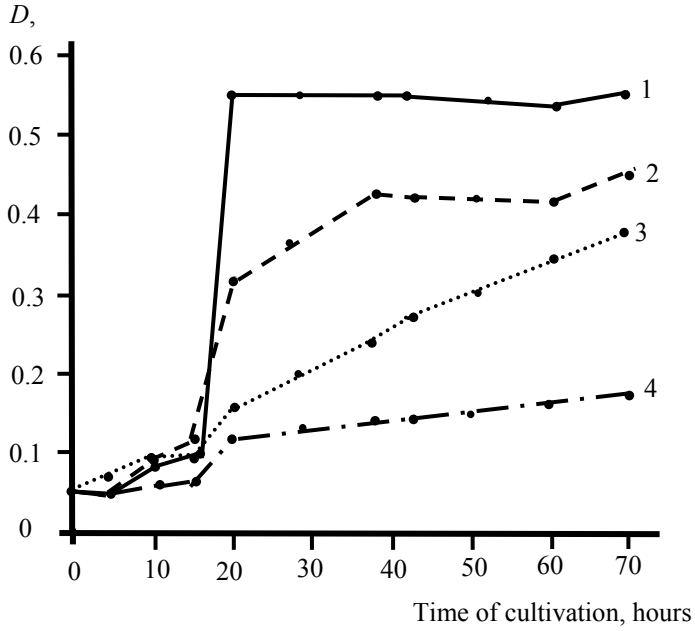


Fig. 5 Biomass growth of *Kocuria carniphila*

1 – copiotrophic conditions; 2 – oligotrophic conditions; 3 – copiotrophic conditions in the presence of 200 ppm of $Cu^{2+}$; 4 – oligotrophic conditions in the presence of 200 ppm of $Cu^{2+}$.

Redox potential value also enables to determine the character of interaction of microorganisms with toxic compounds. Consider the interaction of *K. carniphila* with toxic $Cu^{2+}$, which is chelated by citrate.

Copper(II) compounds are high-potential redox buffers [8, 12]. Calculated potential[2] of $Cu^{2+}$ reduction to insoluble copper oxide $Cu_2O$ is + 385 mV (reaction 1):

$$2Cu^{2+} + H_2O + 2e = Cu_2O + 2H^+ \text{ (Eh = +385, pH = 5.6).} \quad (1)$$

The measured redox potential value in the nutrient medium at copper concentration of 0.003 mol/liter at pH = 5.6 is +330 mV, i.e. close to the calculated value (Fig. 5). The experimentally obtained redox potential value is lower than the theoretical one. It can be explained by presence of sulfur containing amino acids (e.g., cysteine) in the nutrient medium. These amino acids have a low redox potential (-80 ÷ -100 mV) [3]. Therefore, the "summary" redox potential of a medium containing both organic compound (800 mg/l C) and copper(II) is +330 mV. Eh of control medium is 110 mV lower than the Eh of copper(II) containing medium (+220 and +330 mV, respectively). This speaks that copper(II) is high-potential redox buffer.

Two groups of mechanisms define the resistance of microorganisms to toxic metal, and in particular to copper(II).

One group includes mechanisms that lead to metals removing from the medium. They are metal binding with cells compounds, reduction of metals to insoluble compounds, metal precipitation in the form of insoluble hydroxides, and insoluble complexes with microbial exometabolites [6, 7, 10, 13-15].

The other group of mechanisms provides microbial resistance to metals, but does not lead to metals extraction from the medium. Such mechanisms are efflux of metals from cells, conformational changes in the structure of the cytoplasmic membrane, etc. [1, 7, 13, 14].

Obviously, decrease of the concentration of such high potential metals as $Cu^{2+}$ leads to falling of the redox potential of the medium. Thus, the redox potential positively correlates with the copper concentration. The redox potential of medium at 200 ppm of $Cu^{2+}$ was +330 mV at the beginning of the experiment (Fig. 4). The value of the redox potential remained positive throughout the experiment. The Eh fell insignificantly on the 70$^{th}$ hour of cultivation and was +260 mV. Thus, it was assumed that *K. carniphila* during its growth had not removed $Cu^{2+}$ from the medium by none of above mechanisms because Eh had decreased imperceptibly.

To revise this supposition, the concentration of $Cu^{2+}$ in medium was measured. It was confirmed, that $Cu^{2+}$concentration did not fall during the entire experiment, which supported the above supposition. Thus, the concentration of $Cu^{2+}$ at the beginning of the experiment was 200 ppm and remained the same during the 72 hours of cultivation.

[2] The concentration of copper in the medium is 200 ppm, i.e. 0.003 mol/l, and pH = 5.6. In accordance with equation $2Cu^{2+} + H_2O + 2e = Cu_2O + 2H^+$ reaction potential
Eh = 0.203 + 0.0591×pH + 0.0591×lg{$Cu^{2+}$} = 0.203 + 0.0591×5.6 + 0.0591×lg0.003 = 0.203 + 0.330 +
+ 0.0591×(–2.5) = 0.203 + 0.330 + (–0.15) = 0.385 B = +385 mV.

## Conclusion

1. Developed method modification is quick and reliable for Eh and pH of the culture liquid measuring.

2. Insignificantly small amount of culture broth (0.5 ml) is needed for unit measuring of pH and Eh in redox cell. So, multiple serial measurements do not influence the growth of the microbial population.

3. Express method allows determining the influence of extreme factors on the growth of microorganisms and the character of the microbial interaction extreme factors, including toxic metals.

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