Supplementary Information for the article:

Nanostructured copper electrodes - a new step in the development of microbial bioelectrochemical systems

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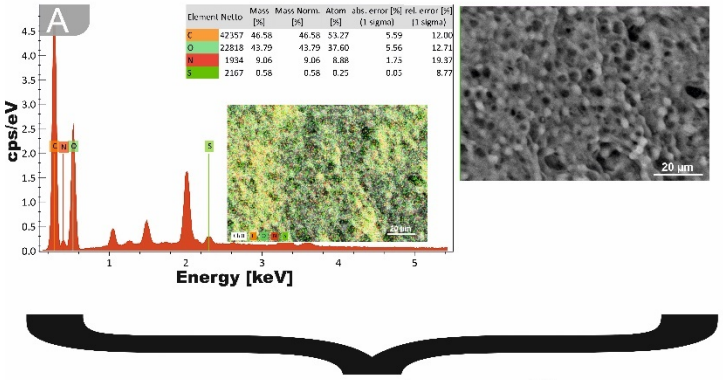
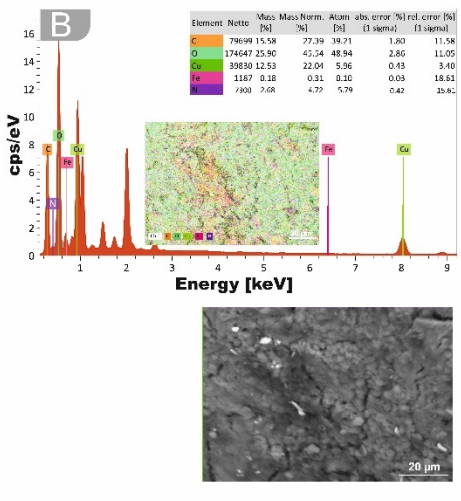
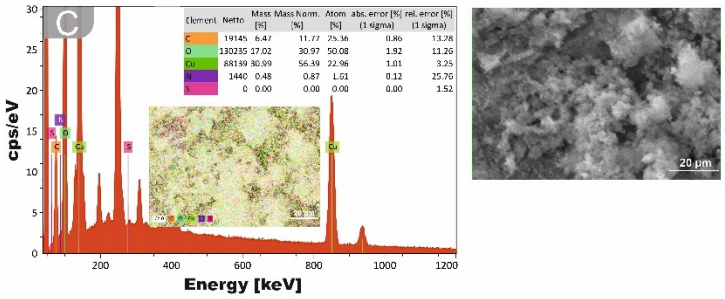
Fabrication of nanostructured copper electrodes as bioelectrode substrates



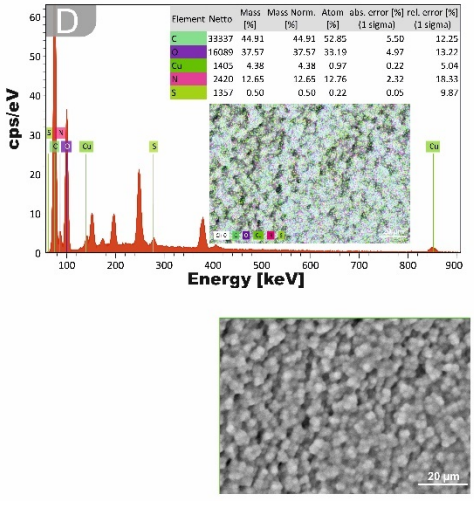
Fc+D. hansenii



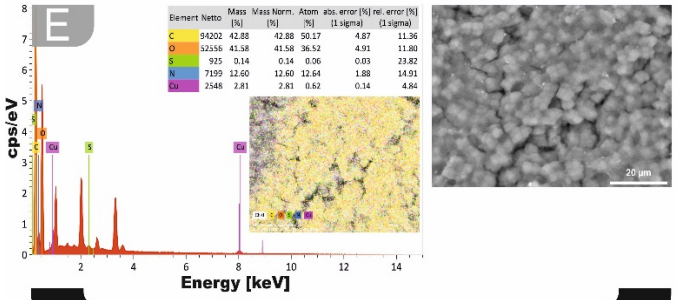
Fc



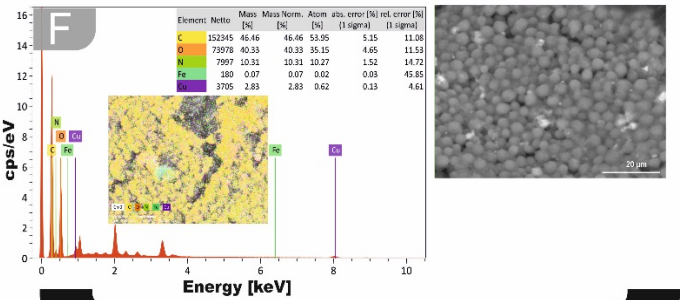
p(NR)



p(NR)
+
P. yeei



p(NR) + P.yeei (post stability test)



Fc + D.hansenii (post stability test)

Figure S1. SEM images of the electrode surface with energy dispersive X-ray spectroscopy (EDS) mapping at different formations: *A) FC/ D. hansenii; B) FC; C) p(NR); D) p(NR)/ P. yeei; E) p(NR)/ P. yeei* post stability test*; F) FC/ D. hansenii* post stability test.

Selection of redox compounds for formation of biosensor receptor system

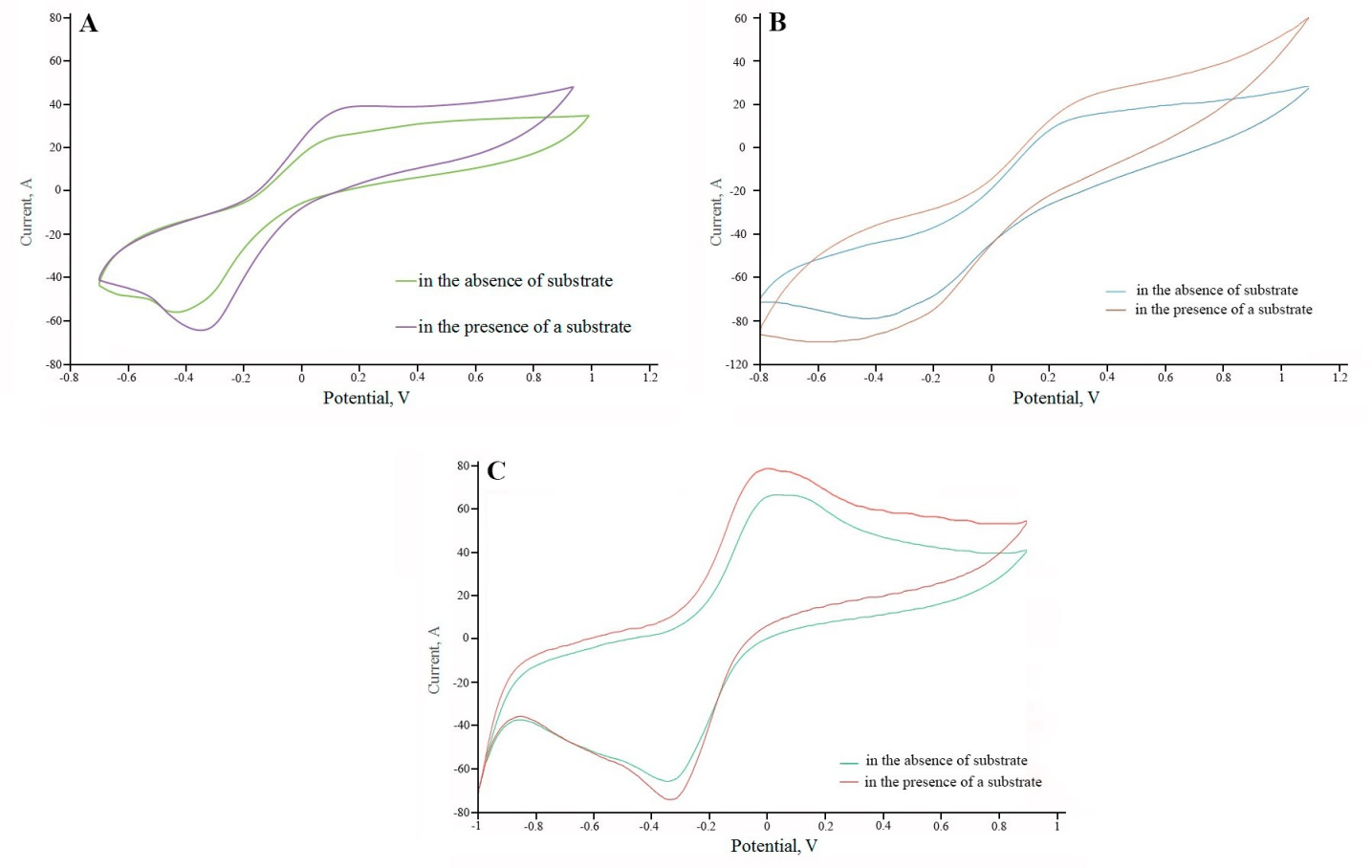


Figure S2. Cyclic voltammetry to determine the interaction constant for systems: A) FC-MB/ *D. hansenii*; B)) p(TN)/ *P. yeei*; C) p(NR)/ *P. yeei*

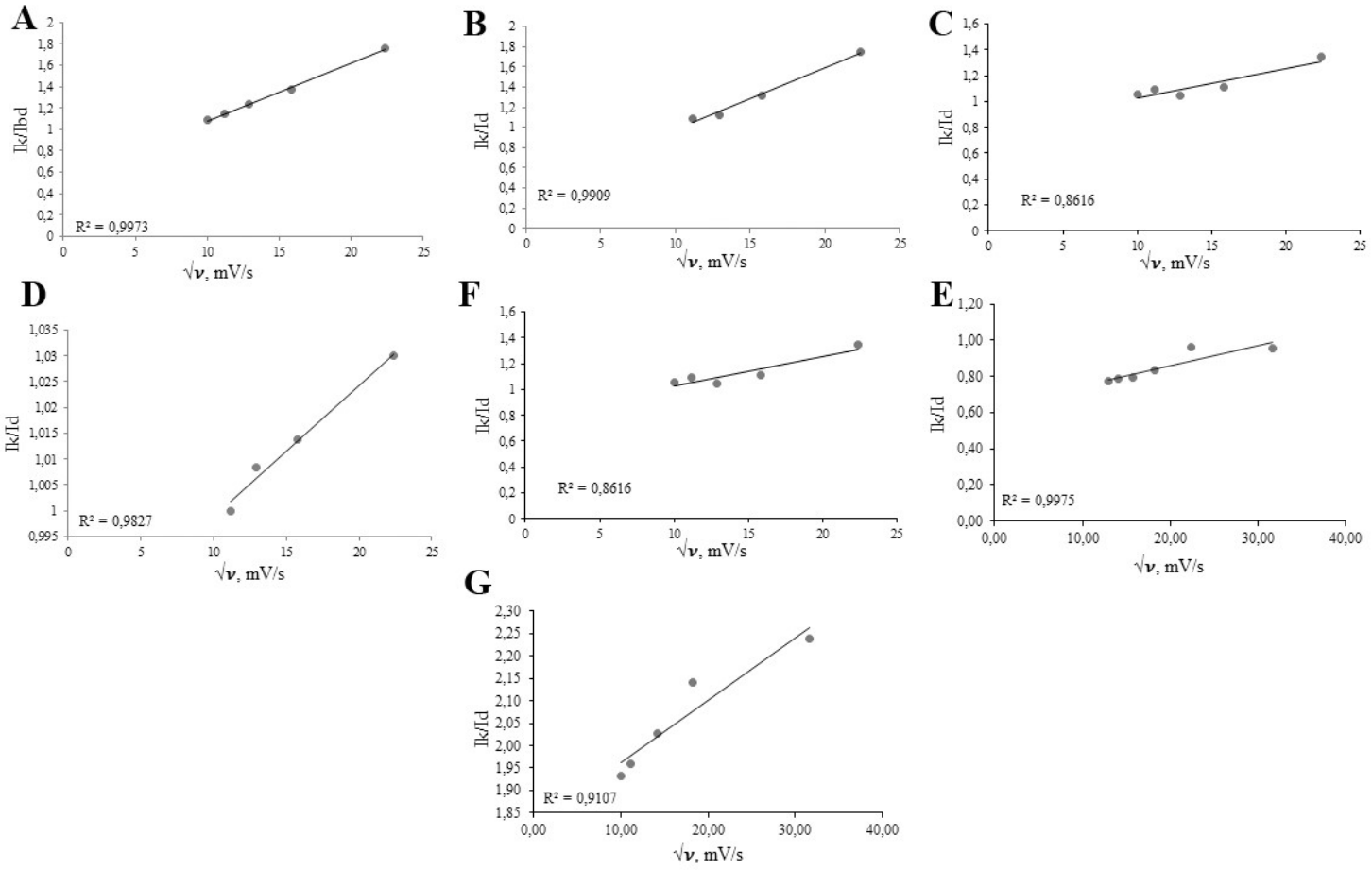


Figure S3. Dependences of the ratio of the limiting anodic current in the presence and absence of a substrate (I_k/I_d) on the value of the inverse scan rate: A) MB; B) NR; C) TN; D) DCPIP; E) FC; F) p(NR); G) p(TN)

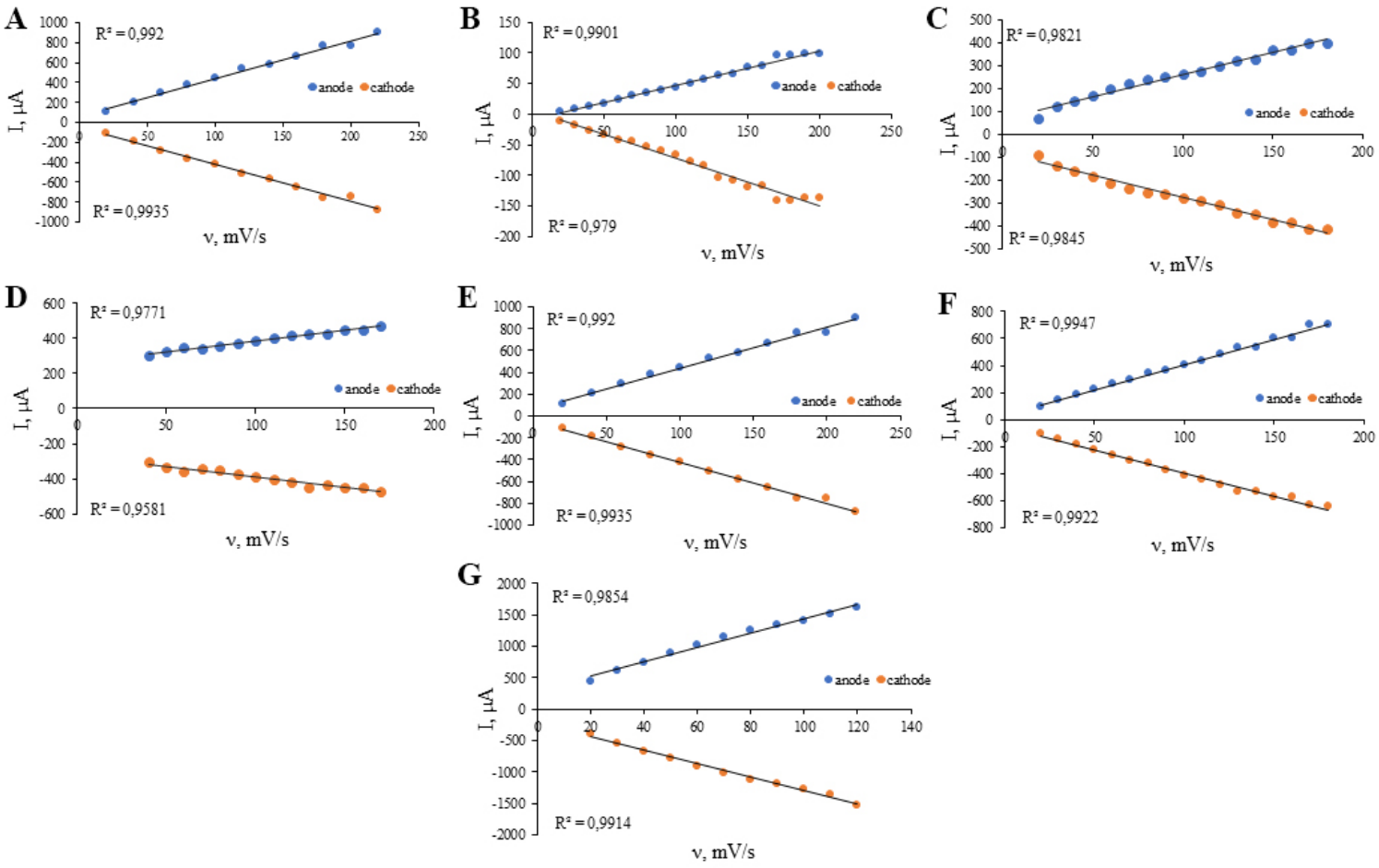


Figure S4. Dependences of I on ν for determining the limiting stage with redox compounds: A) MB; B) NR; C) TN; D) DCPIP; E) FC; F) p(NR); G) p(TN)

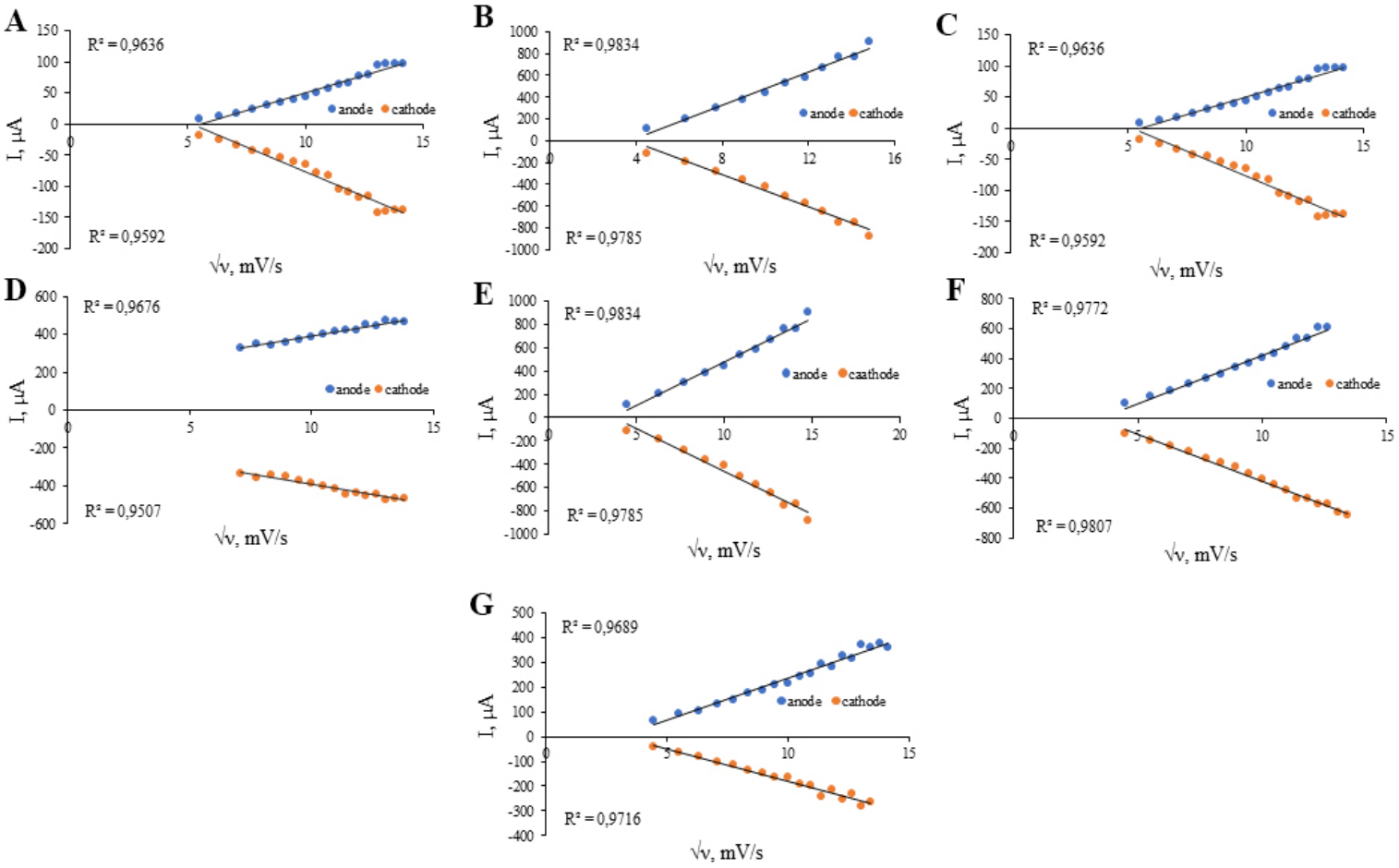


Figure S5. Dependence of I on $\sqrt{\nu}$ for determining the limiting stage with redox compounds: A) MB; B) NR; C) TN; D) DCPIP; E) FC; F) p(NR); G) p(TN)

Testing of the developed bioelectrochemical system for BOD index determination

The calibration dependence of the biosensor response on the substrate concentration is the most important metrological characteristic, since in order to quantify the analytes in a sample, it is necessary to study the dependence of the analytical signal on the concentration.

Based on the data obtained during the experiment, calibration dependences of the analytical signal on the BOD_5 index were constructed for all systems (Figure S6).

Next, the dependence of the sensor response on BOD was approximated based on the Michaelis-Menten equation.

$$V = \frac{V_{max}[S]}{K_M + [S]},$$

where V is the rate of the enzymatic reaction;

V_{max} is the maximum speed of the enzymatic reaction at which the entire enzyme participates in the formation of the enzyme-substrate complex;

K_M - apparent Michaelis constant - substrate concentration at which the reaction rate is half the maximum;

[S] - BOD_5 value.

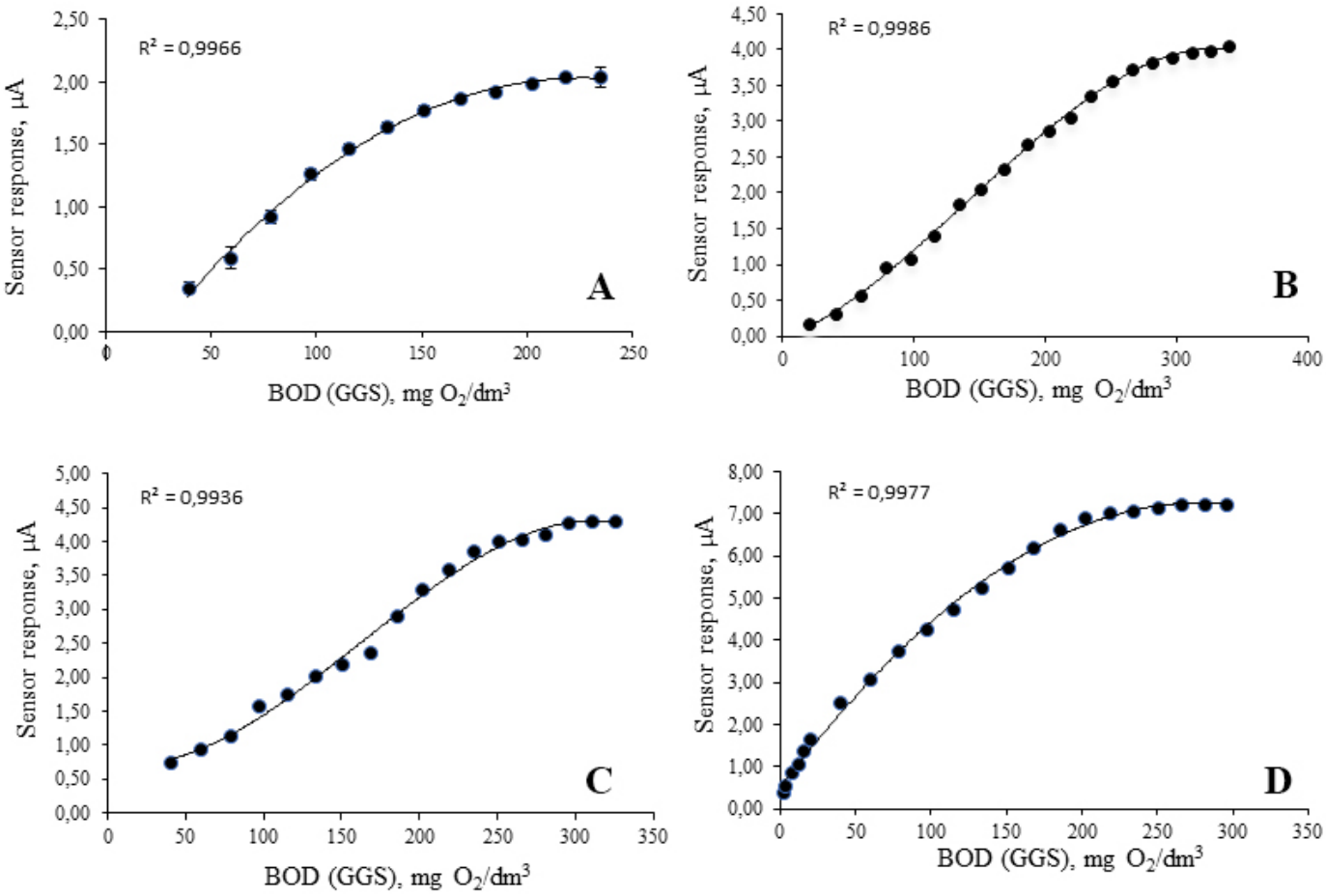


Figure S6. Calibration dependencies for the systems: A) FC/ *D. hansenii*; B) FC-MB/ *D. hansenii*; C) FC/ *P. yeei*; D) p(NR)/ *P. yeei*

Analytical and metrological characteristics of biosenors

1.1.1. Expressiveness of the biosensor

An important analytical characteristic of any method is the duration of the analysis. The time costs of the method are determined primarily by the duration of a single measurement. For a biosensor, this parameter is equal to the sum of the response development time and the recovery time of the activity of the receptor element (sensor washing). For biosensor based on FC/ *D. hansenii*; FC-MB/ *D. hansenii* and FC/ *P. yeei* sensor response time is 2-3 minutes, depending on the concentration of the substrate, the recovery time of the activity of the receptor element after measurement (washing) is 2 minutes, thus the duration of single measurement is approximately 4-5 minutes. And for a biosensor based on p(NR)/ *P. yeei*/, the

sensor response time is 3-4 minutes, the recovery time of the receptor element activity after measurement (washing) is 2-3 minutes, so the duration of single measurement is approximately 5-7 minutes.

1.1.2. ***Operational and long-term stability of biosensors***

The stability of a biosensor is the most important characteristic of its operation. Operational stability is closely related to the metrological characteristic of the method - convergence (repeatability). Figure S7 presents the operational stabilities of biosensors based on FC/ *D. hansenii*; FC-MB/ *D. hansenii*; FC/ *P. yeei* and p(NR)/ *P. yeei*.

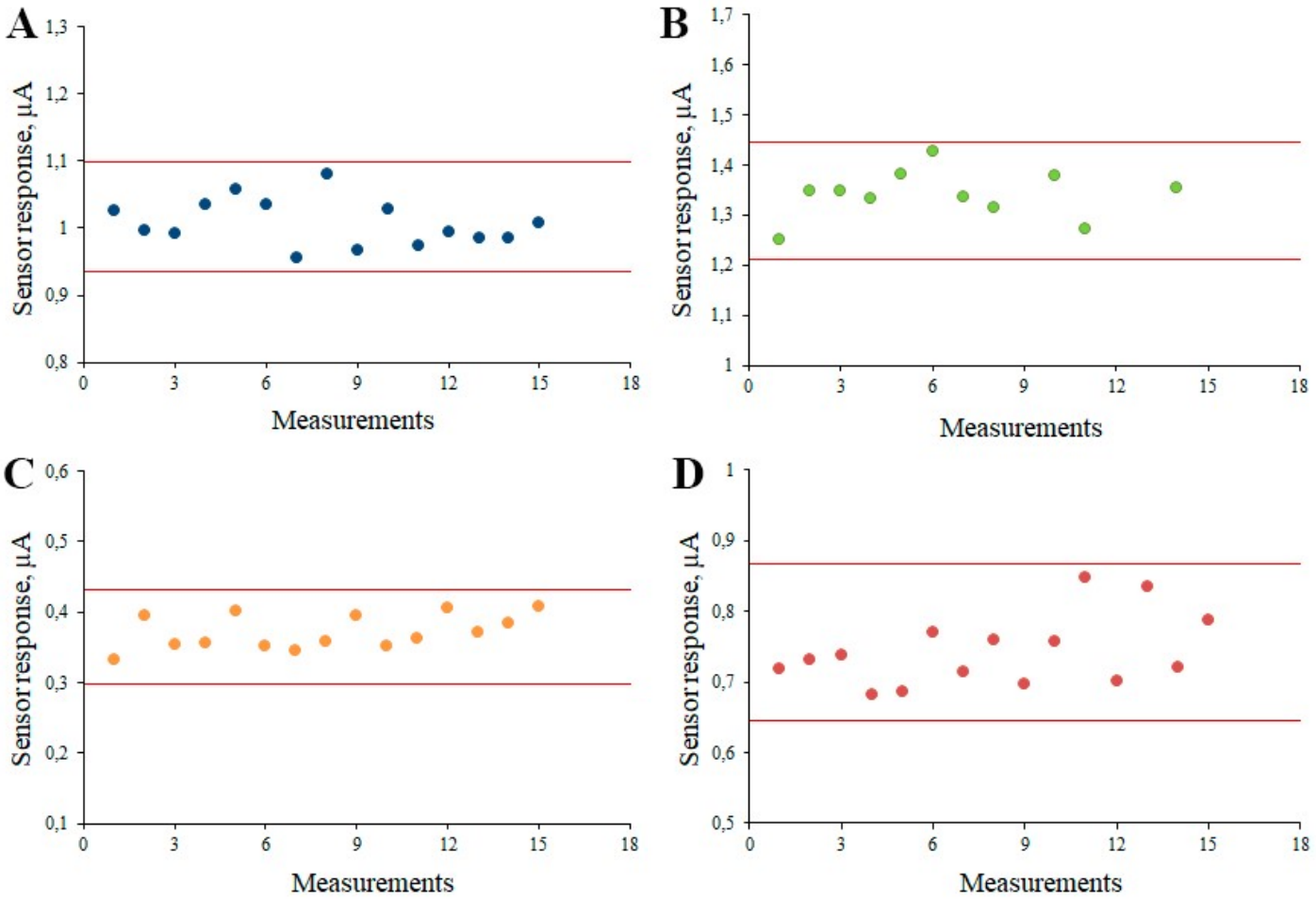


Figure S7. Operational stability of biosensors: *A) FC/ D. hansenii; B) FC-MB/ D. hansenii; C) FC/ P. yeei; D) p(NR)/ P. yeei*

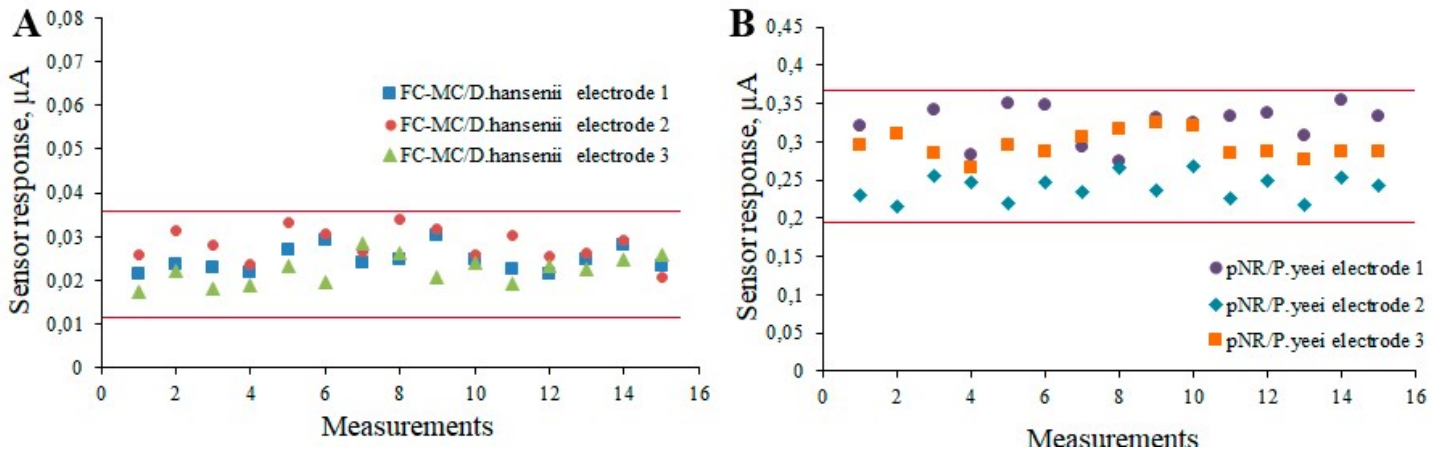


Figure S8. Stability of operation of three biosensors: *A) FC-MB/ D. hansenii; B) p(NR)/ P. yeei*

Long-term stability characterizes the stability of the sensor over a long period of time. The time during which the signal value was at least 50% of the maximum activity is taken as the time of stable operation of the receptor element. Long-term stability was determined by daily measuring the magnitude of the sensor response to the same concentration of a solution of a glucose and glutamic acid mixture. The sensor was stored in a buffer solution at a temperature of +4°C between measurements. The obtained dependencies are shown in Figure S8.

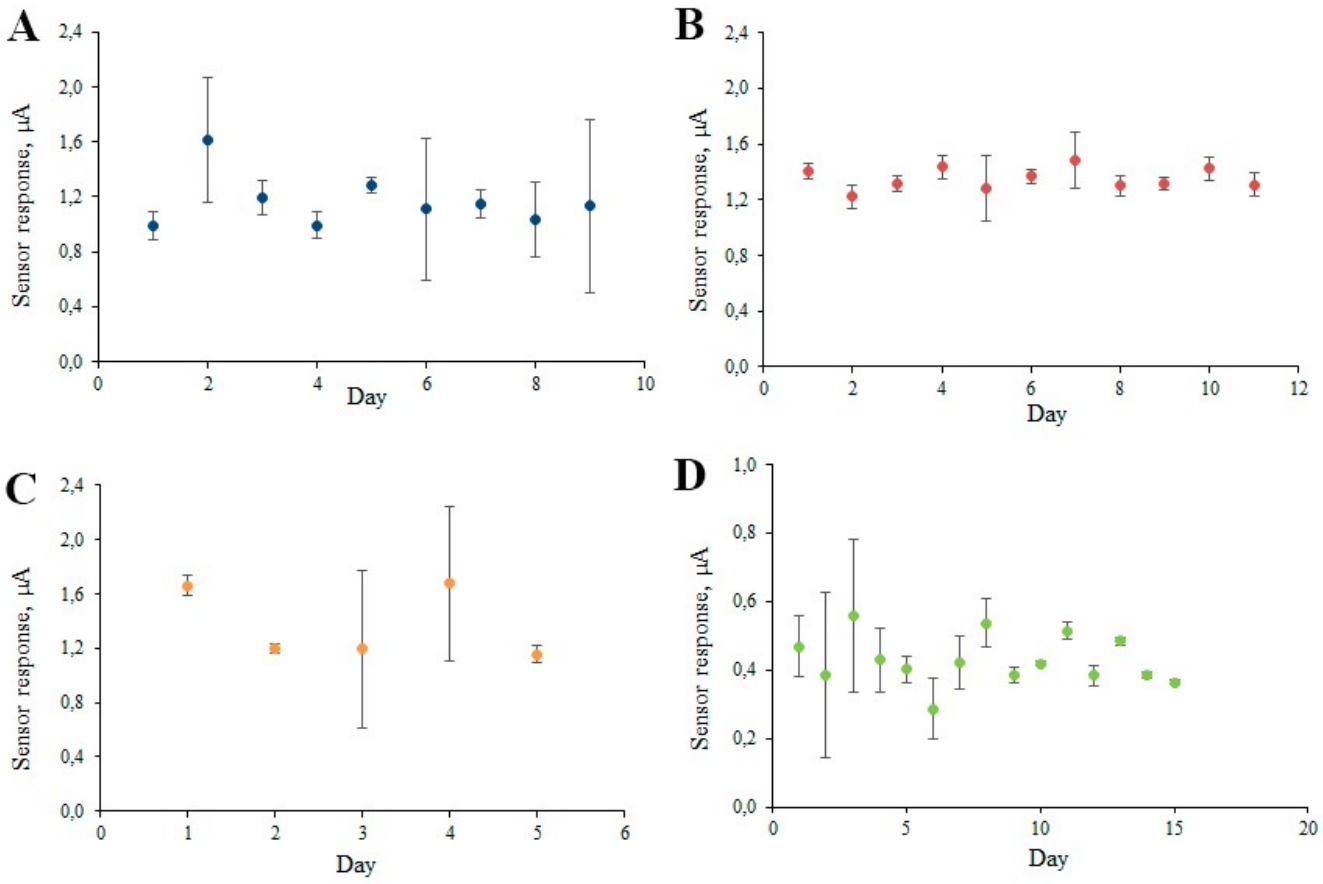


Figure S9. Long-term stability of biosensors: *A) FC/ D. hansenii; B) FC-MB/ D. hansenii; C) FC/ P. yeei; D) p(NR)/ P. yeei.*

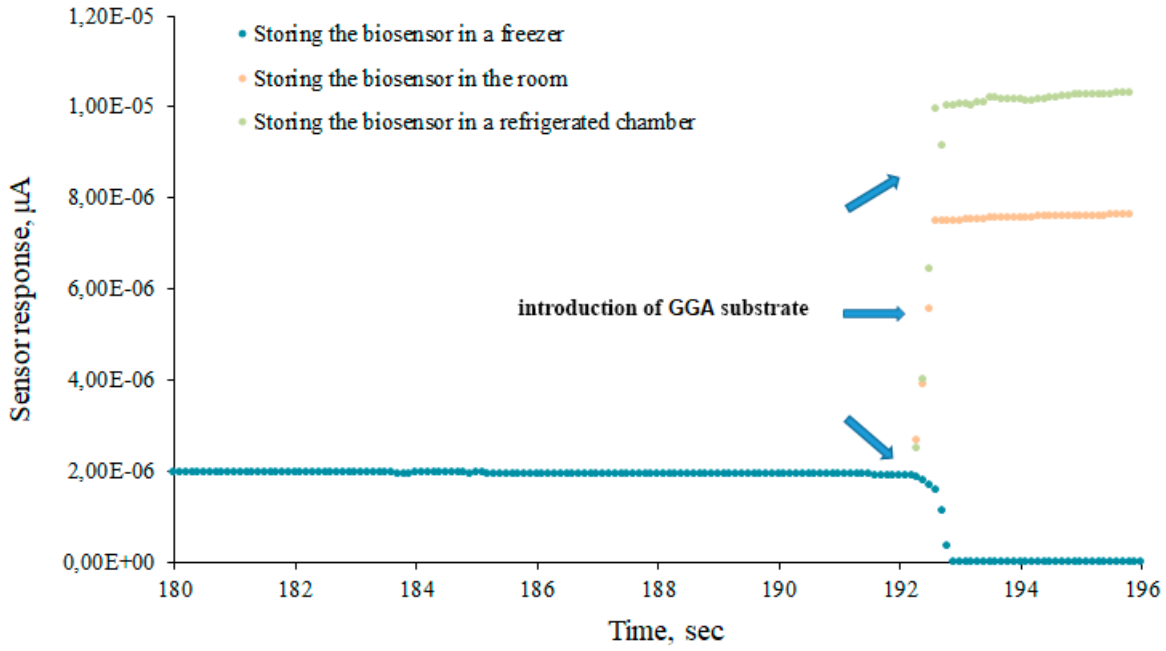


Figure S10. Sensor response under different storage conditions.

Influence of the composition of the studied samples on the oxidative capacity of D. hansenii yeast and baceria P. yeei.

1.1.1. Dependence of oxidative activity of D. hansenii yeast and bacteria P. yeei on solution salinity.

Since wastewater samples may contain substances of inorganic nature that inhibit metabolic processes in cells, it is important to assess the impact of negative environmental factors (salinity and heavy metal ions) on the oxidative activity of microorganisms recovered after lyophilization. The effect of sodium chloride concentration on the oxidative activity of *D. hansenii* yeast and *P. yeei* bacteria was studied (Fig. S11 A-B). The effect of salinity was assessed in the range of sodium chloride concentrations from 0 to 15%.

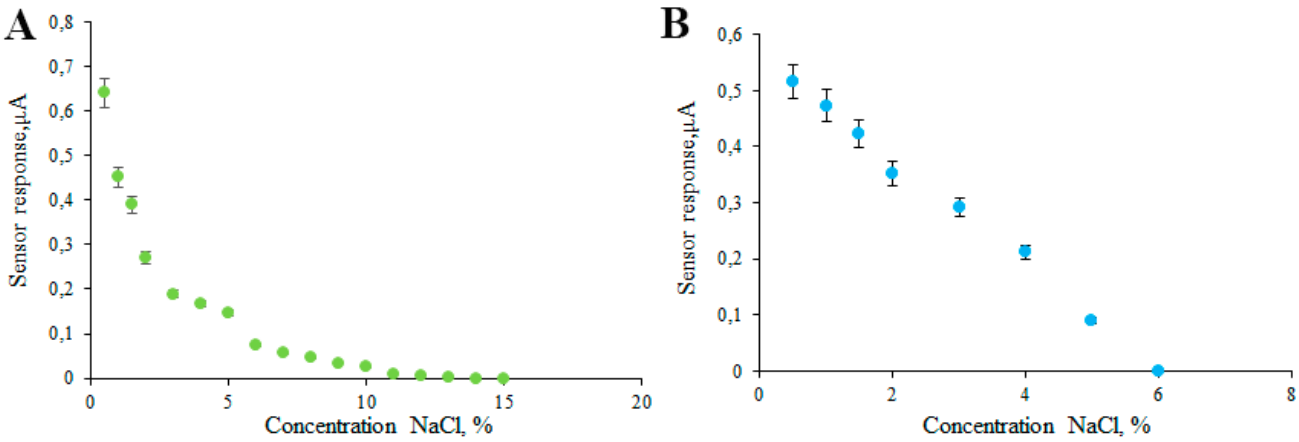


Figure S11. Effect of sodium chloride concentration on oxidative activity: A) FC-MB/ *D. hansenii*; B) p(NR)/ *P. yeei.*

D. hansenii yeast is capable of functioning as part of the biorecognition element of the sensor at a salinity of up to 13%, while the oxidative activity of *P. yeei* bacteria was observed only at a salinity of up to 5%. At a sodium chloride concentration of up to 5%, in both cases the biosensor responses decrease by approximately 22%, which allows these biosensors to be used for BOD assessment in seawater samples.

1.1.2. Influence of pH of the environment

The pH of the medium is one of the factors influencing the activity of cellular enzymes and the sensitivity of the bioreceptor to various substrates. The change in the oxidative activity of yeast *D. hansenii* (Fig. S12 A) and bacteria *P. yeei* (Fig. S12 B) was studied when varying pH from 4.0 to 9.2 (the lower and upper limits of possible pH of the KH_2PO_4/Na_2HPO_4 buffer system). GGA mixture, which is used as a standard in the Russian Federation and in international practice for determining BOD5, was chosen as a substrate.

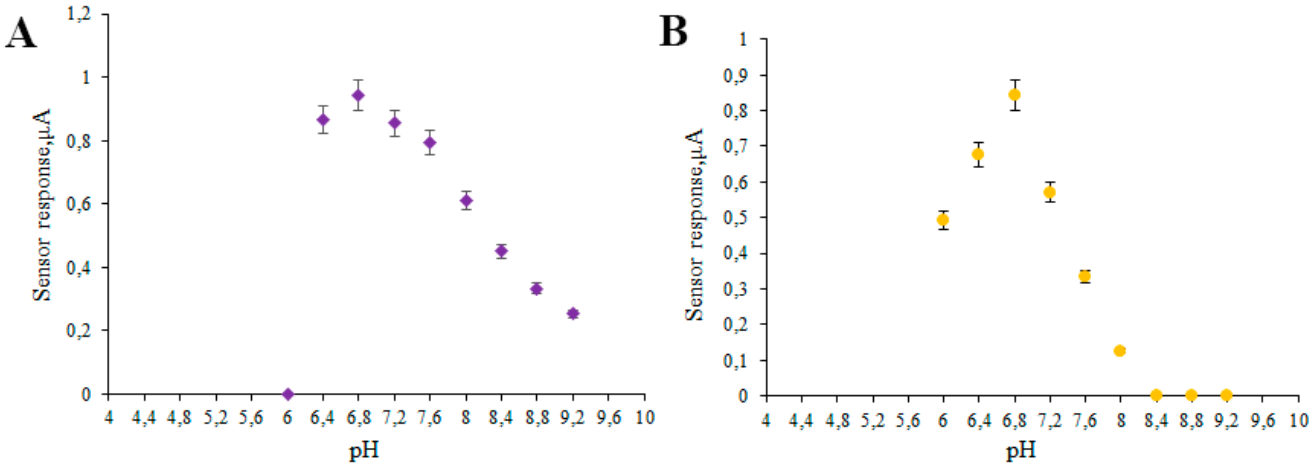


Figure S12. Dependence of the biosensor response on the pH of the environment: A) FC-MB/ *D. hansenii*; B) p(NR)/ *P. yeei.*

The obtained plots show that the maximum response of the biosensor based on *D. hansenii* yeast and *P. yeei* bacteria is observed in the pH range of 6.4-7.2.