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ORIGINAL ARTICLE

Acceptor properties of "carbon nanotubes–redox-active polymer based on bovine serum albumin modified with ferrocenecarboxaldehyde" composite for creating a BOD biosensor with *Blastobotrys adeninivorans* BKM Y-2677 yeast

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Abstract

The possibility of using a composite material based on bovine serum albumin (BSA) covalently bonded with ferrocenecarboxaldehyde and containing carbon nanotubes (CNT) for the immobilization of *Blastobotrys adeninivorans* BKM Y-2677 (*B. adeninivorans*) yeast is discussed. The optimal ratio of ferrocenecarboxaldehyde to BSA for the redox-active polymer synthesis is 1:2, since the heterogeneous electron transfer constant is 0.45 ± 0.01 s^{-1}. When carbon nanotubes (CNTs) are added to this polymer, the heterogeneous electron transfer constant increases: at a CNT specific density of 2.5 μg/mm^2, it reaches a maximum value of 0.55 ± 0.01 s^{-1}. The addition of CNTs into the conducting system leads to increasing of the rate constant of interaction redox species with *B. adeninivorans* yeast by an order: the rate constant of interaction between *B. adeninivorans* yeast and electroactive particles in a redox-active polymer is 0.056 ± 0.005 dm^3/g × s and in a composite material based on CNTs is 0.51 ± 0.02 dm^3/g × s. The yeast specific density at the electrode of 0.1 mg/mm^2 and electrolyte pH of 6.2 was chosen as the working value for the receptor system operation. Immobilized in a composite material, yeast oxidizes a wider range of substrates compared with a similar receptor element based on the ferrocene mediator. The biosensors formed on the basis of hybrid polymers have a high sensitivity with a lower limit of determined concentrations of 1.5 mg/dm^3 with an assay time of 5 min and a high correlation ($R = 0.9945$) with the results of the standard method for determining biochemical oxygen demand (BOD) in nine real surface water samples of the Tula region.

Keywords Redox-active polymer · Bovine serum albumin (BSA) · Biochemical oxygen demand (BOD) · *Blastobotrys adeninivorans*

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Introduction

Rapid pollution assessment, in particular, the organic compounds determination in surface, ground, and waste waters, is a priority task in environmental monitoring, since standard methods are often time consuming. The index of biochemical oxygen demand (BOD) is used for assessment of wastewater pollution with organic substances; by definition, BOD is the amount of oxygen consumed by microorganisms in the process of organic substances biodegradation in 1 dm^3 of a sample. According to sample incubation time, there is BOD_5, taking 5 days of incubation and BOD_{20} taking 20 days (ISO 5815-1:2003 2003; ISO 5815-2:2003 2003). The duration of the standard method leads to a high demand in the biosensor development for BOD assessment (Arlyapov

et al. 2012, 2013; Catterall et al. 2001; Kurbanalieva et al. 2022; Liu et al. 2010; Morris et al. 2001; Pasco et al. 2000, 2004; Yoshida et al. 2000, 2001; Zaitseva et al. 2017).

There are two main approaches for BOD biosensor development. The first one is based on a Clarke electrode, where the rate of oxygen consumption by microorganisms is analytical signal, which is equivalent to the BOD amount (Arlyapov et al. 2012, 2013). This approach has a number of disadvantages, such as oxygen diffusion between the test sample and the atmosphere has an effect on measurement results. A more promising approach is low molecular weight compounds or mediators using electron transport between the microorganisms and the electrode; in this case, analytical signal does not depend on the dissolved oxygen concentration, thereby BOD assay can be carried out under anaerobic conditions (Liu et al. 2010). In addition, the use of a mediator allows to change the applied potential, reducing noise interference appeared due to redox reactions by sample compounds on the electrode (Zaitseva et al. 2017).

Choosing microorganism, which oxidizes a different organic compound, is an important step in mediator BOD biosensor development. Bacteria are often used as a receptor element in mediator BOD biosensors (Catterall et al. 2001; Kurbanalieva et al. 2022; Morris et al. 2001; Pasco et al. 2000, 2004; Yoshida et al. 2000, 2001). Their membrane localization of enzymatic systems provides high rate of interaction with mediators (Baronian et al. 2002). Yeast interaction with mediators is limited by thick (100–200 nm) cell wall containing polysaccharides and proteins (Christwardana and Kwon 2017; Gal et al. 2016). For electrochemical signal, the mediator should pass through the cell wall and interact with intracellular components, such as NAD^+/NADH (Rawson et al. 2012; Kasem et al. 2013); thus, BOD biosensor based on one-mediator systems and yeast is less efficient. On the other hand, yeasts are a promising biomaterial for BOD biosensor development, because they metabolize a broad range of substrates and tolerant to negative factors of the environment (high salt content, temperature, pH) (Arlyapov et al. 2012).

Two-mediator systems can be used for more effective electron transport from yeast cells to the electrode. The most common combination is the *Saccharomyces cerevisiae* yeast and the two-mediator/bi-mediator system potassium hexacyanoferrate (III) (ferricyanide)–vitamin K3 (menadione, 2-methyl-1,4-naphthoquinone) (Zhao et al. 2021). Menadione is able to interact with NADH, which is an element of the respiratory electron transport chain (Rabinowitz et al. 1998), while ferricyanide has good electrochemical properties and does not penetrate into the cell wall due to its hydrophilicity (Trosok et al. 2001). The two-mediator biosensor based on *S. cerevisiae* is possible to estimate BOD in the range of 20–225 mg O_2/dm^3 (Zhao et al. 2021); however, the biosensor has a low sensitivity and does not allow determining BOD in pure surface waters, in which BOD is about 2 mg O_2/dm^3. To provide effective electron transport in a two-mediator system, forming a high sensitive biosensor, both mediators should not compete with each other, it is necessary to provide a difference in the rates of interaction with both the biomaterial and the electrode; the ideal is the implementation of such a scheme, in which one mediator interacted with the electrode at a high rate, and had a low rate of interaction with the biomaterial. In this case, the second mediator should have a high rate of interaction with the biomaterial and a low rate with the electrode (Gao et al. 2017; Ino et al. 2019; Mazloum-Ardakani et al. 2019; Nakamura et al. 2007a,b; Pasco et al. 2005; Zaitseva et al. 2017). This approach was used in our previous study, where two-mediator systems "ferrocene–neutral red" was chosen for *B. adeninivorans* yeast in accordance with kinetics parameters of bioelectrochemical reactions, which results in high sensitive biosensor—the lower limit of the determined concentrations of BOD was 0.16 mg/dm^3, and analysis of ten surface water samples showed that combination of ferrocene and neutral red with *B. adeninivorans* output data had a high correlation ($R = 0.9693$) with the standard method results (Kharkova et al. 2021).

The disadvantage of two-mediator systems is that the analytical system is not reagentless. The mediators must be standardized, and the adding reagents increases the risk of side reactions. Another approach for increasing biosensor sensitivity is applying composite materials, including redox-active polymers and nanomaterials. Redox-active polymers are based on non-conductive, biocompatible polymers; for example, polyvinyl alcohol, bovine serum albumin (BSA), chitosan, alginate, and others (Zamani et al. 2019), which are covalent bonded with a mediator and used for microorganisms immobilization. Redox-active polymers are quite effective, provide sensor stability, reagentless analysis; they can be used to create microbe-based biosensors (Fang et al. 2020). Despite the advantages of redox-active polymers, the limitations of their application for the yeast immobilization occurred due to limiting penetration of a covalently bound mediator into the cell through its membrane to couple biochemical reactions with an electrode; for which reason single-walled and multi-walled carbon nanotubes, nanodots, graphene are added to redox polymers (Lin et al. 2019). When nanoparticles are added to receptor element, the conductivity of the receptor system increases significantly. In addition, a low yield after polymer modification by redox-active compounds is not critical, when there are not enough electroactive particles for the rapid electron transfer to the electrode. Addition of nanomaterials into the system removes the kinetic limiting due to, first, increasing the effective surface area of the electrode, and second, providing conductivity between the covalently bonded redox particles in the polymer, where the electron transfer was not

carried out without nanoparticles. Thus, composite materials based on a redox-active polymer and nanomaterials make it possible to "connect" more redox-active polymer sites, and, consequently, increase the analytical signal and to provide high biosensor sensitivity; therefore, the use of nanomaterials in the microbial biosensor development is current line of research. Our previous study showed the advantage of using composite materials based on the redox-active polymer of bovine serum albumin (BSA) modified with ferrocene for *Paracoccus yeei* VKM B-3302 immobilization and sensitive BOD biosensor development—the lower limit of the determined concentrations of BOD was 0.16 mg/dm^3 and a high correlation ($R = 0.9916$) with the results of the standard method for determining biochemical oxygen demand in surface water samples was observerd (Arlyapov et al. 2021).

In this work, the possibility of using a composite material based on the redox-active BSA polymer modified with ferrocene for the *Blastobotrys adeninivorans* BKM Y-2677 yeast immobilization is discussed. The kinetic limiting connected with the single mediator system of redox polymer is proposed to minimize by nanomaterials, without applying second mediator. The novelty of this research is providing effective charge transfer from the yeast to the electrode by "carbon nanotubes–redox-active polymer based on bovine serum albumin modified with ferrocenecarboxaldehyde" composite and carrying out BOD analysis. Modern research has shown that redox-polymer composite and yeast are promising for biofuel cell design (Andriukonis et al. 2022; Moradian et al. 2022; Verma and Mishra 2021; Zinovicius et al. 2022), but according to our best knowledge, yeast and «redox-polymer–nanomaterial» composite combination is not used for BOD detection.

Experimental

Reagents and materials

Ferrocenecarboxaldehyde (FC) (Sigma-Aldrich, Germany) was used as electron transfer mediators. Bovine serum albumin (Sigma-Aldrich, Germany) was used as the basis of redox polymer. A working carbon-paste electrode was formed using graphite of particle size 75 microns and high purity 99.997% (Fluka, Germany), paraffin oil (Fluka, Germany), and dialysis membrane with an MWCO of 14 kDa (Roth, Germany). Biosensor measurements were carried out in sodium–potassium buffer solution, pH 6.8 (33 mM KH_2PO_4 + 33 mM Na_2HPO_4, Diaem, Russia).

Cultivation of microbial cells

Blastobotrys adeninivorans VKM Y-2677 (*B. adeninivorans*) was provided by the All-Russian Collection of Microorganisms, G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, FRC PCBR RAS (Pushchino).

Microorganisms were grown using ES-20/60 orbital shaker-incubator (BioSan, Latvia); TG16WS (Polikom Ltd, Russia) and MiniSpin plus (Eppendorf, Russia) centrifuges. Biomass was stored in microtubes at a temperature of – 25 °C. The yeast *B. adeninivorans* was cultivated in a medium of the following composition: glucose, 1%; peptone, 0.5%; yeast extract, 0.05%. Cultivation time was 18–20 h at a temperature of 28 °C. Centrifugation was carried out at 7000*g* for 10 min. A phosphate buffer solution, pH 6.8, was used to prepare a cell suspension.

Synthesis of a redox-active polymer based on BSA and mediator ferrocenecarboxaldehyde and formation of a working electrode (BSA–FC)

To form a redox polymer, 0.5 g of BSA was dissolved in a 5-ml buffer solution. Then 0.05 g of ferrocenecarboxaldehyde was dissolved in 5 ml of acetone; this mix was poured into the BSA solution. The produced solution was adjusted to pH 9.3 by adding 5% K_2CO_3. Synthesis was carried out for 1 h at a temperature of 30 ^{0}C. After that, 0.01 g of $NaBH_4$ was added to the mix. The product was stirred using a magnetic stirrer for 10 min and kept at room temperature for 6 h. The pH value of the mix was adjusted to pH 6.5 for the remaining $NaBH_4$ to decompose. Then the pH of the mix was adjusted to pH 8.5 by adding a 0.1-M NaOH solution by 20-µl portions. Further on, the mix was centrifuged at 3000 rpm for 20 min. The supernatant produced was dialyzed by a phosphate buffer solution for 2 days at a temperature of 4 ^{0}C to separate the non-reacting ferrocenecarboxaldehyde. The produced redox-active polymer was dried to a constant weight at a temperature of 60 °C.

To form a working electrode, the redox-active polymer of 0.0035 g was dissolved in 50 µl of phosphate buffer, pH 6.8; then 7.5 µl of glutaraldehyde was added. The product in the amount of 10 µl was applied onto a carbon-paste electrode and left for a complete dry-out.

Modification of redox-active polymers by carbon nanotubes and formation of a working electrode (BSA–FC–CNT)

For modification of redox-active polymer, 100 µl of deionized water was added to 0.5 mg of a suspension (0.5 wt %) of single-walled carbon nanotubes (Carbon Chg, Chernogolovka, Moscow Region, Russia) and left in an ultrasonic bath for 15 min. The produced suspension in the amount of 10 µl was added to redox-active polymer at the preparation of working electrodes.

Immobilization of microorganisms into conducting matrices and formation of biosensor working electrode

To form a biosensor working electrode, 20 µl of a suspension containing *B. adeninivorans* yeast (200 mg wet weight/ml) was applied onto a redox-active polymer-modified electrode and left for complete dry-out. Further on, one more layer of conducting redox-active polymer was applied. After drying, the electrode was coated with a dialysis membrane.

Biosensor measurements

Electrochemical measurements were conducted using an IPC-Micro galvanopotentiostat (OOO NTF Volta, St. Petersburg, Russia). The applied potential was + 250 mV. Measurements were conducted in two electrode system with biosensor working electrode and a saturated silver/silver chloride (Ag/AgCl) reference electrode at a temperature of 20 °C; the volume of the measuring cell was 5 cm^3. The measurements proceeded at the constant stirring of the solution by a magnetic stirrer (300 rpm). After a stable level of current was settled on, a required amount of an investigated compound was introduced into the cell. The cell was washed with a potassium–sodium–phosphate buffer, pH 6.8, after each measurement. The measured parameter of the sensor response was the current amplitude determined as a difference between the final and initial values of current before and after adding analytes into the measuring cuvette.

Current–voltage characteristics

Voltammograms were registered by a three-electrode system using an Ekotest-VA voltammetric analyzer (OOO Ekoniks-Ekspert, Russia) with the working electrode; a platinum electrode was auxiliary and a saturated Ag/AgCl electrode was used as a reference electrode. Cyclic voltammograms were recorded at a scan rate of 20–100 mV/s in a potassium–sodium–phosphate buffer, pH 6.8.

Electron microscopy

Thin layers of a platinum–carbon mix were sputtered onto samples of redox polymer and composite material in a JEE-4X vacuum evaporator (JEOL, Japan). Electron microscopy of the specimens was carried out using a JSM-6510 LV scanning electron microscope (JEOL, Japan) in a high vacuum mode at the registration of secondary electron images.

Determination of BOD by the dilution method

The dilution technique was used as a reference method for determining BOD_5. The analysis was carried out in accordance with the method (ISO 5815-1:2003 2003; ISO 5815-2:2003 2003). The dissolved oxygen content was determined using an Ekspert-001–4.0.1 BOD thermo-oximeter (OOO Ekoniks-Ekspert, Russia).

Results and discussion

Optimization of the ferrocenecarboxaldehyde and BSA ratio in a redox-active polymer

Based on previous studies, BSA modified with FC was chosen as a redox-active polymer, since this system has a high electron transport rate to the electrode: the heterogeneous electron transport rate constant is 0.45 ± 0.01 s^{-1} cm (Arlyapov et al. 2021). To increase the sensitivity of receptor elements, the effect of the mediator amount in the redox polymer on the electron transport rate was studied: for this, redox polymers were synthesized with different ratios of FC to BSA, 1:8; 2:7; 1:2. A further increasing FC content in the polymer (4:5 and 5:4) is impractical, since there are few reactive primary amino groups in BSA (amino groups of lysine react, their content in the protein is 9.8%). The more FC is taken for synthesis, the less free primary amino groups in redox polymer. Due to the lack of a sufficient unmodified primary amino groups amount, the redox polymer does not crosslink with glutaraldehyde, i.e., matrix with a network structure is not formed. For this reason, such redox-active polymer is impossible to use for microorganisms immobilization.

The electrochemical properties of the resulting redox polymers were studied by cyclic voltammetry. In redox-active polymers, two limiting stages of electron transfer can be realized: the "hopping mechanism" diagnosed by cyclic voltammetry when the limiting current is directly proportional to the square root of the scan rate, and the "surface reaction"—the current is proportional to the scan rate (Nicholson 1965). In all the systems under study, the limiting stage is the surface reaction; therefore, to find heterogeneous electron transfer rate constants, the Laviron model (Laviron 1979) was used, which, if the potential difference between the anode and cathode peaks is more than 200 mV, makes it possible to experimentally find the electron transfer coefficient α and the heterogeneous electron transfer constant k_s.

For transfer coefficient determination, plots of the linear dependence of the anodic/cathode peak potential on the logarithm of the scan rate (log*v*) were obtained, the slope is $-2.3RT/\alpha nF$ for the cathode peak dependence and $2.3RT/$

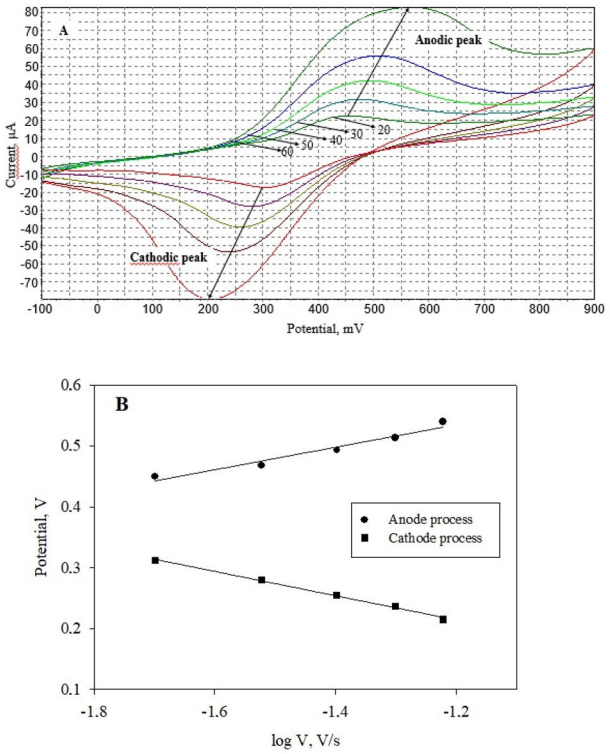

Fig. 1 Determination of the heterogeneous electron transfer constant of BSA–FC redox polymer by cyclic voltammetry. **A** Current–voltage curve at carbon-paste electrode containing modified BSA with FC; FC–BSA molar ratio is 2:7. **B** Calculated plot for the electron transfer coefficients estimation according to the Laviron model

$(1-\alpha)nF$ for the anodic peak dependence. The voltammogram and transfer coefficients plots are presented in Fig. 1.

The calculated values of the transfer coefficients are given in Table 1. Oxidation processes proceed at a much higher rate than reduction processes for all redox-polymer ratio, since the values of the coefficients for anodic processes significantly exceed those cathodic processes. Therefore, all systems are not reversible.

The heterogeneous electron transfer constants were calculated by Eq. (1). Its values are given in Table 1

$$\log\left(k_s\right) = \alpha\log(1-\alpha) + (1-\alpha)\log\alpha - \log\left(\frac{RT}{nFv}\right) - \frac{\alpha(1-\alpha)nF\Delta E}{2,3RT} \quad (1)$$

where k_s is the heterogeneous constant of the rate of the electrochemical system (s^{-1} cm); n, the number of electrons involved; F, the Faraday constant (C/mol); ν, the potential scan rate (V/s); R, the universal gas constant (J mol/K); T, temperature (K); α, the cathodic transfer coefficient; $(1-\alpha)$, the anodic transfer coefficient; ΔE, the potential difference between the anodic and cathodic peaks (V).

The highest electron transfer rate constants were obtained with FC to BSA ratio of 1:2. Therefore, this ratio is the most optimal, and it was chosen for further study of the nanomaterials effect on the electrochemical properties of the composite material. Obtained heterogeneous constants are lower than for polymers applied for energy storage (Patil et al. 2021; Claire et al. 2018), but can be used for biosensor development.

Choosing the CNTs' amount

After electrode modification with a redox-active polymer by CNTs, the effective surface area of the electrode increases. In addition, nanotubes connect covalently bonded mediators in the redox polymer (Fig. 2), and increase the electron transport rate.

The structures of composite materials were studied by scanning electron microscopy. Their images are shown in Fig. 3. The pore sizes make it possible to immobilize microorganisms.

To determine the optimal ratio of CNTs, cyclic current–voltage dependences were recorded for BSA–FC redox polymer using different amounts of CNTs: specific density

Table 1 Selection of the FC–BSA ratio for the formation of a redox-active polymer, taking into account the electrochemistry of electron transport processes

The FC–BSA ratio	Transfer coefficient (α)		Heterogeneous constant k_s, s^{-1}
	Anode process	Cathode process	
1:8	0.9835 ± 0.0001	0.0163 ± 0.0002	0.42 ± 0.02
2:7	0.9796 ± 0.0003	0.0147 ± 0.0004	0.43 ± 0.03
1:2	0.9827 ± 0.0003	0.0114 ± 0.0003	0.45 ± 0.01
$[Mo_2(isonicotinate)_4]^{0/-1}$ (Claire et al. 2018)	0,5	0,5	1.49 (Claire et al. 2018)
Poly(catechol) copolymer (Patil et al. 2021)	–	–	47.5 (Patil et al. 2021)
Poly(catechol) homopolymer (Patil et al. 2021)	–	–	8,6

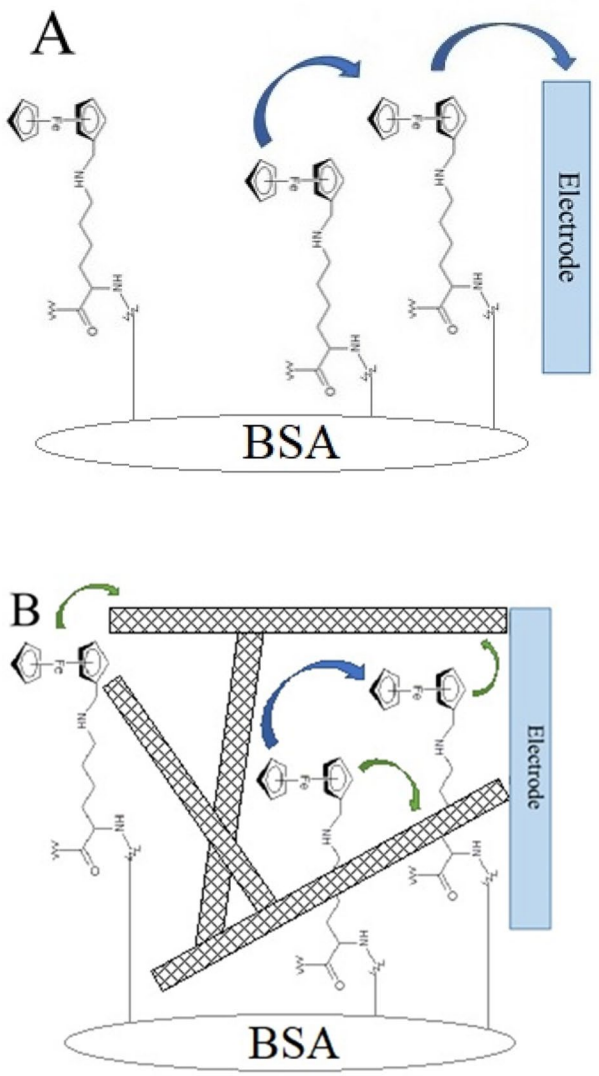

Fig. 2 The mechanism of electron transfer to the electrode: **A** in a redox-active polymer; **B** in the "redox-active polymer–CNT" composite

of CNTs at the electrode: 0.2, 1.2, 2.5, 3.7, and 5.0 μg/mm^2. The current–voltage plots for composite with different amounts of CNT are shown in Fig. 4.

Adding different CNTs amounts, the nature of the limiting stage does not change, which made it possible to apply the Laviron model to calculate the transfer coefficients and the heterogeneous rate constant according to Eq. (1). The obtained values are presented in Table. 2.

After adding CNTs, the heterogeneous electron transfer constant increases, since nanotubes connect covalently bonded mediator molecules in the polymer (Liang et al. 2011). The constant increases up to 0.55 ± 0.01 s^{-1} cm, and then it starts to decrease. This is explained by the fact that the amount of the mediator is limited, and after all, possible connections between mediator have already been created, CNTs disrupt the matrix structure. The rate constants for other composite are given in Table 2, modification with CNTs has insignificant kinetic limitations comparing other studies (Wang et al. 2019; Niu et al. 2018). The highest value of the heterogeneous electron transfer rate constant is observed at the specific density of CNTs of 2.5 μg/mm^2. Therefore, this amount is the most optimal for the microorganisms immobilization and microbial biosensors creation.

Yeast immobilization into the developed composite material

The *B. adeninivorans* BKM Y-2677 strain is quite promising for BOD assessment, since it has a wide substrate specificity and high salt tolerance (Chan et al. 1999; Kharkova et al. 2021). To reveal the general regularities of electron transport by redox-active particles to the working surface of the indicator electrode, the efficiency of bioelectrocatalytic oxidation of glucose by *B. adeninivorans* microorganisms in the presence of a ferrocene mediator (Kharkova et al. 2021), a redox-active polymer, and a composite material was estimated. The proposed mechanism of electron transport from substrate (analyte) to the electrode in receptor system based on immobilized *B. adeninivorans* yeast in BSA–FC–CNT composite is shown in Fig. 5.

Yeast oxidizes substrate (analyt) by Eq. (2). Redox-active particles interact with the yeast by Eq. (3) and transfer electrons to the electrode by CNTs according to Eq. (4).

$$S\,(analyt) + E_{ox}(B.\,adeninivorans) \rightarrow P + E_{red}(B.\,adeninivorans) \quad (2)$$

$$E_{red}(B.\,adeninivorans) + M_{ox}(\text{redox} - \text{active polymer}) \overset{k_{int}}{\Rightarrow} M_{red}(\text{redox} - \text{active polymer}) + E_{ox}(B.\,adeninivorans) \quad (3)$$

$$M_{red}(\text{redox} - \text{active polymer}) \rightarrow M_{ox}(\text{redox} - \text{active polymer}) + n\bar{e} \quad (4)$$

where S (analyte) is substrate (glucose); P, product; E_{ox} (*B. adeninivorans*) and E_{red} (*B. adeninivorans*), oxidized and reduced forms of the enzymes of the yeast *B. adeninivorans*; M_{red} (redox-active polymer) and M_{ox} (redox-active polymer), oxidized and reduced forms of the mediator—redox-active particles of polymer; $n\bar{e}$, the number of electrons transferred to the electrode; k_{int}, constant of the rate of interaction between the mediator and biomaterial.

The electron transport efficiency after yeast immobilization in a composite material was studied by cyclic voltammetry (Fig. 6A) and the constant redox-active particle interaction with yeast k_{int} in Eq. (3) was calculated using Nicholson and Shain method (Nicholson and Shain 1964).

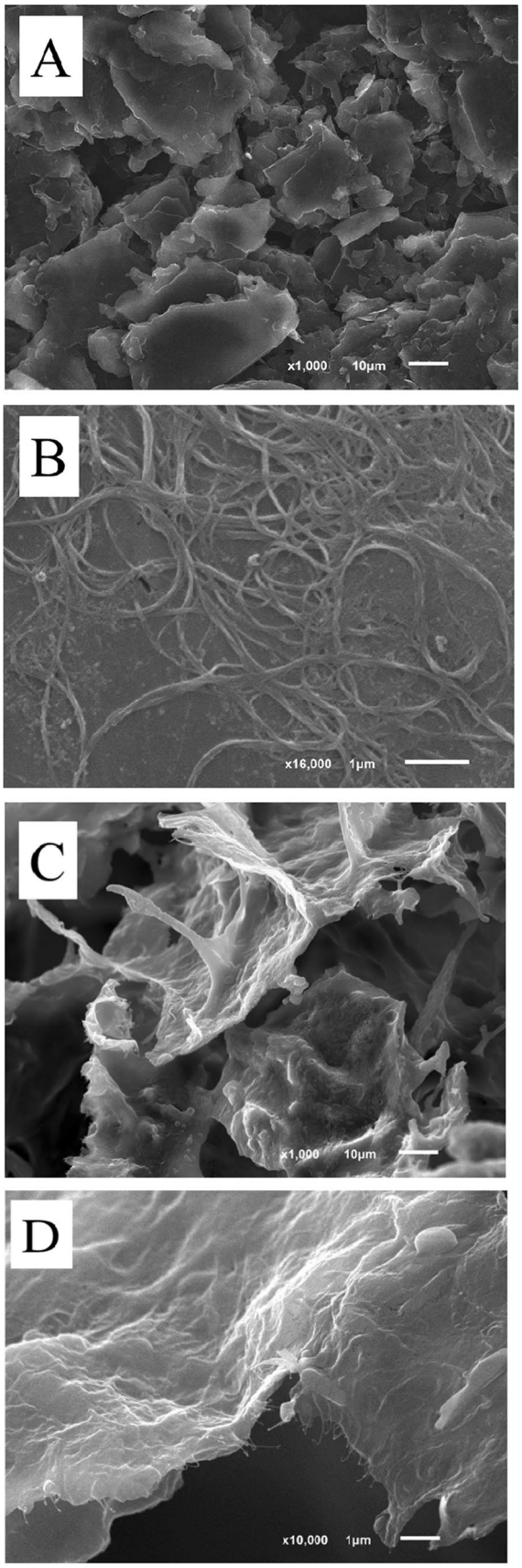

Fig. 3 Scanning electron micrographs of **A** carbon-paste electrode; **B** ▸ CNTs; **C** FC–BSA redox polymer; **D** BSA–FC–CNT composite

The high value of rate constant provides successfully compete redox-active particles with the dissolved oxygen, as natural electron acceptor, deaeration during the biosensor analysis is not required. For constant determination, all experiments were carried out under substrate concentration excess conditions: the final concentration of glucose was 50 mM, and in this case, reaction (2) is not rate-limiting. Linear dependence of the limiting current on the root of the scan rate $v^{1/2}$ in the electrode–composite material/redox-active polymer–microorganisms–substrate system (Fig. 6B) indicates that the generated current is diffusion limited. Thus, the Nicholson–Shine model is applicable to the studied systems and the constant of interaction between yeasts and the redox-active particles of polymer can be found using Eq. (5) by slope of the linear regression the dependences of the ratio of the limiting anodic currents in the presence and absence of substrate, (I_k/I_d), on the value of $1/\nu^{1/2}$.

$$\frac{I_k}{I_d} = \sqrt{\frac{k_{int}[E]RT}{nF\nu}}, \tag{5}$$

where I_k is the limiting current in the presence of substrate; I_d, the limiting current in the absence of substrate (A); k_{int}, the rate constant of the interaction of the polymer redox-active particles and yeast (dm^3/mg s); R, the universal gas constant (J/mol k); T, temperature, degrees Kelvin (K); [E], the titre of cells (mg/dm^3); ν, the potential scan rate (V/s); n, the number of transferred electrons; F, Faraday constant (C/mol).

The obtained values of the interaction constants of the mediator ferrocene, redox-active polymer BSA–FC, composite material BSA–FC–CNT with *B. adeninivorans* yeast are presented in Table 3.

Based on the obtained constants, it should be noted that the *B. adeninivorans* yeast immobilization into a redox-active polymer and a composite material is promising for further use in mediator bioelectrocatalysis. The resulting interaction constants exceed similar constants for a system based on *P. yeei* bacteria (Arlyapov et al. 2021); after adding nanomaterials to the systems, the interaction constant increases by an order, probably it is due to the ability of CNTs to penetrate into the cell (Mohanta et al. 2019), and as the result, the efficiency of electron transport increases in the receptor element. Thus, for the BOD receptor system formation, the BSA–FC–CNT composite material was chosen, since, in this system, high rate of interaction with eukaryotes and a high rate of interaction with the electrode were achieved.

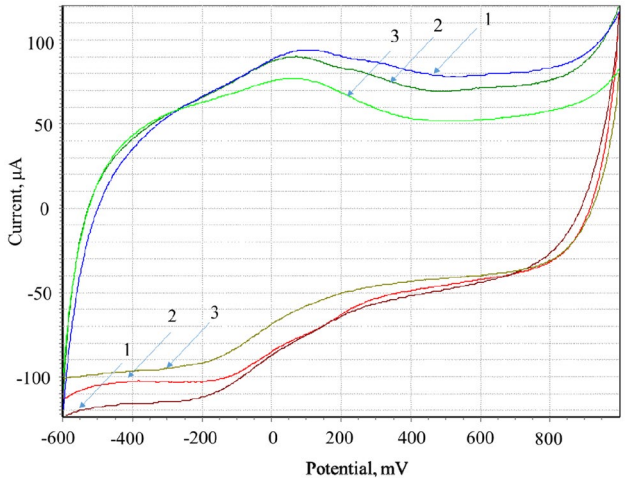

Fig. 4 Current–voltage curve for carbon-paste electrode containing modified BSA with FC, FC–BSA molar ratio is 1:2, and CNTs with a specific density of 2.5 (curve 1), 1.2 (curve 2), 0.2 (curve 3) μg/mm^2

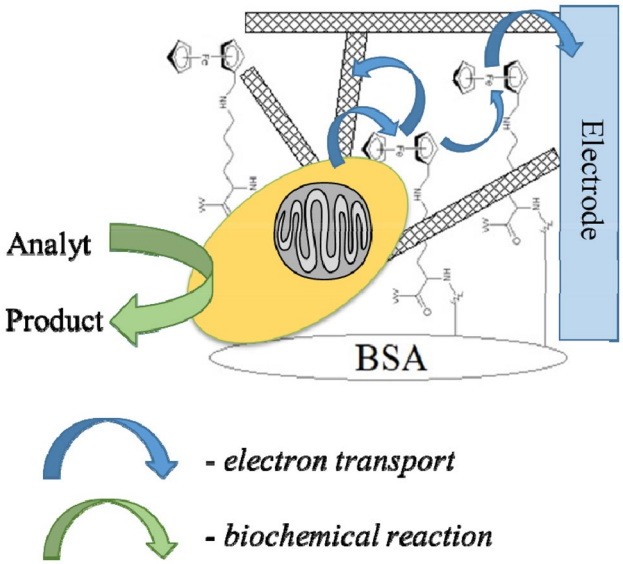

Fig. 5 Proposal mechanism of electron transport from substrate (analyte) to the electrode in receptor system based on immobilized *B. adeninivorans* yeast in BSA–FC–CNT composite

Selection of yeast specific density at the electrode and electrolyte pH for the receptor system operation

The yeasts specific density at the electrode and electrolyte pH impact on the biosensor current. The higher the analytical signal, the higher the sensitivity of the biosensor. Operation value of yeast specific density was determined as value corresponding to maximal analytical signal by dependences of biosensor responses on this parameter (Fig. 7A). Biosensor responses increase, when the yeast specific density on the electrode surface grows from 0.05 up to 0.1 mg/mm^2. This fact is corresponding to systems with the enzyme electrocatalytic oxidation, where current is directly proportional to the concentration of the enzymes per electrode surface area unit (Ikeda et al. 1996). If the yeast specific density on the electrode surface is more than 0.1 mg/mm^2, the biosensor responses decrease. The thickness of the biocatalytic layer has increased and lead to a diffusion limitation for the glucose transport to the enzyme active sites (Akyilmaz et al. 2020). Thus, 0.1 mg/mm^2 was chosen as the working value for the biomass specific density.

Electrolyte pH affects the yeast enzymatic system activity, therefore has an impact on biosensor response. In research (Babkina et al. 2006) was noted that the optimum electrolyte pH for the mediator biosensor depends not only microorganism, but also on the chosen mediator. Operation value of electrolyte pH was determined as value corresponding to maximal analytical signal by dependences of biosensor responses on this parameter in the pH range from 5.6 to 7.8 (Fig. 7B).

The electrode reaction with ferrocene in composite material occurs without the protons' participation, its effect on the shift of pH optimum is insignificant: an optimum is reached at pH equal to 6.2. It is the same for ferrocene system (Kharkova et al. 2021) and mediatorless biosensor (Yudina et al. 2015)

Table 2 Choosing the optimal amount of CNTs for the formation of a composite material based on a redox-active polymer and nanoparticles, taking into account the electrochemistry of electron transfer processes

Specific density of CNTs μg/mm^2	Transfer coefficient (α)		Heterogeneous transfer constant ks, s^{-1}
	Anode process	Cathode process	
0.2	0.9798 ± 0.0001	0.0225 ± 0.0002	0.49 ± 0.02
1.2	0.9810 ± 0.0002	0.0174 ± 0.0001	0.51 ± 0.04
2.5	0.9837 ± 0.0003	0.0119 ± 0.0001	0.55 ± 0.01
3.7	0.9826 ± 0.0004	0.0154 ± 0.0001	0.43 ± 0.02
5.0	0.9855 ± 0.0003	0.0144 ± 0.0001	0.42 ± 0.02
Gold nanotriangles modification of a carbon ionic liquid electrode (Niu et al. 2018)	0.49	–	1.01 (Niu et al. 2018)
Graphene–graphene oxide nanocomposite (Wang et al. 2019)	0.52	–	3.5 (Wang et al. 2019)

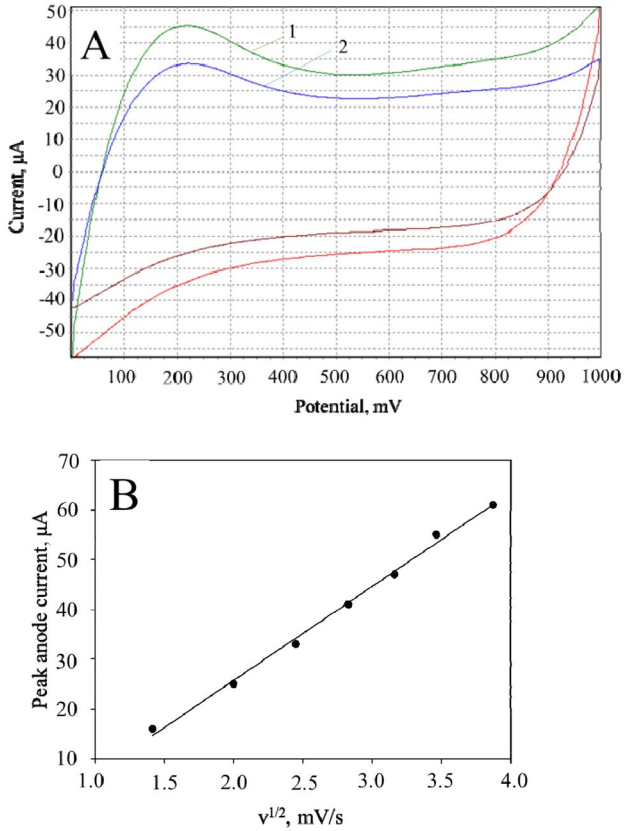

Fig. 6 Determination of the mediator interaction with *B. adeninivorans* yeast rate constant by cyclic voltammetry. **A** Current–voltage curve electrode with immobilized *B. adeninivorans* yeast in "BSA–FC–CNT" composite at a scan rate of 10 mV/s: in the presence (curve 1) and absence (curve 2) of glucose; **B** determination of the limiting stage according to the dependence of the peak current on the root of the scan rate $\nu^{1/2}$ in the system "yeast *B. adeninivorans*–"BSA–FC–CNT" composite–glucose"

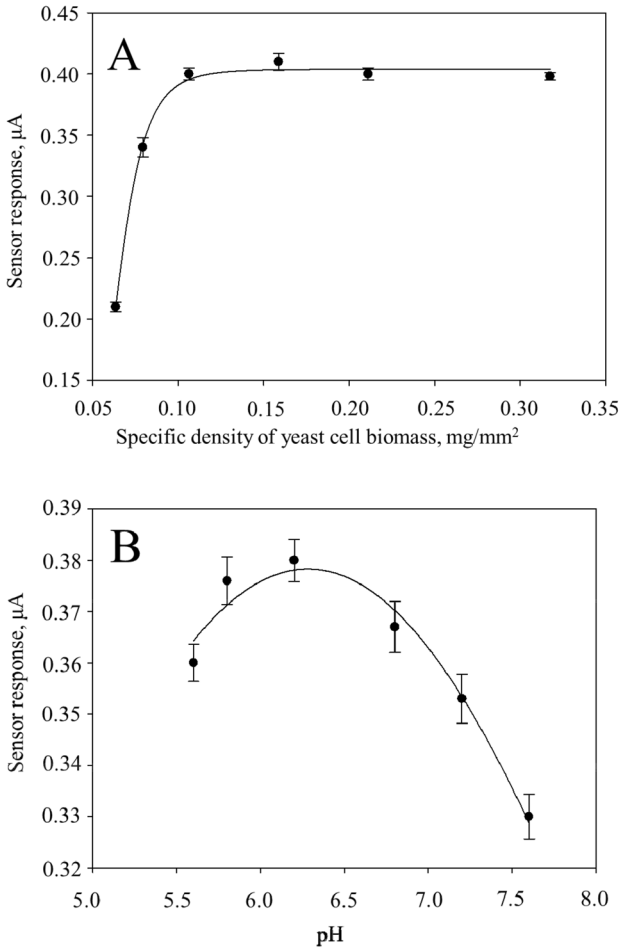

Fig. 7 Dependence of the sensor response in the system of *B. adeninivorans* yeast, immobilized in the composite material "BSA–FC–CNT" on **A** the specific density of the biomass on the electrode; **B** pH of the electrolyte solution

based on *B. adeninivorans* yeast. Thus, the working value of the pH of BSA–FC–CNT biosensors depends on the nature of used biocatalyst—the yeast *B. adeninivorans*.

Analytical and metrological characteristics of the BSA–FC–CNT biosensor for BOD determination

For BOD biosensor analysis, it is necessary that the developed system registers oxidations of as a broad range of organic substrates; therefore, substrate specificity of the receptor element based on BSA–FC–CNT composite was studied and compared with ferrocene system (Kharkova et al. 2021). Substrate specificity is shown on Fig. 8.

It should be noted that the amount of registered substrates does not decrease after changing of yeast immobilization, which indicates the composite material is the most biocompatible and allow registering of the wide range of organic compounds oxidation by *B. adeninivorans* yeast, which ensures the correlation with standard BOD_5 method.

To quantify measurement of the analyzed substances content in the sample,a hyperbolic dependence of the biosensor response on BOD_5 was obtained (Fig. 9), which was approximated by the Michaelis–Menten equation (Eq. 6).

$$\Delta I = \frac{\Delta I_{\max} * [S]}{K_M + [S]} \quad (6)$$

where ΔI is the rate of the enzymatic reaction, μA; $\Delta I_{\max}$ is the maximal rate of the enzymatic reaction, μA; K_M is the apparent Michaelis constant, mg/dm^3; [S] is the initial concentration of the substrate, mg/dm^3.

The metrological characteristics were determined from the calibration dependence of the analytical signal on BOD_5 (Fig. 9). The Michaelis constant was 19 ± 1 mg O_2/dm^3, the lower limit of the determined concentrations was 1.5 mg O_2/

Table 3 Rate constants of interaction of electron transport mediators with *P. yeei* bacteria (Nicholson 1965), and mediators with yeast *B. adeninivorans* founded in this work

Conducting system	Constant of the rate of interaction mediators with microorganisms, $dm^3/(g\ s)$	
	B. adeninivorans yeast	*P. yeei* bacteria
BSA–FC	0.056 ± 0.005	0.051 ± 0.004 (Arlyapov et al. 2021)
BSA–FC–CNT	0.51 ± 0.02	0.055 ± 0.001 (Arlyapov et al. 2021)
Ferrocene	0.011 ± 0.005 (Kharkova et al. 2021)	0.023 ± 0.001 (Kharkova et al. 2020)

dm^3. All the characteristics of the developed biosensor based on the *B. adeninivorans* yeast immobilized in a composite material and analogues BOD biosensors are shown in the Table 4.

According to the obtained data, it can be noted that the lower limit of the determined BOD_5 concentrations of the developed biosensor based on BSA–FC redox-polymer and CNTs exceeds many some analogues. Despite the fact that the lower limit of the developed biosensor is higher than some development model, the sensitivity of the proposed device allows sample analysis that contain lower than maximum allowable concentration BOD_5. CNTs make it possible to increase the efficiency of electron transfer by increasing the heterogeneous electron transfer constant and the interaction constant, providing better acceptor properties from eukaryotic cells, while the use of a second mediator is not required, which simplifies the analysis.

Analysis of real water samples by the developed biosensor and by the standard BOD_5 detection method

Biosensor based on *B. adeninivorans* yeast immobilized in BSA–FC–CNT composite was tested on nine real samples of the Tula region's surface waters (Fig. 10). Sampling procedure and BOD_5 determination by the standard method were carried out according to standard protocols (ISO 2003a,b).

Modified Student's *t* test showed that the data obtained by both methods (developed biosensor and standard technic) differed insignificantly. The biosensors formed on BSA–FC redox-polymer and CNTs have a high correlation (R = 0.9945) with the results of the standard method for BOD determination. The developed biosensor can be used as an alternative to the standard assay.

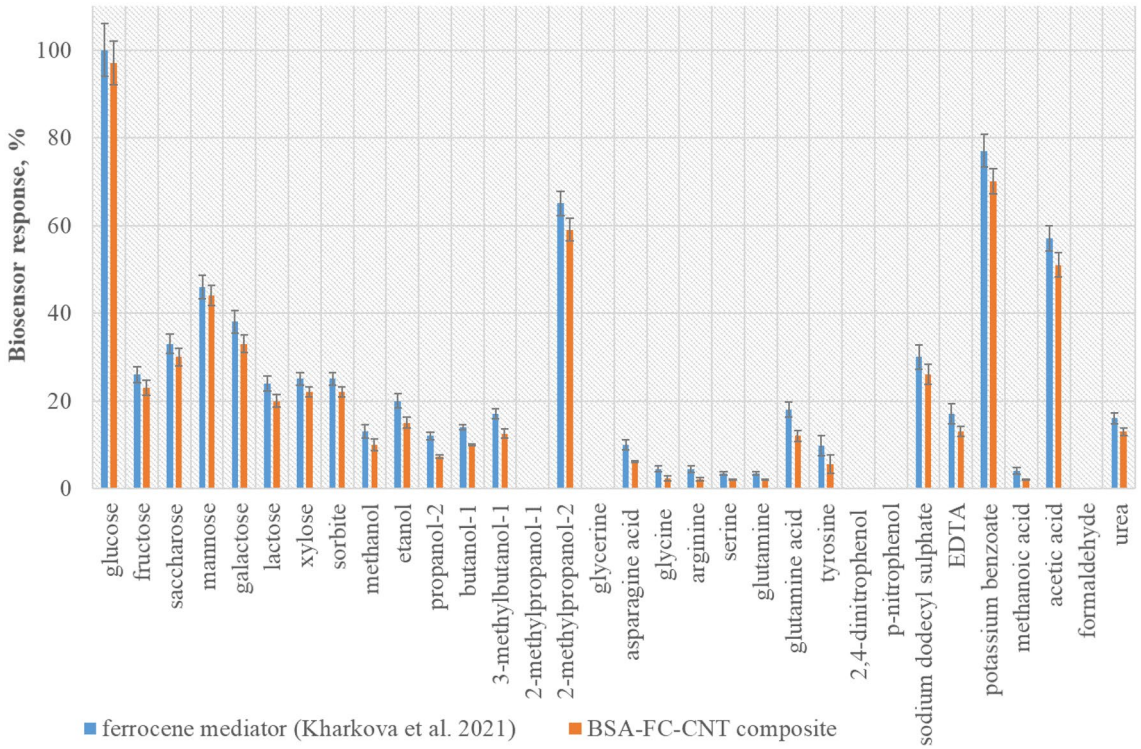

Fig. 8 Substrate specificity of *B. adeninivorans* yeast immobilized in the composite and in the presence of ferrocene mediator (Kharkova et al. 2021)

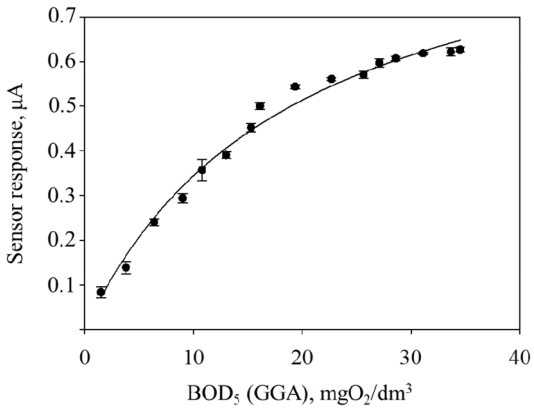

Fig. 9 Calibration dependence of the sensor response on the concentration of BOD_5 for the biosensor based on the *B. adeninivorans* yeast immobilized in a composite

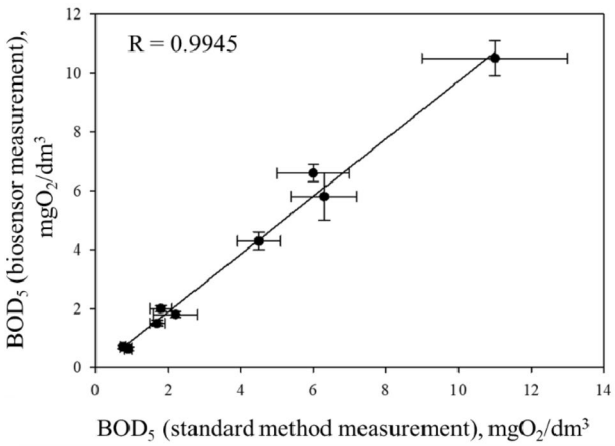

Fig. 10 Linear dependence of the results of BOD_5 determination obtained by the standard method and using the developed biosensor

Conclusion

The study proposes applying composite material based on BSA modified with FC for the immobilization of the *B. adeninivorans* yeast. On the basis of electrochemical studies, the optimal ratio for obtaining a redox-active polymer was found—the ratio of FC to BSA 1:2, since the heterogeneous electron transfer constant is 0.45 ± 0.01 s^{-1}. When carbon nanotubes are added to this polymer, the heterogeneous electron transfer constant increases since the nanotubes act as bridges between mediator covalently bonded with the BSA. The largest constant (0.55 ± 0.01 s^{-1}) corresponds to the CNT specific density of 2.5 μg/mm^2. The rate constants for developed composite show some insignificant kinetic limitations comparing other studies (Wang et al. 2019; Claire et al. 2018; Niu et al. 2018; Patil et al. 2021).

Addition of nanomaterials, not only increases the rate of electron transport to the electrode, but also the rate of interaction with eukaryotes (the interaction constant with the biomaterial is 0.51 ± 0.02 dm^3/(g s)), which makes it possible to effectively use this biomaterial in a single mediator system. It is shown that the composite material does not change the optimal pH value for yeast, i.e., 6.2.

The effective use of the developed receptor element for the determination of BOD_5 was shown: the BOD_5 linear range is 1.5–19 mg/dm^3, which allows analysis to be carried out within the maximum allowable concentration BOD_5. The limit of detection is 1,5 mg/dm^3 that is less than it is for literature analogues: 20 mg/dm^3 (Zhao et al. 2021), 3 mg/dm^3 (Hu et al. 2016), 4 mg/dm^3 (Hu et al.

Table 4 Comparative analytical and metrological characteristics of mediator biosensors

Biomaterial	Conducting system	BOD_5 linear range, mg/dm^3	Assay time, min	References
B. adeninivorans	BSA–FC–CNT	1.5–19	5	This work
P. yeei	BSA–FC–CNT	0.1–2	5	Arlyapov et al. (2021)
B. adeninivorans	Ferrocene–neutral red	0.16–2.7	12	Kharkova et al. (2021)
S. cerevisiae	Potassium hexacyanoferrate (III)–vitamin K3	20–225	20	Zhao et al. (2021)
B. subtilis	Potassium hexacyanoferrate (III)–graphene–polypyrrole	4–60	15	Hu et al. (2017)
P. aeruginosa	Potassium hexacyanoferrate (III)–polypyrrole	5–100	10	Hu et al. (2015)
P. aeruginosa	Poly-neutral red–polypyrrole	3–100	20	Hu et al. (2016)
Active sludge	Methylene blue–graphene	1–100	9	Niyomdecha et al. (2017)
C. violaceum	Potassium hexacyanoferrate (III)	20–225	30	Khor et al. (2015)
Biofilm	-	20–800	7	Liu et al. (2014)
B. subtilis	Carboxyl graphene	2–15	3	Li et al. (2017)

2017). Assay time is 5 min in contrast to the one shown in the literature where the time of analysis is 20–30 min (Zhao et al. 2021; Hu et al. 2016; Khor et al. 2015). Analysis of water samples showed that the use of immobilized *B. adeninivorans* yeast in a composite material allows obtaining data that have a high correlation with the results of the standard method ($R = 0.9945$). The proposed combination of microorganisms and a conducting system can be used in the future to create a prototype BOD biosensor, the sensitivity of which will not be inferior to known analogues.

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Author contributions ASK: Formulation of the research goal, execution of experiments data, writing the manuscript. The provision of financial support. Formulation of the purpose of the study. Formulation of further research plans. VAA: Execution of experiments data, writing the manuscript. Discussion of the results, formulation of conclusions, setting the goal of the work, writing the manuscript. DVP: Experimental studies related to the study of bioelectrocatalytic characteristics of microbial cells. AVM: Performing analytical procedures related to the use of electron microscopy.

Availability of data and material The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Research involving human participants and/or animals Not applicable because the research was not conducted on human participants and/or animals.

Informed consent Not applicable because the research was not conducted on human participants.

References

Akyilmaz E, Guvenc C, Koylu H (2020) A novel microbial biosensor system based on *C. tropicalis* yeast cells for selective determination of L-Ascorbic acid. Bioelectrochemistry. https://doi.org/10.1016/j.bioelechem.2019.107420

Andriukonis E, Reinikovaite V, Ramanavicius A (2022) Comparative study of polydopamine and polypyrrole modified yeast cells applied in biofuel cell design. Sustain Energy Fuels. https://doi.org/10.1039/D2SE00634K

Arlyapov V, Kamanin S, Ponamoreva O, Reshetilov A (2012) Biosensor analyzer for BOD index express control on the basis of the yeast microorganisms *Candida maltosa*, *Candida blankii*, and *Debaryomyces hansenii*. Enzyme Microb Technol. https://doi.org/10.1016/j.enzmictec.2012.01.002

Arlyapov VA, Yudina NYu, Asulyan LD, Alferov SV, Alferov VA, Reshetilov AN (2013) BOD biosensor based on the yeast *Debaryomyces hansenii* immobilized in poly (vinyl alcohol) modified by N-vinylpyrrolidone. Enzyme Microb Technol. https://doi.org/10.1016/j.enzmictec.2013.05.004

Arlyapov VA, Kharkova AS, Kurbanaliyeva SK, Kuznetsova LS, Machulin AV, Tarasov SE, Melnikov PV, Ponamoreva ON, Alferov VA, Reshetilov AN (2021) Use of biocompatible redox-active polymers based on carbon nanotubes and modified organic matrices for development of a highly sensitive BOD biosensor. Enzyme Microb Technol. https://doi.org/10.1016/j.enzmictec.2020.109706

Babkina E, Chigrinova E, Ponamoreva O, Alferov V, Reshetilov A (2006) Bioelectrocatalytic oxidation of glucose by immobilized bacteria *Gluconobacter oxydans*. Evaluation of water-insoluble mediator efficiency. Electroanalysis 15:10. https://doi.org/10.1002/elan.200603608

Baronian K, Downard A, Lowen R, Pasco N (2002) Detection of two distinct substrate-dependent catabolic responses in yeast cells using a mediated electrochemical method. Appl Microb Biotechn. https://doi.org/10.1007/s00253-002-1108-3

Catterall K, Morris K, Gladman C, Zhao H, Pasco N, John R (2001) The use of microorganisms with broad range substrate utilisation for the ferricyanide-mediated rapid determination of biochemical oxygen demand. Talanta. https://doi.org/10.1016/S0039-9140(01)00527-6

Chan C, Lehmann M, Tag K, Lung M, Kunze G, Riedel K, Gruendig B, Renneberg R (1999) Measurement of biodegradable substances using the salt-tolerant yeast *Arxula adeninivorans* for a microbial sensor immobilized with poly(carbamoyl)sulfonate (PCS) Part II: application of the novel biosensor to real samples from coastal and island regions. Biosens Bioelectron. https://doi.org/10.1016/S0956-5663(98)00110-9

Christwardana M, Kwon Y (2017) Yeast and carbon nanotube based biocatalyst developed by synergetic effects of covalent bonding and hydrophobic interaction for performance enhancement of membraneless microbial fuel cell. Bioresour Technol. https://doi.org/10.1016/j.biortech.2016.11.051

Claire FJ, Tenney SM, Li MM, Siegler MA, Wagner JS, Hall AS, Kempa TJ (2018) Hierarchically ordered two-dimensional coordination polymers assembled from redox-active dimolybdenum clusters. J Am Chem Soc. https://doi.org/10.1021/jacs.8b06331

Fang D, Gao G, Yang Y, Wang Y, Gao L, Zhi J (2020) Redox mediator-based microbial biosensors for acute water toxicity assessment: a critical review. ChemElectroChem. https://doi.org/10.1002/cssc.201802126

Gal I, Schlesinger O, Amir L, Alfonta L (2016) Yeast surface display of dehydrogenases in microbial fuel-cells. Bioelectrochemistry. https://doi.org/10.1016/j.bioelechem.2016.07.006

Gao G, Fang D, Yu Y, Wu L, Wang Y, Zhi J (2017) A double-mediator based whole cell electrochemical biosensor for acute biotoxicity assessment of wastewater. Talanta. https://doi.org/10.1016/j.talanta.2017.01.081

Hu J, Gao G, Xia S (2015) Development of a mediator-type bioelectrochemical sensor based on polypyrrole immobilized ferricyanide and microorganisms for biochemical oxygen demand fast detection. Int J Electrochem Sci 10:9695–9705

Hu J, Gao G, Xia S (2016) A mediated BOD microsensor based on poly (Neutral Red) and bacteria modified interdigited ultramicroelectrode array. Int J Electrochem Sci 15:10. https://doi.org/10.20964/2016.07.07

Hu J, Li Y, Gao G, Xia S (2017) A mediated BOD biosensor based on immobilized *B. subtilis* on three-dimensional porous graphene-polypyrrole composite. Sensors 15:10. https://doi.org/10.3390/s17112594

Ikeda T, Kurosaki T, Takayama K, Kano K, Miki K (1996) Measurements of oxidoreductase-like activity of intact bacterial cells by an amperometric method using a membrane-coated electrode. Anal Chem. https://doi.org/10.1021/ac950240a

Ino K, Onodera T, Fukuda M, Nashimoto Y, Shiku H (2019) Combination of double-mediator system with large-scale integration-based amperometric devices for detecting NAD (P) H: quinone oxidoreductase 1 activity of cancer cell aggregates. ACS Sens. https://doi.org/10.1021/acssensors.9b00344

ISO 5815-1:2003 (2003) Water quality-determination of biochemical oxygen demand after N days (BODn)—part 1: dilutionand seeding method with allylthiourea addition. https://www.iso.org/standard/31090.html

ISO 5815-2:2003 (2003) Water quality—determination of biochemical oxygen demand after N days (BODn)—part 2: method for undiluted samples. https://www.iso.org/standard/31091.html

Kasem ET, Tsujiguchi T, Nakagawa N (2013) Effect of metal modification to carbon paper anodes on the performance of yeast-based microbial fuel cells part I: in the case without exogenous mediator. Key Eng Mater. https://doi.org/10.4028/www.scientific.net/KEM.534.76

Kharkova AS, Arlyapov VA, Turovskaya AD, Shvets VI, Reshetilov AN (2020) A mediator microbial biosensor for assaying general toxicity. Enzyme Microb Technol. https://doi.org/10.1016/j.enzmictec.2019.109435

Kharkova A, Arlyapov V, Ilyukhina A, Ponamoreva O, Alferov V, Reshetilov A (2021) A kinetic approach to the formation of two-mediator systems for developing microbial biosensors as exemplified by a rapid biochemical oxygen demand assay. 3 Biotech. https://doi.org/10.1007/s13205-021-02709-8

Khor BH, Ismail AK, Ahamad R, Shahir S (2015) A redox mediated UME biosensor using immobilized *Chromobacterium violaceum* strain R1 for rapid biochemical oxygen demand measurement. Electrochim Acta. https://doi.org/10.1016/j.electacta.2015.07.089

Kurbanalieva S, Arlyapov V, Kharkova A, Perchikov R, Kamanina O, Melnikov P, Popova N, Machulin A, Tarasov S, Saverina E, Vereshchagin A, Reshetilov A (2022) Electroactive biofilms of activated sludge microorganisms on a nanostructured surface as the basis for a highly sensitive biochemical oxygen demand biosensor. Sensors. https://doi.org/10.3390/s22166049

Laviron E (1979) General expression of the linear potential sweep voltammogram in the case of diffusionless electrochemical systems. J Electroanal Chem. https://doi.org/10.1016/S0022-0728(79)80075-3

Li Y, Sun J, Wang J, Bian C, Tong J, Li Y, Xia S (2017. A rapid and sensitive BOD biosensor based on ultramicroelectrode array and carboxyl graphene. In: 2017 IEEE 12th International Conference on nano/micro engineered and molecular systems (NEMS). https://doi.org/10.1109/NEMS.2017.8017024

Liang RP, Fan LX, Huang DM, Qiu JD (2011) A label-free amperometric immunosensor based on redoxactive ferrocene-branched chitosan/multiwalled carbon nanotubes conductive composite and gold nanoparticles. Electroanalysis. https://doi.org/10.1002/elan.201000534

Lin Z, Wu G, Zhao L, Lai KWC (2019) Carbon nanomaterial-based biosensors: a review of design and applications. IEEE Nanotechnol Mag. https://doi.org/10.1109/MNANO.2019.2927774

Liu L, Shang L, Liu C, Liu C, Zhang B, Dong S (2010) A new mediator method for BOD measurement under non-deaerated condition. Talanta. https://doi.org/10.1016/j.talanta.2010.01.062

Liu C, Zhao H, Ma Z, An T, Liu C, Zhao L, Yong D, Jia J, Li X, Dong S (2014) Novel environmental analytical system based on combined biodegradation and photoelectrocatalytic detection principles for rapid determination of organic pollutants in wastewaters. Environ Sci Technol. https://doi.org/10.1021/es4031358

Mazloum-Ardakani M, Barazesh B, Moshtaghioun S, Sheikhha M (2019) Designing and optimization of an electrochemical substitute for the MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) cell viability assay. Sci Rep. https://doi.org/10.1038/s41598-019-51241-6

Mohanta D, Patnaik S, Sood S, Das N (2019) Carbon nanotubes: evaluation of toxicity at biointerfaces. J Pharm Analysis. https://doi.org/10.1016/j.jpha.2019.04.003

Moradian JM, Yang F-Q, Xu N, Wang J-Y, Wang J-X, Sha C, Ali A, Yong Y-C (2022) Enhancement of bioelectricity and hydrogen production from xylose by a nanofiber polyaniline modified anode with yeast microbial fuel cell. Fuel. https://doi.org/10.1016/j.fuel.2022.125056

Morris K, Catterall K, Zhao H, Pasco N, John R (2001) Ferricyanide mediated biochemical oxygen demand–development of a rapid biochemical oxygen demand assay. Anal Chim Acta. https://doi.org/10.1016/S0003-2670(01)01133-3

Nakamura H, Abe Y, Koizumi R, Suzuki K, Mogi Y, Hirayama T, Karube I (2007a) A chemiluminescence biochemical oxygen demand measuring method. Anal Chim Acta. https://doi.org/10.1016/j.aca.2007.08.050

Nakamura H, Suzuki K, Ishikuro H, Kinoshita S, Koizumi R, Okuma S, Gotoh M, Karube I (2007b) A new BOD estimation method employing a double-mediator system by ferricyanide and menadione using the eukaryote *Saccharomyces cerevisiae*. Talanta. https://doi.org/10.1016/j.talanta.2006.10.019

Nicholson RS (1965) Theory and application of cyclic voltammetry for measurement of electrode reaction kinetics. Anal Chem. https://doi.org/10.1021/ac60230a016

Nicholson RS, Shain I (1964) Theory of stationary electrode polarography. Single scan and cyclic methods applied to reversible, irreversible, and kinetic systems. Anal Chem. https://doi.org/10.1021/ac60210a007

Niu Y, Liu J, Chen W, Yin C, Weng W, Li X, Wang X, Li G, Sun W (2018) A direct electron transfer biosensor based on a horseradish peroxidase and gold nanotriangle modified electrode and electrocatalysis. Anal Methods. https://doi.org/10.1039/C8AY01980K

Niyomdecha S, Limbut W, Numnuam A, Asawatreratanakul P, Kanatharana P, Thavarungkul P (2017) A novel BOD biosensor based on entrapped activated sludge in a porous chitosan-albumin cryogel incorporated with graphene and methylene blue. Sens Actuators B Chem. https://doi.org/10.1016/j.snb.2016.10.102

Pasco N, Baronian K, Jeffries C, Hay J (2000) Biochemical mediator demand–a novel rapid alternative for measuring biochemical oxygen demand. Enzyme Microb Technol. https://doi.org/10.1007/s002530051666

Pasco N, Baronian K, Jeffries C, Webber J, Hay J (2004) MICREDOX—development of a ferricyanide-mediated rapid biochemical oxygen demand method using an immobilised *Proteus vulgaris* biocomponent. Biosens Bioelectron. https://doi.org/10.1016/j.bios.2004.02.016

Pasco N, Hay J, Scott A, Webber J (2005) Redox coupling to microbial respiration: an evaluation of secondary mediators as binary mixtures with ferricyanide. Aust J Chem. https://doi.org/10.1071/CH05001

Patil N, Palma J, Marcilla R (2021) Macromolecular engineering of poly (catechol) cathodes towards high-performance aqueous zinc-polymer batteries. Polymers. https://doi.org/10.3390/polym13111673

Rabinowitz J, Vacchino J, Beeson C, McConnell H (1998) Potentiometric measurement of intracellular redox activity. J Am Chem Soc. https://doi.org/10.1021/ja973560f

Rawson F, Gross A, Garrett D, Downard A, Baronian H (2012) Mediated electrochemical detection of electron transfer from the outer

surface of the cell wall of Saccharomyces cerevisiae. Electrochem Commun. https://doi.org/10.1016/j.elecom.2011.11.030

Trosok SP, Driscoll BT, Luong JHT (2001) Mediated microbial biosensor using a novel yeast strain for wastewater BOD measurement. Appl Microbiol Biotechnol. https://doi.org/10.1007/s002530100674

Verma M, Mishra V (2021) Recent trends in upgrading the performance of yeast as electrode biocatalyst in microbial fuel cells. Chemosphere. https://doi.org/10.1016/j.chemosphere.2021.131383

Wang B, Wang X, He Z, Zhao X, Wang L (2019) Direct electrochemistry of glucose oxidase on a graphene-graphene oxide nanocomposite-modified electrode for a glucose biosensor. Int J Electrochem Sci 5:10. https://doi.org/10.20964/2019.08.34

Yoshida N, Yano K, Morita T, McNiven SJ, Nakamura H, Karube I (2000) A mediator-type biosensor as a new approach to biochemical oxygen demand estimation. Analyst. https://doi.org/10.1039/B005995L

Yoshida N, Hoashi J, Morita T, McNiven S, Nakamura H, Karube I (2001) Improvement of a mediator-type biochemical oxygen demand sensor for on-site measurement. J Biotechnol. https://doi.org/10.1016/S0168-1656(01)00282-6

Yudina NYu, Arlyapov VA, Chepurnova MA, Alferov SV, Reshetilov AN (2015) A yeast co-culture-based biosensor for determination of waste water contamination levels. Enzyme Microb Technol. https://doi.org/10.1016/j.enzmictec.2015.06.008

Zaitseva AS, Arlyapov VA, Yudina NYu, Alferov SV, Reshetilov AN (2017) Use of one- and two-mediator systems for developing a BOD biosensor based on the yeast *Debaryomyces hansenii*. Enzyme Microb Technol. https://doi.org/10.1016/j.enzmictec.2016.12.005

Zamani FG, Moulahoum H, Ak M, Demirkol DO, Timura S (2019) Current trends in the development of conducting polymers-based biosensors. Trends Anal Chem 15:10. https://doi.org/10.1016/j.trac.2019.05.031

Zhao C, Wang G, Sun M, Cai Z, Yin Z, Cai Y (2021) Bacterial cellulose immobilized *S. cerevisiae* as microbial sensor for rapid BOD detection. Fibers Polym. https://doi.org/10.1007/s12221-021-0650-5

Zinovicius A, Rozene J, Merkelis T, Bruzaite I, Ramanavicius A, Morkvenaite-Vilkonciene I (2022) Evaluation of a yeast–polypyrrole biocomposite used in microbial fuel cells. Sensors. https://doi.org/10.3390/s22010327