RESEARCH ARTICLE

Biosensor for maltose quantification and estimation of maltase activity

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Abstract: The aim of this study was to create a laboratory model of an amperometric microbial biosensor for maltose quantification in the presence and absence of starch and to estimate the use of the model in the study of maltase activity of the culture-receptor. The biosensor for maltose was developed on the basis of a Clark-type oxygen electrode, coupled with a bioreceptor, which contained bacterial cells immobilized on the membrane. The determination of maltose concentration was based on measuring the rate of electrode current change in response to addition of the analyte. The detection limit of the biosensor was 1 μM maltose, a linear interval of standard curve was observed from 14 μM up to 1.9 mM of maltose. The microbial biosensor demonstrated good sensitivity to maltose, 36.02 nA (M·s)$^{-1}$. Combination of bioreceptors on the basis of fungus and bacterium allowed of using the biosensor for quantification of maltose in the presence of starch. Changes in metabolism of the culture-receptor had an effect on the biosensor response. It indicated that the developed model was a tool of simple construction and easy-to-use in the study of maltase activity of the immobilized culture-receptor.

Keywords: Amperometric microbial biosensor, Clark-type oxygen electrode, maltose determination, maltase, starch

1 Introduction

Maltose (malt sugar) is natural disaccharide composed of two glucose residues. Maltose is present in large amounts in malt (germinated seeds of barley, rye and other cereals).

Biosynthesis of maltose is known only in some yeast.[1] In the body maltose is formed under enzymatic hydrolysis of starch and glycogen. Further hydrolysis of maltose is catalyzed by the maltase enzyme.

The enzyme maltase (α-D-glucosidase, EC 3.2.1.20) is involved in the hydrolyses of 1,4-α-linkage in the oligosaccharides which remain after degradation of starch by amylases. This enzyme originates from different sources, which include plants, seaweeds, protozoa, fungi, bacteria, vertebrates, and invertebrates.[2]

The enzymes have been purified and characterized from bacteria, yeasts and moulds, and differ in their substrate specificities. The enzymes from *Bacillus* strains (*B. amylolyticus, B. cereus, B. megaterium, B. subtilis*)

have been termed "maltases" since they have a high activity towards maltose.[3] Maltase catalyzes the intracellular hydrolysis of maltose to two glucose molecules:

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 2C_6H_{12}O_6$$
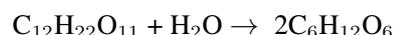
In food industry maltose is used as maltose syrup, which is produced under enzymatic starch hydrolysis. Disaccharide is used for manufacture of baby food products, bakery products, in brewing and distilling industry, for preparation of nutrient media in microbiology.

In the human body maltose can be easily digested. This compound is split by maltase. However, as a result of a genetic defect that causes a deficiency of this enzyme in the intestinal mucosa, hereditary maltose intolerance may occur. Individuals, who want to stay well, need to avoid products that contain glycogen, starch and maltose or eat these products simultaneously taking the enzyme. Of great importance is a rapid quantitative analysis of maltose because of wide application of starch and maltose.

In addition to conventional techniques (gas and liquid chromatography) fast methods of maltose detection may use sensor analyzers as well as microbial sensors. Furthermore, a biosensor method of analysis provides an easy variation in selectivity of detection.

Different types of enzymatic sensors, which can be used for maltose detection, are developed. In a number of sensors the enzymes were immobilized on the surface of electrodes.[4–10] A comparison of enzymatic

biosensors for maltose was presented by Pyeshkova, *et al.*[9] But a microbial sensor can be used not only for quantitative determination of maltose but also for estimation of the activities of enzymes involved in disaccharide metabolism and features of maltose metabolism in the biological object used as a bioreceptor.

Amperometric biosensors are employed to detect almost 80 various substances including monosaccharides, disaccharides. Enzyme amperometric sensors for maltose were described, in some of these studies a Clark-type oxygen electrode was used as a transducer.[11–15] One of amperometric microbial biosensors, which consisted of immobilized cells of yeast and an electrochemical transducer (amperometric oxygen electrode) allowing detection of culture response to maltose, was proposed by Riedel, *et al.*[16]

Early it was found that the enzyme maltase was unstable after 10-12 fold purification and was inactivated after a few days at -15 °C.[17] Only stabilizers were able to retain the activity of the enzyme. So, the use of a biosensor approach for study of the response of maltase-containing culture-receptor to maltose allows for estimation of stable activity of the enzyme without enzyme isolation.

The objective of this work was to create a laboratory model of the amperometric microbial biosensor for maltose quantification in the presence and absence of starch and to estimate the use of the model in the study of maltase activity of the immobilized culture-receptor.

2 Materials and Methods

2.1 Microorganisms

The study was performed using the wild strains of bacterium (*Pseudomonas*-B-4c/3) and fungus (*Mucor*-F-6/2).[18] The bacterium was isolated from the wastewater of cardboard paper production (Polotnyanyi Zavod, Kaluga region, Russia). The bacterial culture was maintained on peptone-tryptone agar slants at +4°C and transferred every 6 months. The fungus was isolated as an accompanying culture of the wood-rotting basidiomycete. This isolate was maintained on malt agar slants at +4°C and transferred every 6 months.

2.2 Immobilization of microbial cells

To obtain biomass, B-4c/3 bacterium was grown on peptone-tryptone agar slants at +28°C for 18 h. Obtained biomass was suspended in a 50 mM K-Na phosphate buffer (pH 7.4) to a concentration of 100 mg wet cells per milliliter. The suspension was stored at +4°C during 12 h and then used for manufacturing receptor elements.

To prepare a receptor element, the obtained suspension of bacterial cells was immobilized on paper (as a support matrix) by the method of physical adsorption: 10 μL of the cell suspension were spotted onto the paper (a spot of 3-4 mm in diameter). The prepared receptor element was air-dried for 30 min and then used as a receptor element immediately or stored at +4 °C before its application.

To obtain fungal biomass, F-6/2 fungus was grown on malt agar in Petri dishes at +28°C for 6 days. The fungal film was then peeled from malt agar surface, stored at +4 °C during 24 h and then used as receptor element of biosensor.

2.3 Creation of amperometric microbial biosensor for maltose

The receptor element obtained as described above was used for preparation of a microbial electrode. For this purpose the receptor element was fixed on the measuring surface of the Clark-type electrode by means of nylon net. For amperometric studies the obtained microbial electrode was placed in an open measuring cell with a working volume of 5 mL containing the measuring solution (buffer) and equipped with a magnetic mixer. The oxygen electrode was used together with "Ingold 531 O_2 amplifier" (Switzerland-USA). The signal was recorded with a two-coordinate "XY Recorder-4103" (Czech Republic).

The registered parameter (the response of the amperometric biosensor) was the maximum change of the electrode current (the first derivative of the change in the electrode current in response to addition of the substance), which was proportional to the rate of oxygen concentration change.

2.4 Principle of proposed biosensor and measurements

A kinetically controlled sensor was proposed. In the created model metabolism of the receptor's bacterial cells and the rate of oxygen consumption by the bioreceptor altered under maltose action. The Clark oxygen electrode coupled with the bioreceptor converted a chemical signal (oxygen concentration) into an electric one. The determination of maltose concentration was based on measuring the rate of electrode current change (the rate of a change of oxygen consumption by the bioreceptor) in response to addition of the substrate. Addition of different amounts of maltose led to changes in oxygen consumption rate, i.e. proportional changes of current. The biosensor response was measured in pA s^{-1}.

Measurements were carried out at +20-22°C in a 50 mM K-Na-phosphate buffer solution (pH 7.4) saturated

with air at continuous mixing. The base steady-state current (baseline) implied oxygen saturation of buffer solution in the absence of the substrate of respiration (maltose). When the baseline was stabilized, a sample solution was injected into a measuring cell and response was recorded as change of the Clark oxygen electrode current. After signal registration, the system was washed with a 50 mM K-Na-phosphate buffer (pH 7.4) to reach the baseline, and the biosensor was ready for next sample measurement.

3   Result and Discussion

3.1   Influence of the type of membrane for immobilization of microbial cells

To immobilize B-4c/3 bacterial cells, membranes with different densities and pore diameters from various companies were used. They were Millipore filter papers (AA, AP_{pre}, DA, GS, LS, PH), Whatman glass fiber papers (GF/A, GF/C) and Russian filter papers "colored ribbon" ("black, yellow, white, red and blue ribbons"). Bacterial cells were immobilized by physical adsorption without chemical treatment of cells. In the case of adsorption, membrane mechanically supports the microbes. Microorganisms are not damaged by this immobilization procedure.[19] Constants of the hyperbolic dependence of Michaelis–Menten type (the dependence of response rate to maltose V, which is the response of the biosensor, on maltose concentration S), V-S, were calculated. With the Millipore filters the best results were obtained using PH filter paper: V_{max} = 478.8 pA s^{-1} and $S_{0.5}$ = 4.9×10^{-3} M (where V_{max} is the maximal rate of response to maltose, $S_{0.5}$ is maltose concentration, when V = 1/2V_{max}). The best constants of the rate of a reaction between maltose and cells immobilized on the Whatman papers were derived for GF/C: V_{max}= 134.7 pA s^{-1} and $S_{0.5}$ = 1.5×10^{-3} M. As to Russian filter papers the result was better for a "white ribbon" filter: V_{max}= 33.0 pA s^{-1} and $S_{0.5}$ = 2.7×10^{-3} M. Figure 1 represents plots of the dependence of the response rate to maltose (the biosensor response) on substrate concentration when different membranes were used.

Using the diffusion equation for digital simulation, the influence of the thickness of enzyme membrane on the biosensor response was investigated.[20] The equation for calculation of the maximum thickness of the membrane, on which the enzymes were immobilized, at σ dimensionless diffusion module when $1/\sigma$ = 0.664 was given. Maximum rates of the sensor response were calculated when the thickness of the membrane was less than 0.1 mm.

The author considered a particular case of Tile module, Ψ,[21] when $\Psi = 1/\sigma$, to assess the impact of diffusion on the formation of the sensor response (see below). Maximum values of Ψ, 0.24-0.18, were obtained in our study for the membranes with the thickness of 0.25-0.1 mm, with different density and different pore size.

Whatman GF/C paper is glass fiber filters manufactured from 100% borosilicate glass. These filters are widely used for isolation of cells from culture liquid. Pore size of Whatman GF/C is 1.2 μm. "White ribbon" filter is a medium-density paper manufactured from ultra-thin fiber, pore size of "white ribbon" filter is 5-8 μm. The pore size of Millipore PH filters is 0.3 μm. Taking into account that pseudomand cell sizes are 1.0-1.5×0.5 μm, it is clear why the results obtained with Millipore PH filter and Whatman GF/C paper in this study were better than those using "white ribbon" filter.

The permanence of the membrane used for immobilization of bacterial cells was of great importance. In our case the permanence was the ability of the filter to keep the form under repeated placing on the electrode not breaking into the fragments when the membrane is present in the mixed buffer solution for a long time. At the same time, the membrane should be rather plastic to contact the working surface of the electrode closely enough but it should not stick or crumble. Whatman GF/C paper met all these requirements.

3.2   Response to maltose and calibration of the maltose biosensor

Calibration of B-4c/3 biosensor was carried out by a kinetic method. This signifies that measurements should not be limited by substrate diffusion.

Using different membranes for immobilization of B-4c/3 bacterium, the influence of diffusion (diffusion of maltose molecules up to cells-receptors) on the value of biosensor responses was estimated by author earlier.[22] The Tile module, Ψ, was used as a criterion for estimation of diffusion influence. It is known that diffusion has a considerable effect on the rate of the process, when Ψ > 1.[21] For Ψ < 1, the process does not depend on diffusion. Under our conditions, for GF/C paper, when Ψ = 0.23, it can be concluded that the process of the biosensor response to maltose injection was not limited by substrate diffusion, but controlled by substrate diffusion into cells of the culture-receptor.

Electrode current in the absence of maltose reflected the endogenous respiration rate of B-4c/3 bacterial cells. After injection of maltose into the measuring cell, immobilized bacterial cells interact with substrate leading to an increase in respiration rate. The maximum rate of current change was proportional to the maltose concen-
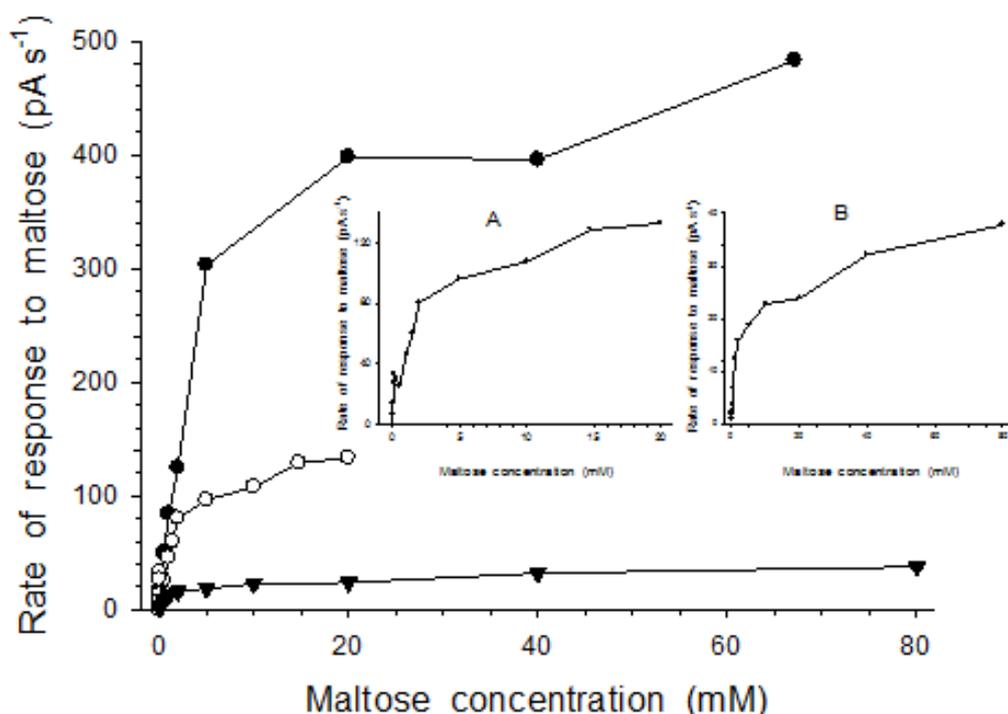
Figure 1. Dependence of the rate of the biosensor response on maltose concentration for B-4c/3 cells immobilized on different membranes: PH, closed circles, GF/C, open circles (insert A),"white ribbon", closed triangles (inset B).

tration. The response of the amperometric biosensor to maltose at different concentrations of maltose is shown in Figure 2a. A linear relationship between the rate of current change and maltose concentration was observed from 14 μM up to 1.9 mM of maltose. The linear dependence is depicted in Figure 2b.

The results obtained in this study are close to those received for the enzyme sensor for maltose which was described by Pyeshkovaet, *et al.*[9] But for the B-4c/3 biosensor formation more easily produced biological receptor (bacterial cells) was used. In our case the dependence of the biosensor response on the maltose concentration was described by the linear equation: $\Delta I \cdot \Delta t^{-1}$ (pA·s^{-1}) = 12.4 + 36.0×C, where C was the maltose concentration (mM) below 1.9 mM. The detection limit of the biosensor was 1 μM maltose.

Due to the kinetic method of the biosensor response determination, the registration of the biosensor response was finished in 2 min after sample addition. The total duration of the assay including the system washing was no longer than 15 min. The use of the laboratory model of the biosensor for academic research of maltose metabolism in the microorganism provides the possibility to vary easily experimental conditions and obtain quickly the information about changes in metabolism.

3.3 Influence of pH and type of buffer

To determine the optimum buffer and pH for the quantification of maltose, their influences on the response of B-4c/3 biosensor were investigated for $9.4×10^{-4}$ M of maltose. Different buffer solutions were used: 50 mM MES buffer, 50 mM K-Na phosphate buffer, 50 mMMOPS buffer and 50 mMTris/HCl buffer. The pH value was investigated varying from 5.5 to 9.5 (Figure 3). The best results were obtained for a 50 mM K-Na phosphate buffer. The optimum pH was within 7.4-7.8.

The obtained results were examined for the biosensor with the B-4c/3 receptor element after 15-day air-storage at +4°C (for three maltose concentrations: $9.4×10^{-5}$, $9.4×10^{-4}$ and $9.4×10^{-3}$ M). The results were recorded at pH 7.4 and 7.7. The responses were higher for pH 7.7 than for pH 7.4 (Figure 4).

It is known that the rate of the response of immobilized cells to a substrate is caused by two processes: substrate metabolism by cell enzymes and processes of substrate transport into the cell.[23] It is obvious that in the absence of the enzyme that initiates substrate metabolism the cell response to a substrate is caused by the processes of substrate transport into the cell. In the presence of the initiating enzyme both processes contribute to the formation of the response. It has been shown earlie[24]

(a)

(b)
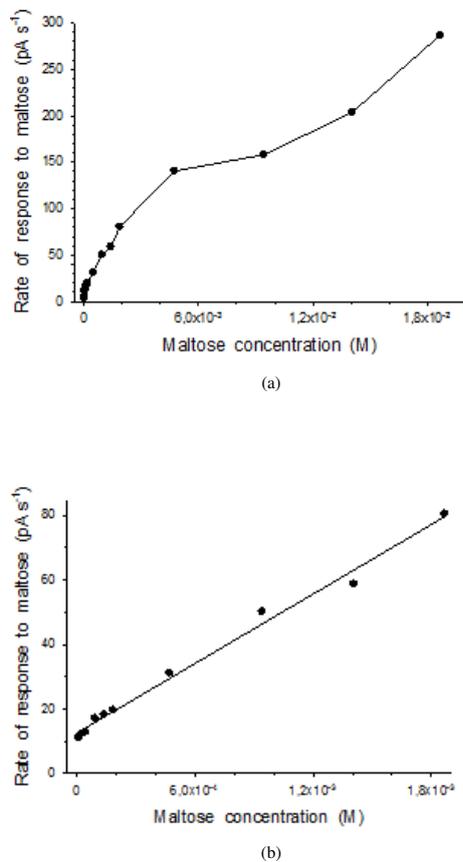
Figure 2. Dependence of the maximum change in the current on maltose concentration (a) and standard curve (b) for the maltose B-4c/3 biosensor.
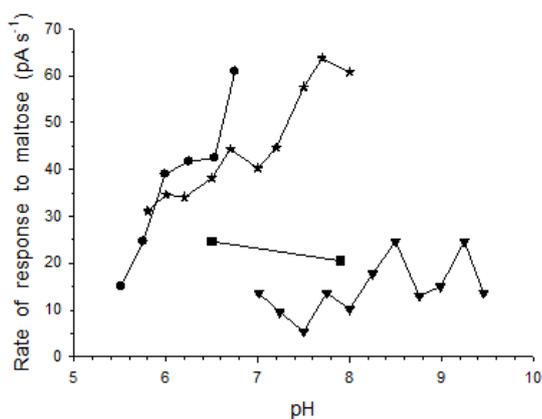
Figure 3. Influence of pH and type of buffer on the B-4c/3 biosensor response (response to maltose at 9.4×10^{-4} M). The type of buffer: MES, closed circles; K-Na phosphate, closed stars; MOPS, closed squares; Tris/HCl, closed triangles.

that the response of intact cells to a substrate depends on the presence of the cell enzymes that initiate substrate metabolism.

The responses of intact B-4c/3 bacterial cells to three maltose concentrations, 9.4×10^{-5}, 9.4×10^{-4} and 9.4×10^{-3} M, were tested. The obtained responses were 4.1, 5.9 and 27.3 pA·s^{-1}, respectively. It indicates that the enzyme, the substrate of which is maltose, was present in the cells of the culture used for the formation of the receptor.

The provided by Wang and Hartman comparison of the properties of maltases previously reported showed that optimum pH for maltase varied 4.6 to 7.3 depending on the culture: bacteria, yeast or fungi.[25] If pH optimum for the enzyme from Escherichia coli was 6.9,[25] similar optimal pH, 6.5, was obtained for another Escherichia coli maltase.[26] Nevertheless, the maltase from the yeast Saccharomyces cerevisiae had pH optimum had pH optimum between 7.0 and 7.5,[17] but for enzyme of *Saccharomyces carlsbergensis* the pH optimum was found to be between pH 6.7 and 6.8.[27] The purified maltase of *Bacillus subtilis* was relatively stable at pH values of 5.5 to 6.6,[25] and pH stability range of partially purified enzyme from *Bacillus brevis* was from 5.0 to 7.0.[28] But 90% activity of *Bacillus liheniformis* maltase was obtained at pH 7.0.[3]
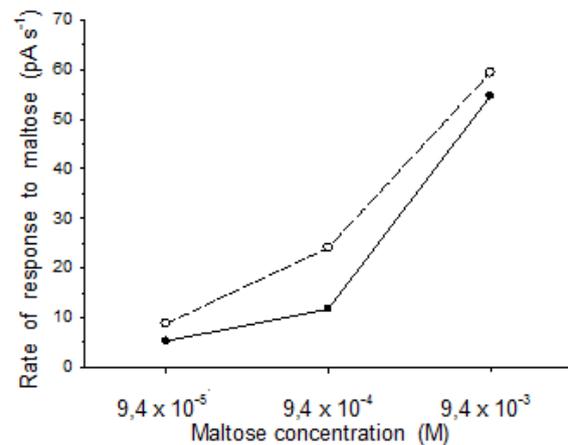
Figure 4. Rate of the B-4c/3 biosensor response to maltose at different pH of a K-Na phosphate buffer (pH 7.4, closed circles, and 7.7, open circles).

It should be noted that in this study pH optimum was determined not for the enzyme but for the cells able to metabolize maltase substrate (maltose). Therefore, the pH optima measured for B-4c/3 can be considered similar to the values reported for microbial maltases.

In study of Mfomber and Senwo various buffers have been used to assay maltase activity from microbes iso-

lated from soil and soil extracts; the authors pointed out that the phosphate buffers have been the most used.[2] In next study Tris buffer inhibited B-4c/3 cells response to maltose as Tris inhibited maltase from the yeast and other sources as reported by Guffanti and Corpe.[29] In contrast to greater Tris inhibition of the pseudomonas enzyme at alkaline than at acid pH,[29] inhibition of the B-4c/3 cells response to maltose by Tris was more pronounced at neutral than at alkaline pH.

Using distilled water as the measuring solution, results for three concentrations of maltose were greater than those obtained using buffer solution when both the receptor element, which constantly worked and new dry-stored receptor kept at +4°C, were applied. At low concentrations of maltose the differences between the responses in the buffer solution and water were insignificant but they increased when maltose concentration elevated. Similar dynamics was observed for both the already used and not yet employed (kept in a dry state in the refrigerator) receptor elements. Therefore, the cells of the culture-receptor were stable to hypotonic working solutions. This refers to the cells which were both adopted (receptor elements worked in the buffer solution) and non-adopted (not yet employed receptor elements stored in dried state in the refrigerator) to some tonicity.

3.4 Effect of ionic strength

Tests of the solutions with different ionic strength varied by NaCl were undertaken. The influence of ionic strength of the buffer solution on the biosensor response was investigated (Figure 5) in a 50 mM K-Na phosphate buffer, pH 7.4, at maltose concentration of 9.4×10^{-4} M. Change in ionic strength, ΔI, was equal to NaCl concentration in M, $\Delta I = m_{salt}$.[30] The increase in ionic strength of a 50 mM buffer by 100 mM was accompanied by the raise of the B-4c/3 biosensor response almost by 50%. However, further increase in sodium chloride content in the buffer solution led to a considerable decrease in the sensor sensitivity to maltose. Therefore, it is not desirable to use the biosensor for analysis of products that contain high NaCl concentration.

3.5 Stability of biosensor

Stability is an important characteristic for the biosensor system. For operational stability in a series of 10 samples of the maltose solution containing 9.4×10^{-4} M of maltose the response was reproducible within $\pm 7\%$. The long-time operational stability of the B-4c/3 biosensor was not tested.

For storage stability, biosensor responses were examined for one receptor element, which was stored and
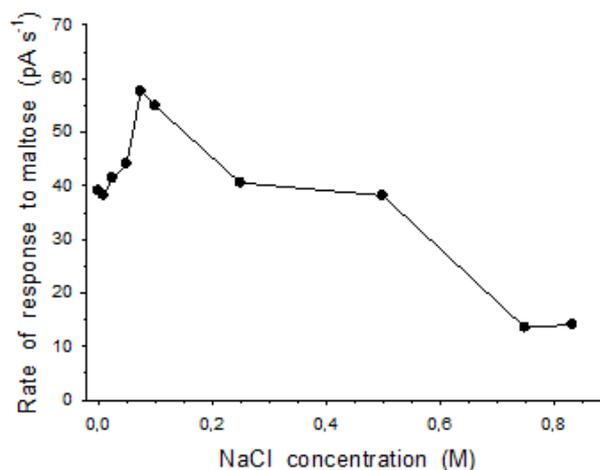
Figure 5. Influence of the ionic strength of buffer solution on the B-4c/3 biosensor response.

worked in a phosphate buffer at room temperature, and for the receptor element stored in air-dry state at +4 °C (new for each measurement). Measurements of samples of 9.4×10^{-5} M, 9.4×10^{-4} M and 9.4×10^{-3} M maltose solutions were performed. Figure 6 shows the results of measurements.

During the first 24 hours of the biosensor operation (for the receptor element stored in a phosphate buffer), the kinetic response to maltose increased significantly for high maltose concentrations. For those substrate concentrations, the opposite effect was then observed during the next 24-140 hours, but then up to 13 days of storage the measured responses were almost constant. It should be noted that for the samples solution containing 9.4×10^{-5} M of maltose, the response was practically stable for all 13 days. Therefore, during about half a month of storage, the biosensor gave stable response.

For the receptor element stored in air-dry state at +4 °C, the similar effects were registered for 27 days. But a decrease in the biosensor response was higher while measuring 9.4×10^{-3} M maltose.

3.6 Induction of B-4c/3 response to maltose

For the kinetically controlled biosensor with low "microbe loading", the sensitivity of the biosensor is mostly determined by "cell activity".[19] In the presence of the initiating enzyme both substrate metabolism and processes of substrate transport contribute to the formation of the biosensor response.

For the majority of maltase, data on which are given above, it is known, that these enzymes are inducible. The activity of maltase of strain SB15 of *Pseudomonas* was much higher in maltose medium than in glucose or galactose medium (inducible enzyme); although maltase ac-
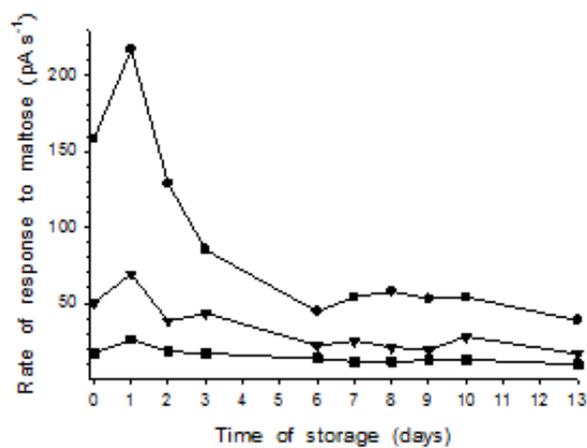
(a)
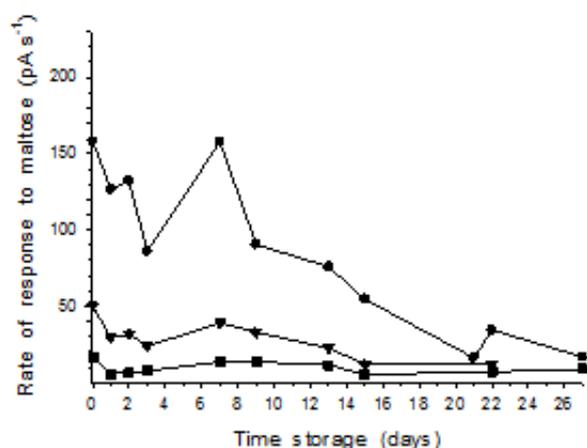
(b)

Figure 6. Stability of the maltose B-4c/3 biosensor: the receptor element stored in phosphate buffer at room temperature (a) and the receptor element stored in an air-dry state at +4°C, new for each measurement (b). Response to maltose concentration: 0.0094 M, closed circles; 0.00094 M, closed triangles; 0.000094 M, closed squares.

(a)

(b)

Figure 7. Influence of the medium for B-4c/3 growth on the B-4c/3 biosensor response (50 mM phosphate buffer, pH 7.4). (a) Growth of B-4c/3 bacterium on malt agar. (b) Growth of B-4c/3 bacterium on peptone-tryptone agar.

tivities of mutant strain MS1 (constitutive enzyme) were high in all media.[31]

Induction of enzymes, required for maltose detection, in the cells of the culture-receptor depended on the composition of the medium used for culture growth (Figure 7). The B-4c/3 bacterium was grown on malt (MA) or peptone-tryptone (PTA) agar. For the biosensor based on B-4c/3 culture grown on malt agar, the angle of the standard curve's slope (the interval of linear relationship) was greater. Within that interval the biosensor with B-4c/3 grown on PTA instead of MA showed only 25% of the sensitivity to maltose compared to MA-grown cells biosensor. The main components of malt extract are carbohydrates (up to 90% of dry weight), among which the

maltose content is the highest one. The higher response to maltose for B-4c/3 cells grown on MA than that for PTA-grown cells can be explained by maltose induction of the desirable enzymes.

The response to maltose was recorded for intact cells of B-4c/3 grown on PTA. The result obtained indicated that the constitutive enzymes initiated the response of intact cells to maltose. However, the response to maltose for immobilized PTA grown B-4c/3 cells (biosensor response) could be caused not only by a constitutive nature of the enzymes able to metabolize a substrate but also by initiation of maltose transport into the cells.

To enhance the sensitivity of the biosensor, an incubation approach was used. The enzymes of B-4c/3 bacterium were induced by cells incubation with maltose. The suspensions of B-4c/3 bacterium grown on malt or peptone-tryptone agar were incubated in nongrowing condition (in buffer solution) in the presence of maltose. The responses to maltose for incubated suspensions are

presented in Figure 8. Induction of MA-grown B-4c/3 cells by maltose led to a 2.3-fold increase in response of the bacterial suspension to 9.4×10^{-3} M maltose. At the same time, the effect was not so significant to lower maltose concentration, but negligible to 9.4×10^{-5} M maltose.
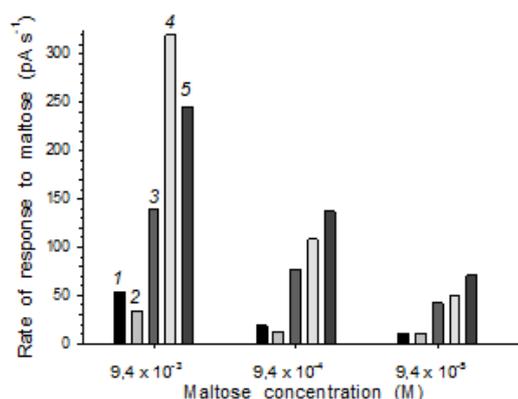
Figure 8. Effect of pre-conditioning of suspension of B-4c/3 cells on response of bacterial suspension to maltose: 1 is suspension (PTA) before induction; 2 is suspension (PTA) after induction; 3 is suspension (MA) before induction; 4 is suspension (MA) after 1-st induction; 5 is suspension (MA) after 2-nd induction.

The response of immobilized cells to maltose also depends on substrate transport into the cells. The maltose transport system is able to transport substrate efficiently even at very low external substrate concentrations. At 10^{-6} M concentrations, bacteria are able to maintain a ratio of internal to external concentrations of maltose of approximately 8×10^{4}, as reported by Shuman and Beckwith.[32] The chemoreceptor of the maltose transport system is the periplasmic maltose binding protein which is involved in active transport processes, which can occur against the concentration gradient.[33] Transporter-bound protein facilitates the acquisition of the sugar at low concentrations.[34] But when the maltose concentration exceeds the transport capacity, the protein captures maltose and dissociates from the transporter. Dissociation from the transporter limits the rate of transport.

In this study, the undertaken induction of B-4c/3 by maltose most likely led to the enhancement of protein affinity for maltose resulting in a decrease of transport rate at low concentrations of maltose.

In contrast to MA-grown cells, for PTA-grown B-4c/3 cells after induction by maltose not only an increase in the response was not recorded but even a decrease in response to maltose at all range of concentrations studied was seen. Similarly, it was shown that *Pseudomonas fluorescens* W cells grown on DL-alanine exhibited lower levels of maltase than those grown on maltose.[35]
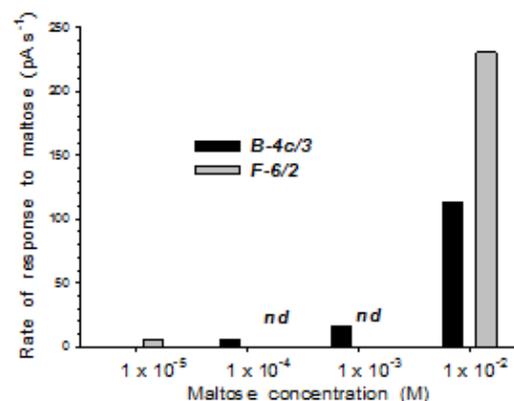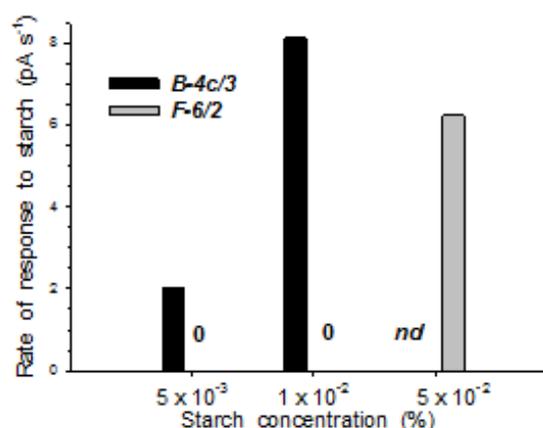
Figure 9. Responses of B-4c/3 (A) and F-6/2 (B) biosensors to maltose and starch. ***nd*** -- not determed.

3.7 Maltose determination in the presence of starch

The specificity of the biosensor is important for analyte detection. Maltose is mainly used as maltose syrup which is produced under enzymatic starch hydrolysis. Of the studied cultures two were selected: bacterial (B-4c/3) and fungal (F-6/2). Those cultures showed different sensitivities to maltose and starch (Figure 9). The biosensor based on F-6/2 fungus demonstrated high specificity to maltose with no response to starch at its concentration lower than $1 \times 10^{-2}\%$, but it was inapplicable for a long period of use. B-4c/3 biosensor was highly sensitive to maltose and also showed low sensitivity to starch. It was reported by Yoshigi, *et al.*[36] that *Bacillus cereus* NY-14 cells, probably, like bacteria B-4c/3, digested soluble starch to yield oligosaccharides which were then hydrolyzed to glucose by one of cell-bound α-glucosidases. Combination of the receptors on the basis of two cultures

(B-4c/3 and F-6/2) provided the possibility of detecting maltose in the presence of starch.

4   Conclusion

Thus, the biosensor based on a Clark-type oxygen electrode and B-4c/3 bacterium has allowed rapid quantification of maltose. Recording of the biosensor response began in 60-100 s after analyte injection and ended in 10-60 s. After washing the cell, the system can be used for the next testing in 6.5-11 min. For a linear interval of the standard curve the biosensor showed a stable response for 10 days when the bioreceptor was both in buffer solution at room temperature and kept in an air-dry state at +4°C. The biosensor demonstrated good sensitivity to maltose, 36.02 nA $(M·s)^{-1}$. Changes associated with metabolic rearrangements in the culture-receptor had an effect on the biosensor response. It indicates that the developed model is a tool of simple construction and easy-to-use for the study of maltose metabolism in the culture immobilized on electrode.

5   Conflict of Interest and Funding

The author declares no conflict of interest.

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