# MICROBIOLOGY AND IMMUNOLOGY

# Membrane-Bound Dehydrogenases of *Gluconobacter Oxydans*: Sensors for Measuring Sugars, Alcohols, and Polyoles

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The activity of membrane-bound dehydrogenases of immobilized *Gluconobacter oxydans* was used in ampero- and potentiometric biosensors for measuring glucose, ethanol, and glycerol. An amperometric biosensor was used for detection of glucose in human blood, glycerol in fermentation media, and ethanol vapors in the air. The results indicate that biosensors for practical use in medicine and biotechnology can be created on the basis of this cells culture.

**Key Words:** *bacterial biosensor; Gluconobacter oxydans; carbohydrates; ethanol; glycerol*

Development of accurate, rapid, and reliable methods for measuring organic compounds in various specimens is a pressing task of medicine, biotechnology, and ecology. Analytical potentialities of biosensors based on bacterial cells holds good promise [8].

*Gluconobacter,* a bacterium that rapidly oxidizes at least 80 organic compounds and accumulates the products of their incomplete oxidation in the medium [1,4], can be used as bacterial biosensors for detecting alcohols, carbohydrates, and polyoles. Aldoses, alcohols, and polyoles are oxidized by dehydrogenases localized in the plasma membrane of bacteria [5,9]. These enzymes can be effectively used in biosensors on the basis of *Gluconobacter* cells.

Here we describe bacterial sensors containing immobilized *G. oxydans* in the receptor element. Generation of analytical signal in sensors is due to activity of membrane-bound dehydrogenases (aldose dehydrogenase responsible for glucose and xylose sensitivity, alcohol dehydrogenase responsible for ethanol sensitivity, and glycerol dehydrogenase responsible for glycerol sensitivity). Sensitivity of the sensors and the dependence of signals on the conditions of measuring was investigated.

## MATERIALS AND METHODS

*G. oxydans* subsp. *industrius* cells from the Bank of Microorganisms (Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences) served as the basis of bioreceptor. The bacteria were maintained and cultured as described previously [2]. In order to form a biosensor, cells in a preset concentration (0.01 mg wet weight/mm$^2$ for potentiometric and 0.1 wet weight/mm$^2$ for amperometric detection) were immobilized by direct adsorption on the surface of potentiometric electrode, a pH-sensitive field transistor (Avangard, St. Petersburg), or on chromatographic paper (GF/A, Whatman) which was fixed on the surface of amperometric Clark's electrode (5313/10, Ingold). Potassium phosphate buffer (20 mM, pH 6.5) was the

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solution for amperometric detection and 15 mM NaCl, pH 7.0, for potentiometric detection. Substrates in required concentrations were added to the measuring cuvette.

## RESULTS

The relationship between bacterial sensor signals and substrate concentration (calibration curves) for amperometric registration of substrate oxidation is shown in Fig. 1. The sensor had high sensitivity to glucose and ethanol and relatively low sensitivity to glycerol and xylose. According to the calibration curve, ethanol and glucose could be measured at glycerol concentrations 0.05-5 mM and xylose concentrations 0.5-50 mM. The time of measurements was no more than 30-90 sec.

With pH-sensitive transistor as the recorder electrode, the sensitivity was virtually the same as that shown in Fig. 1: minimum detectable concentrations 0.03 and 0.5 mM for glucose and xylose, 0.1-0.2 mM for glycerol, and 0.01 mM for ethanol.

The accuracy of measurements was assessed using glucose as the substrate. Signals from amperometric sensor were stable and well reproducible during repeated addition of glucose (1 mM), the standard deviation being 0.01 nA/sec or 6.7% of the mean value of the signal equal to 0.15 nA/sec (10 measurements). Standard deviation of this order of magnitude (5-8%) characterized changes in glucose concentrations in the 0.1-10 mM range for models with both amperometric and potentiometric transistors.

Standard deviation in a series of 12 receptor elements prepared from the same lot of cells was 0.03 nA/sec (23% of the mean 0.13 nA/sec), i. e., the differences in the measured parameters were negligible.

Study of the relationship between the amplitude of sensor signals and surface concentration of cells on the electrode helped determine their optimal concentration: 0.01 mg wet weight/mm$^2$ for potentiometric and 0.1 mg/mm$^2$ for amperometric detection. A higher surface concentration of cells in amperometric detection is explained by a longer distance of cell layer from the surface of amperometric electrode than in potentiometric detection, and therefore the recording is less effective.

A drawback of biosensor detection with pH-sensitive field transistors is the dependence of the output signal on buffer concentration in measured solution [6,7]. We studied the effect of buffer concentration on sensor response with transistors and amperometric electrodes. As expected, the potentiometric sensor signals depended on the content of the buffer component and were 7 times lower if

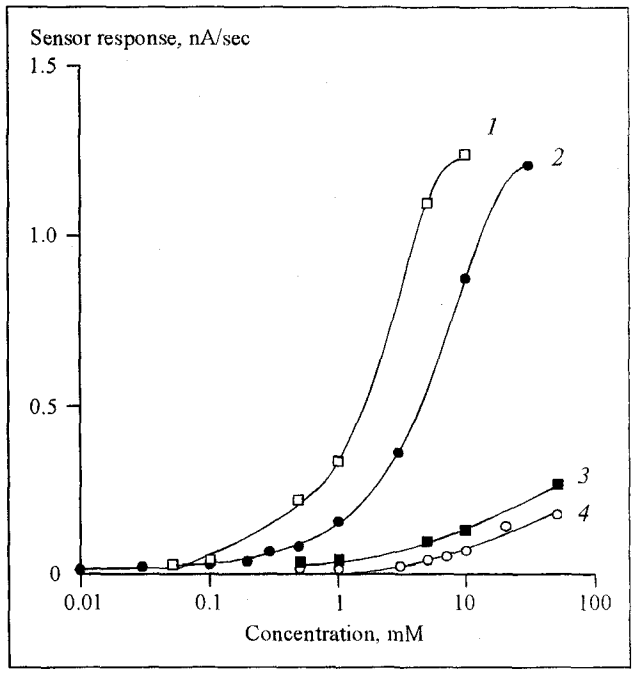


**Fig. 1.** Calibration curves of amperometric sensor for ethanol (*1*), glucose (*2*), glycerol (*3*), and xylose (*4*). Maximum rate of signal alteration during addition of a substance was measured.

phosphate buffer concentration was increased from 1 to 3 mM (detection of 1 mM glucose). Such a sensitivity of the potentiometric sensor to the concentration of buffer components in the medium deteriorates its practical value. In amperometric registration the relationship between signals and buffer capacity of the medium was nonmonotonous. Increase of buffer concentration from 1 to 20 mM enhanced the signals by 10%; further increase in buffer concentration to 250 mM slightly (by 20%) suppressed the responses, i. e., amperometric detection proved to be far less sensitive to the buffer component of the medium.

For estimating the effect of environmental conditions on sensor signals, we investigated their relationship with pH, temperature, and osmolarity. The pH optimum of sensor responses (in ampero- and potentiometric detection) was 5-6. The effects of temperature and osmolarity were studied for amperometric sensors. In the temperature range 20-50°C the maximum amplitude of responses was observed at 40°C. Ionic strength of solution was modified by NaCl content in the medium. Maximum signals (120% of signals in 5 mM NaCl) were observed at a concentration of 20 mM; at 500 mM the responses decreased by 25% in comparison with responses in 5 mM solution. The relationships between signals and pH, temperature, and salt concentration in measured solution indicate that the sensor parameters are stable.

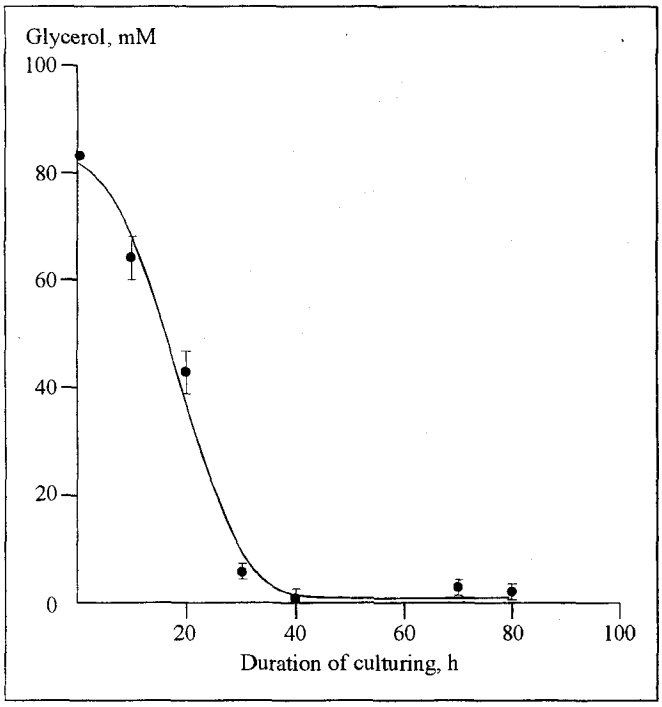


**Fig. 2.** Time course of glycerol concentration in fermentation medium during amperometric detection.

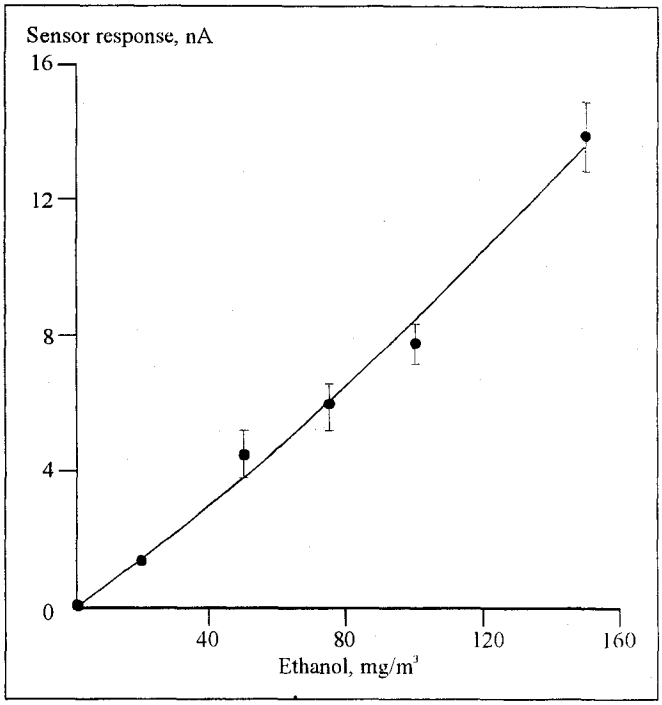


**Fig. 3.** Calibration curve for detection of ethanol vapors with a bacterial sensor.

Previously we assessed glucose concentration in the serum by an anthropometric bacterial sensor [3]. A random sample included specimens from diabetics and normal subjects. Results of analysis performed using an Eksan-G glucose analyzer and the bacterial sensor were in good correlation: $r$=0.97. Abnormal glucose concentrations can be detected in the serum with the use of the sensor.

Measurement of glycerol concentration is a difficult task in biotechnology. The activity of *G. oxydans* glycerol dehydrogenase was used for measuring glycerol concentration in fermentation medium during biotransformation of sitosterol into androstanedione by *Mycobacterium sp.;* the analyzed medium contained no components oxidized by *G. oxydans* except glycerol. The kinetics of glycerol consumption by cells is shown in Fig. 2. The heterophasic composition of fermentation medium requires pretreatment of samples for traditional glycerol analysis and, therefore, biosensor detection of glycerol is convenient and simple.

Alcohol dehydrogenase activity of *G. oxydans* permitted rapid analysis of ethanol in liquid and gaseous mixtures. The relationship between signals and content of ethanol vapor in the air (Fig. 3) shows that the sensor sensitivity threshold is 20 mg/m$^3$ and time of sensor response no more than 2-5 min.

Our models demonstrate the possibility and efficacy of using the dehydrogenase activity of *G. oxydans* in ampero- and potentiometric sensors. Amperometric sensors were highly sensitive and were successfully used to determine the concentration of glucose in human serum, of glycerol in fermentation media containing no other substrates oxidized by this bacterium, and of water phase ethanol and its vapors in the air. Therefore, these cells can be used for designing biosensors for medicine and biotechnology.

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