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SHORT COMMUNICATIONS

Strain *Alcaligenes xylosoxydans* subsp. *denitrificans* TD2 as the Basis of a Biosensor for Determination of Thiodiglycol

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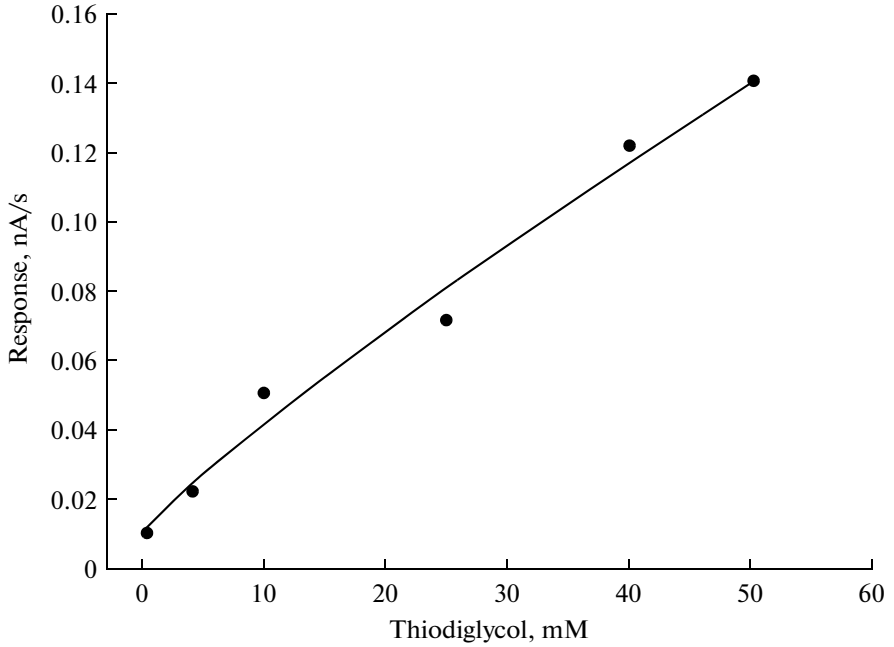
Thiodiglycol (β,β-dioxydiethylsulfide, TDG) is the hydrolysis product of mustard gas (yperite), a chemical warfare agent with a blistering effect [1]. TDG biodegradation is carried out by the representatives of several taxonomic groups of microorganisms, mainly representatives of the genera *Alcaligenes* and *Pseudomonas* [2–4]. Thiodiglycolic acid and bis(2-hydroxyethyl)sulfoxide were shown to be the products of TDG degradation by the strain *Alcaligenes xylosoxydans* subsp. *denitrificans* TD2 [5]. The latter compound was formed when TDG was oxidized by molecular oxygen [2]. Capacity of the microorganisms to oxidize low-molecular organic compounds with the consumption of molecular oxygen can be used for analytical purposes.

The aim of the present work was to develop a biosensor model for determination of thiodiglycol using the immobilized cells of *Alcaligenes xylosoxydans* subsp. *denitrificans* TD2 as a receptor and the Clark-type oxygen electrode as a converter.

The strain *A. xylosoxydans* subsp. *denitrificans* TD2 was obtained by selection of the strain *A. xylosoxydans* subsp. *denitrificans* TD1 isolated from the soil contaminated with mustard gas reaction masses. Strain TD2 had better growth characteristics: a higher specific growth rate and a short lag phase. The bacteria were grown in flasks on a shaker in liquid mineral



Calibration curve for the sensor using immobilized cells of *A. xylosoxydans* subsp. *denitrificans* TD2 depending on TDG concentration.

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medium with TDG [5]. At the end of the exponential growth phase, the biomass was separated by centrifugation and washed off with 30 mM potassium phosphate buffer (pH 7.8). The cells were immobilized using the method of physical adsorption on Whatman GF/A chromatographic glass paper (1.0–1.5 mg of raw biomass/receptor, 3 mm^2). The bioreceptor was attached to the measuring surface of the oxygen electrode. The measurements were made in the buffer (pH 7.8) at 20–22°C in the open-type cell. The maximal rate of a change in the output signal (dI/dt)nA/s, which is proportional to the rate of a change in the concentration of oxygen consumed was the parameter recorded (the sensor response). The figure shows the calibration curve of the sensor response depending on TDG concentrations. The TDG detection range was 0.5 mM. The immobilized cells responded to TDG, thioglycolic acid, glutamic acid, and ethanol. The maximal sensor response was observed at pH 7.8. Duration of the analysis was 15 min; the operational stability was 72 h. Thus, a biosensor of the amperometric type was developed for TDG determination on the basis of immobilized cells of *A. xylosoxydans* subsp. *denitrificans* TD2.

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