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EXPERIMENTAL ARTICLES

Thiodiglycol Metabolism in *Alcaligenes xylosoxydans* subsp. *denitrificans*

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Abstract—The investigation of the degradation of thiodiglycol (the major product of mustard gas hydrolysis) by *Alcaligenes xylosoxydans* subsp. *denitrificans* strain TD2 showed that thiodiglycol is metabolized through the oxidation of its primary alcohol groups and the subsequent cleavage of C–S bonds in the intermediate products, thiodiglycolic and thioglycolic acids. The end products of these reactions are SO_4^{2-} ions and acetate, the latter being involved in the central metabolism of strain TD2. The oxidation of the sulfur atom gives rise to diglycolsulfoxide, which is recalcitrant to further microbial degradation. Based on the data obtained, a metabolic pathway of thiodiglycol transformation by *A. xylosoxydans* subsp. *denitrificans* strain TD2 is proposed.

Key words: mustard gas, thiodiglycol, metabolism, transformation, intermediate oxidation products, *Alcaligenes xylosoxydans.*

Thiodiglycol (TDG) is a persistent water-soluble compound, which is the major product of mustard gas hydrolysis. In spite of the fact that it is not so toxic as mustard gas, TDG is to be destroyed along with chemical warfare agents, as specified by the Chemical Weapons Convention. TDG can conveniently be mineralized by biotechnological methods, which provide for the high ecological safety of detoxication processes. Such methods can also be used for bioremediation of soils contaminated with the detoxification products of chemical warfare agents.

Zhang *et al.* [1] and Dave *et al.* [2] devised biocatalytic methods for the detoxication of organophosphorus chemical warfare agents through the cleavage of P–O and P–F bonds by organophosphorus hydrolases. Other researchers search for microorganisms that have high activities of C–P lyase, the enzyme that cleaves C–P bonds in the molecules of methylphosphonic acid and its derivatives, the products of the enzymatic hydrolysis of the neurotoxic chemical warfare agents sarin and soman [3, 4].

TDG can be mineralized by the microorganisms that are able to break the C–S bond. There are microorganisms, such as *Rhodococcus rhodochrous*, *Gordona aichiensis*, and *Brevibacterium* sp., which cleave this bond in the molecule of dibenzothiophene and utilize this compound as the sulfur source [5–7].

It was found that TDG can be used by *Alcaligenes xylosoxydans* subsp. *denitrificans* strain TD2 [8], *Alcaligenes xylosoxydans* subsp. *xylosoxydans* strains SH42 and SH91 [9], and *Pseudomonas* sp. 8-2 [10] as the source of carbon and by *Rhodococcus rhodochrous* IGTS8 (ATCC 53968) as the source of sulfur in a medium without this element [11]. The *Alcaligenes xylosoxydans* subsp. *xylosoxydans* utilizes TDG with the formation of diglycolsulfoxide (DGSO), 2-hydroxyethylthioglycolic acid, and thiodiglycolic acid (TDGA) [12]. *Pseudomonas* sp. utilizes TDG, forming only these two acids and no DGSO. *Rhodococcus rhodochrous* utilizes 2-chloroethyl ethyl sulfide (a chlorinated analogue of TDG) as the sulfur source with the formation of 2-chloroethanesulfinic acid. All these sulfur compounds contain a C–S bond, which must be cleaved for these compounds to be used as the sources of carbon or sulfur.

The aim of the present work was to study the mechanism of TDG oxidation in the *Alcaligenes xylosoxydans* subsp. *denitrificans* strain TD2.

MATERIALS AND METHODS

Alcaligenes xylosoxydans subsp. *denitrificans* strain TD2 was obtained by means of a long-term selection of strain TD1, which was isolated earlier from soil contaminated with products of mustard gas detoxication. Strain TD2 was cultivated in a mineral medium [8] in shaken flasks at an aeration rate of 0.5 g O_2/(l h) at pH 7.0–7.5 (the pH of the medium mas maintained at this level by adding NaOH) or at pH 5.0–7.5 (the pH of the medium was not controlled). Alternatively, the strain was grown in a fermentor at 30% oxygen saturation at

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pH 7.5. Cells for inoculation were grown on an agar mineral medium containing 2 g/l TDG.

Bacterial growth in liquid media was monitored by measuring the culture turbidity at 560 nm in a Specol spectrophotometer. The culture turbidity (or optical density, OD) was converted to the dry biomass (DB) weight using an experimentally obtained coefficient of 0.5 g DB/OD unit.

Cell respiration was measured using an LP-7 polarograph (Czech Republic) equipped with a Clark-type oxygen electrode. Cells for these measurements were grown in a fermentor to the exponential growth phase, harvested by centrifugation, washed, and suspended in 50 mM phosphate buffer (pH 7.3).

Strain TD2 was grown using TDG, TDGA, DGSO, thioglycolic acid (TGA), and sulfoacetic acid as the carbon sources. DGSO and TDGA were synthesized as described earlier [8]. TGA was purchased from Reakhim (Russia). The disodium salt of sulfoacetic acid was synthesized as follows: A solution containing 13.9 g anhydrous Na_2SO_3 in 50 ml water was added dropwise at 50°C to a solution of sodium monochloroacetate (this was obtained by mixing 9.5 g monochloroacetic acid with a solution containing 5.5 g sodium carbonate in 36 ml water). The mixture was heated to 60–70°C and stirred for 2 h, after which it was completely evaporated in a porcelain cup. The residue was purified by twofold crystallization from water solutions. The product yield was 1.7 g. The NMR spectrum of the product recorded at 80 MHz in D_2O had one singlet peak at 4.06 ppm. The chemical analysis of the product showed the presence of 12.93% C and 0.98% H. The calculated content of C and H was found to be 13.05 and 1.09%, respectively.

TDG and the intermediate products of its oxidation were quantitatively analyzed by high-performance liquid chromatography (HPLC) and mass spectrometry. HPLC was carried out using an LKB-2150 chromatograph equipped with an LKB-2151 UV detector. Chromatographic peaks were identified by comparing their elution times with those of the respective authentic samples (the elution times of TDG, TDGA, DGSO, and TGA were 2.24, 1.54, 1.26, and 1.59 min, respectively). Gas chromatography–mass spectrometry was carried out using an HP-5793 mass spectrometer combined with an HP-6890 gas chromatograph equipped with a 50-m capillary column with ULTRA-2 phase. The temperature of the column was raised from 40 to 250°C at a rate of 10°C per min.

The concentration of SO_4^{2-} ions was determined gravimetrically [13].

RESULTS

The pathways of TDG oxidation in *Alcaligenes xylosoxydans* subsp. *denitrificans* strain TD2 was investigated through (1) the analysis of intermediate products formed from TDG by cells growing under different cultivation conditions (pH, pO_2, and substrate concentration) and by washed intact cells; (2) the cultivation of cells on the intermediates of TDG oxidation (TDGA, TGA, DGSO, acetate, and sulfoacetic acid) as the sole sources of carbon; and (3) the measurement of the respiratory activity of cells oxidizing TDG and its intermediates.

When strain TD2 was grown on TDG as the sole source of carbon, the culture liquid was found to contain two compounds, DGSO and TDGA. These compounds were not detected in the mineral medium incubated without cells or with inactivated cells. With the increasing initial concentration of TDG, the amount of detected DGSO and TDGA, as well as the SO_4^{2-} ions produced from TDG, increased. In this case, the SO_4^{2-} ions comprised 80–90% of the sulfur present in the TDG consumed [8].

The accumulation of DGSO in the medium began in the exponential growth phase of the culture (Fig. 1a). The concentration of DGSO in the medium increased as the concentration of TDG decreased and remained at a constant level in the stationary growth phase, when TDG was exhausted in the medium. A similar dynamics of DGSO was observed during the cultivation of strain TD2 in the fermentor at the controlled values of pH and aeration (Fig. 1c) and in the medium without pH maintenance (Fig. 1b).

The accumulation pattern of TDGA in the culture liquid was mainly determined by cultivation conditions. When the pH of the culture was maintained at the level of 7.0–7.5, TDGA began accumulating in the exponential growth phase, reached a maximum in the retardation phase, and was exhausted by the onset of the stationary phase (Fig. 1a). In the case of uncontrolled pH, its decrease to unfavorable values of 5.0–5.5 resulted in the absence of TDGA consumption (Fig. 1b). During cultivation in the fermentor at optimal values of pH and pO_2, the culture liquid contained TDGA in trace amounts (Fig. 1c).

When the initial concentration of TDG in the medium was high (39.3 g/l), this compound was gradually oxidized to TDGA by inoculated cells without their noticeable growth. However, as the TDG concentration in the medium decreased to 25 g/l (in this case, the concentration of the TDGA produced reached 6.6 g/l), the cells began to grow with the specific growth rate in the exponential phase equal to 0.004 h^{-1}. It should be noted that the specific growth rate was several times higher (0.015 h^{-1}) when the initial concentration of TDG in the medium was 26.4 g/l. Culture growth and TDGA accumulation continued until TDG was depleted in the medium. In this case, the rate of TDGA accumulation decreased from 0.045 g/(l h) in the lag phase to 0.01 g/(l h) in the phase of active growth. DGSO accumulated in the culture liquid in detectable amounts (0.3 g/l) only in the phase of active growth (Fig. 2).

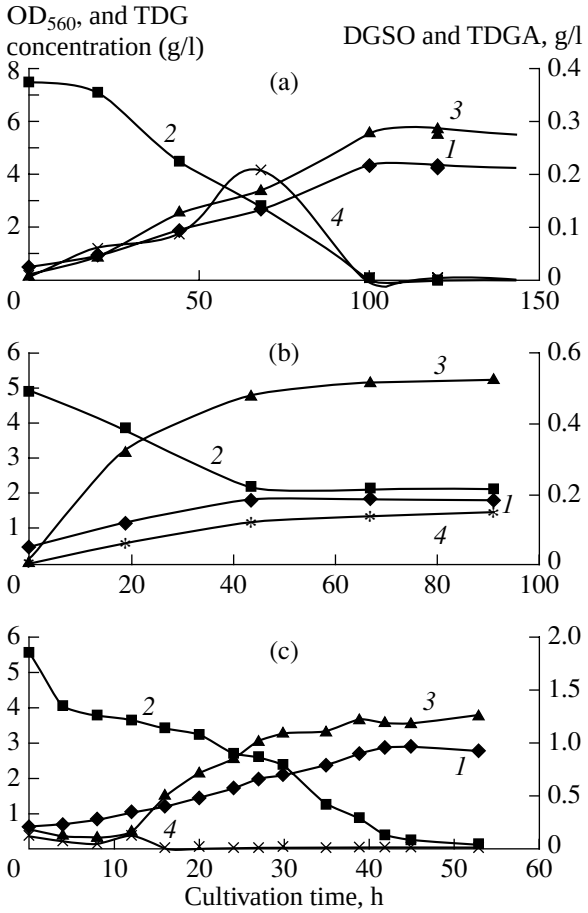


Fig. 1. (*1*) Growth of *A. xylosoxydans* strain TD2 and the accumulation of (*3*) DGSO and (*4*) TDGA in a medium with TDG (b) at pH varying from 7.5 to 5.5 in the flask experiments, (a) at pH controlled at a level of 7.0–7.5 in the flask experiments, and (c) at pH maintained at a level of 7.5 in the fermentor experiments. Curves *2* show the TDG decline in the cultivation medium.

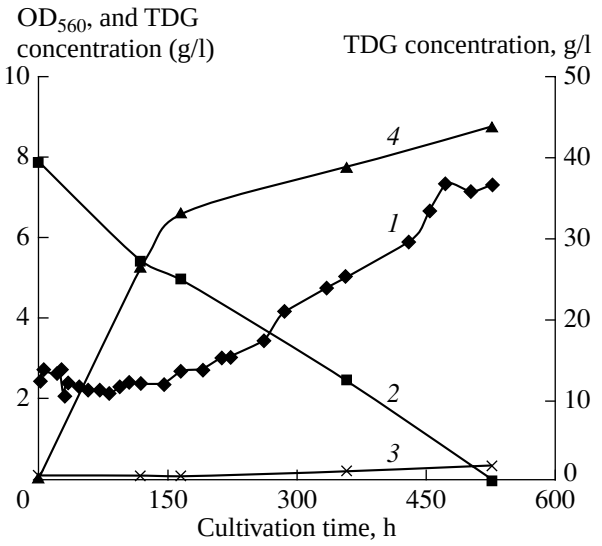


Fig. 2. Dynamics of growth of *A. xylosoxydans* strain TD2 and the accumulation of intermediate products of TDG oxidation in the medium with a high initial concentration of TDG. Curve designations as in Fig. 1.

Experiments showed that DGSO could not be used as the source of carbon by strain TD2 and did not inhibit its growth when added to the medium together with TDG. In experiments with washed TDG-grown cells, they neither oxidized DGSO, nor accumulated it during the oxidation of TDG. Sulfoacetic acid, the possible product of TDG oxidation containing an oxidized sulfur atom, was not utilized by growing cells. Nor was it oxidized by washed cells (Table 1).

Unlike DGSO, TDGA could be used by strain TD2 as the carbon source for growth, the growth parameters being dependent on the initial concentration of TDGA in the medium. At optimal values of pH and aeration, strain TD2 began to grow in the medium with 0.8 g/l TDGA at a specific growth rate of 0.008 h^{-1} after an extended 72-h lag phase. TDGA at an initial concentration of 2.0 g/l completely inhibited the growth of the strain. In the medium with TDG, the addition of TDGA at a concentration of 0.2 g/l did not influence the growth of the strain, but extended the lag phase to 48 h and decreased μ_{max} to 0.018 h^{-1} when added at a concentration of 2.0 g/l. For comparison, in the medium with 3.0 g/l TDG without added TDGA, the lag phase was as short as 4 h and the specific growth rate was 0.04 h^{-1}. TDGA was readily oxidized by washed cells, which exhibited a maximum respiratory rate on this substrate equal to 172 natom O_2/(mg cells min) at an oxygen concentration of 10 mM (Table 1).

The gas chromatography–mass spectroscopy of the intermediates of TDG oxidation by washed cells using untreated samples and those methylated with diazomethane revealed the presence of TGA, thiodiglycolic aldehyde, DGSO, the dimethyl ester of TDGA, and the methyl ester of acetic acid (Table 2).

The oxidation of TDGA by washed cells was accompanied by the formation of TGA, acetate, and SO_4^{2-} ions. In this case, TDGA was oxidized almost completely, so that the content of sulfur in the sulfate ion produced and in the TDGA oxidized was equal to 6.14 and 6.27 mM, respectively.

TGA was oxidized by strain TD2 at a rate comparable with that of TDGA oxidation. Acetate was readily metabolized by both growing and washed nongrowing cells (Table 1).

DISCUSSION

The data presented in this paper show that strain TD2 can transform TDG through the oxidation of its sulfur atom with the formation of DGSO. At the same

Table 1. Growth parameters and respiratory activity of *A. xylosoxydans* TD2 cells utilizing TDG and the possible products of its oxidation as the carbon sources

Substrate	Growth parameters				Respiration	
	substrate concentration		μ_{max}, h^{-1}	lag phase, h^{-1}	substrate concentration, mM	rate, natom O_2/(mg cells min)
	g/l	mM				
Thiodiglycol	3.0	24.0	0.04	4	40.0–160.0	350–450
Diglycolsulfoxide	0.5–1.5	3.6–10.8	No growth		0.7–3.6	0
Thiodiglycol	3.0	24.0				
			0.04	4	–	–
+ diglycolsulfoxide	0.5–1.0	3.6–7.2				
Thiodiglycolic acid	0.8	5.3	0.008	72	3.3–10.0	120–172
	2.0	13.2	No growth		16.0	100% inhibition
Thiodiglycol	3.0	24.0				
					–	–
+ thiodiglycolic acid	0.2	1.32	0.04	4		
	2.0	13.2	0.018	48		
Thioglycolic acid	0.5–1.7	5.5–18.6	No growth		1.1–11.0	340–240
					30.0	100% inhibition
Acetic acid	1.0	17.0	0.08	2	10.0–34.0	150–200
Sulfoacetic acid	0.5–1.0	2.7–5.4	No growth		2.7	0

Note: The symbol "–" stands for "not determined."

Table 2. Analysis of the intermediate products of TDG oxidation by *A. xylosoxydans* TD2 cells by combined gas chromatography–mass spectrometry

Sample	Retention time, min	Molecular ion M^+, *m*/*z*	Fragment peaks, *m*/*z*	Metabolite
1	0.51	92	74 ($M^+ - H_2O$), 47 (M^+ – COOH), 46 (M^+–H_2O–CO)	HS–CH_2–COOH
2	12.03	118	89 (M^+ – CHO), 74 ($M^+ - CH_2$=CH–OH)	$S(CH_2CHO)_2$
			60 (M^+ – CHO–CHO), 46 ($M^+ - CH_2$=CH–OH–CO),	
			45 ($M^+ - CH_2$=CH–OH–CHO)	
3	19.3	138	94 ($M^+ - C_2H_4O$), 76 ($M^+ - C_2H_4O - H_2O$)	$O{=}S(CH_2{-}CH_2OH)_2$
			63 ($M^+ - C_2H_4O - CH_2OH$), 48 ($M^+ - C_2H_4O - H_2O - C_2H_4$)	
			46 ($M^+ - C_2H_4O - CH_2OH$–OH)	
4	16.47	178	146 ($M^+ - CH_3OH$), 132 ($M^+ - CH_2S$), 131 (M^+–CH_2S–H),	$S(CH_2{-}COOCH_3)_2$
			119 (M^+ – $COOCH_3$), 118 ($M^+ - CH_3OH$–CO)	
			104 ($M^+ - CH_2C(OH)OCH_3$), 87 ($M^+ - CH_3OH - COOCH_3$)	
5	4.81	74	59 ($M^+ - CH_3$)	CH_3–$COOCH_3$

time, this strain can metabolize TDG through the oxidation of its primary alcohol groups and the subsequent cleavage of C–S bonds in the intermediate products.

The cleavage of the rather strong C–S bond (its energy is equal to 64 kcal/mol [14]) is the key reaction of the microbial degradation of sulfur-containing compounds, since the resultant products of this reaction can be assimilated by microorganisms via central or peripheral metabolism.

The cleavage of the C–S bond is well studied for the polycyclic aromatic compound dibenzothiophene. The initial stage of microbial attack at the dibenzothiophene molecule is the oxidation of its sulfur atom by dibenzothiophene oxygenase with the successive formation of sulfoxide, sulfone, and 2-hydroxybiphenyl-2-sulfinate. The cleavage of the C–S bond by 2-hydroxybiphenyl-2-sulfinate lyase is accompanied by the elimination of the sulfur atom in the form of a sulfate ion and by the

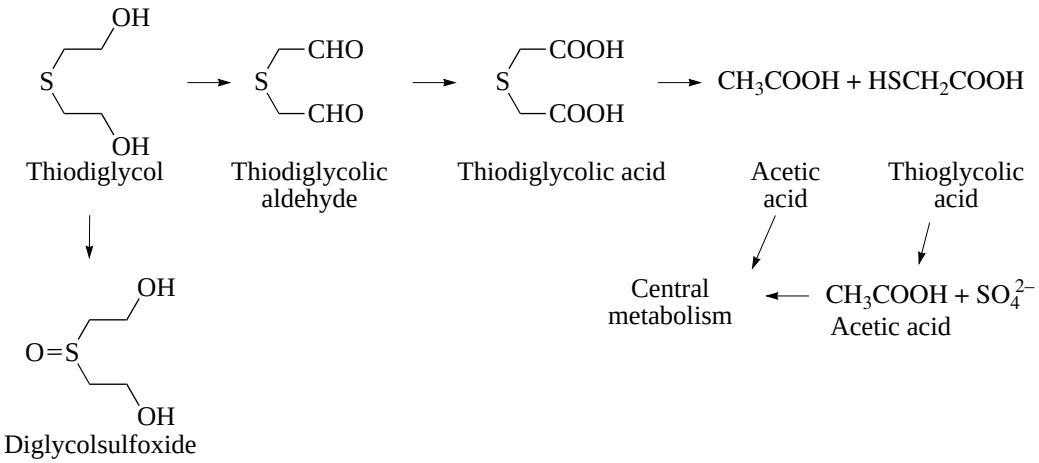
formation of 2-hydroxybiphenyl in the case of *Rh. rhodochrous* and *G. aichiensis* [6, 15] or benzoate in the case of *Brevibacterium* sp. [7]. The enzymatic complexes of *Alcaligenes* sp. [16] and *Pseudomonas putida* [17], which include dioxygenases with a broad substrate specificity, cleave the C–S bonds of aromatic sulfonates.

Taking into account these data, we can suggest that the oxidation of TDG to DGSO in the *Alcaligenes xylosoxydans* subsp. *denitrificans* strain TD2 is catalyzed by an oxygenase with a broad substrate specificity. This enzyme is probably inactive toward DGSO and sulfoacetic acid, which contain more oxidized sulfur atoms. The transformation of TDG to DGSO in both *Alcaligenes xylosoxydans* subsp. *xylosoxydans* strain SH91 [12, 18] and *Alcaligenes xylosoxydans* subsp. *denitrificans* strain TD2 is a deadlock in their metabolism. Such processes take place during the biodegradation of xenobiotics and occur with the involvement of enzymes with a broad substrate specificity.

The data obtained show that the formation and utilization of TDGA is strongly regulated by cultivation conditions (pH and pO_2 values and the initial TDG concentration). Under unfavorable cultivation conditions (low pH values and high initial TDG concentrations), TDGA is accumulated in the medium. However, under conditions optimal for growth, the concentration of TDGA in the medium is very low. The use of TDGA for constructive metabolism can explain the 4.5-fold slower accumulation of this acid during the active growth of strain TD2 as compared with its accumulation in the lag phase in media with high initial concentrations of TDG (Fig. 2). This implies that it is the subsequent conversion of TDGA that is the key reaction of TDG metabolism.

The oxidation of TDG to TDGA may involve an alcohol dehydrogenase, such as the NAD-dependent butanol dehydrogenase catalyzing the successive formation of 2-hydroethylthioglycolic acid and TDGA from TDG [12]. The sulfur atom of TDGA is covalently bound to two carbon atoms, as in the methionine molecule, whose C–S bond can be cleaved by methionine gamma-lyase with the formation of 2-ketobutyrate and methanethiol [19]. The detection of TDA, acetate, and sulfate ions as the intermediate products of TDGA oxidation by washed *A. xylosoxydans* TD2 cells allows the suggestion to be made that a similar mechanism is functioning in these cells.

The data presented in this paper allow the following pathway of TDG metabolism in the *Alcaligenes xylosoxydans* subsp. *denitrificans* strain TD2 to be proposed.



Thus, the primary reactions of TDG metabolism are the oxidation of TDG to TDGA and the subsequent cleavage of the C–S bonds of TDGA and TGA with the formation of acetate, which is involved in central metabolism, providing cells with carbon and energy sources. This inference is supported by the fact that sulfate ions are formed from TDG in equimolar amounts.

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REFERENCES

1. Zhang, Y., Autenrieth, R.L., Bonner, J.S., Harvey, S.P., and Wild, J.R., Biodegradation of Neutralized Sarin, *Biotechnol. Bioeng.*, 1999, vol. 64, no. 2, pp. 221–231.
2. Dave, K.I., Miller, C.E., and Wild, J.R., Characterization of Organophosphorus Hydrolases and the Genetic Manipulation of the Phosphotriesterase from *Pseudomo-*

nas diminuta, *Chem.-Biol. Interact.*, 1993, vol. 87, pp. 55–68.

3. DeFrank, J.J., Guelta, M.A., Harvey, S.P., Fry, I.J., Earley, J.P., and Lupton, F.S., Biodegradation of Hydrolyzed Chemical Warfare Agents by Bacterial Consortia, *NATO Advanced Study Institute: Enzymes in Heteroatom Chemistry*, The Netherlands, 1999, p. 29.
4. Yakovleva, G.M., Kim, S.K., and Wanner, B.L., Phosphate-Independent Expression of the Carbon–Phosphorus Lyase Activity of *Escherichia coli*, *Appl. Microbiol. Biotechnol.*, 1998, vol. 49, no. 5, pp. 573–578.
5. Kayser, K.J., Bielaga-Jones, B.A., Jackowski, K., Odusan, O., and Kilbane, J.J., Utilization of Organosulphur Compounds by Axenic and Mixed Cultures of *Rhodococcus rhodochrous* IGTS8, *J. Gen. Microbiol.*, 1993, vol. 139, pp. 3123–3129.
6. Finkel'shtein, Z.I., Baskunov, B.P., Golovlev, E.L., and Golovleva, L.A., Desulfurization of 4,6-Dimethyldibenzothiophene and Dibenzothiophene by *Gordona aichiensis* 51, *Mikrobiologiya*, 1999, vol. 68, no. 2, pp. 187–190.
7. van Afferden, M., Schacht, S., Klein, J., and Trüper, H.G., Degradation of Dibenzothiophene by *Brevibacterium* sp. DO, *Arch. Microbiol.*, 1990, vol. 153, pp. 324–328.
8. Tikhonova, E.B., Ermakova, I.T., Slepen'kin, A.V., Kashparov, K.I., Starovoitov, I.I., and Boronin, A.M., Bioutilization of Thiodiglycol (a Product of Mustard Gas Detoxification): Isolation of Degrading Strains and Investigation of Biodegradation Conditions, *Mikrobiologiya*, 2002, vol. 71, no. 2.
9. Sines, B.J., Teather, E.W., Harvey, S.P., and Weigand, W.A., Investigation of Biological Reactor Designs for Treatment of Methanol and Thiodiglycol Waste Streams, *Appl. Biochem. Biotechnol.*, 1994, vol. 45/46, pp. 881–895.
10. Medvedeva, N.G., Zaitseva, T.B., Polyak, Yu.M., and Gridneva, Yu.A., Utilization of Thiodiglycol by a *Pseudomonas* Culture, *Biotekhnologiya*, 1998, no. 2, pp. 89–92.
11. Kilbane, J.J. and Jakowski, K., Biocatalytic Detoxification of 2-Chloroethyl Ethyl Sulfide, *J. Chem. Technol. Biotechnol.*, 1996, vol. 65, pp. 370–374.
12. Lee, T.-S., Weigand, W.A., and Bentley, W.E., Observations of Metabolite Formation and Variable Yield in Thiodiglycol Biodegradation Process: Impact on Reactor Design, *Appl. Biochem. Biotechnol.*, 1997, vol.s 63–65, pp. 743–757.
13. Lur'e, Yu.Yu. and Rybnikova, A.I., *Khimicheskii analiz proizvodstvennykh stochnykh vod* (Chemical Analysis of Industrial Waste Waters), Moscow: Khimiya, 1974, pp. 83–84.
14. March, J., *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, McGraw-Hill Series in Advanced Chemistry, New York: McGraw-Hill, 1968. Translated under the title *Organicheskaya Khimiya*, Moscow: Mir, 1987, p. 41.
15. Grey, K., Dibenzothiophene Desulfurization Map, *The University of Minnesota Biocatalysis/Biodegradation Database: Microorganisms, Genomics, and Prediction*, 2000. (http://www.labmed.umn.edu/umbbd/dbt/dbt_map.html).
16. Thurnheer, T., Zurrer, D., Hoglinger, O., Leisinger, T., and Cook, A.M., Initial Steps in the Degradation of Benzene Sulfonic Acid, 4-Toluene Sulfonic Acids, and Orthanilic Acid in *Alcaligenes* sp. Strain O-1, *Biodegradation*, 1990, vol. 1, pp. 55–64.
17. Balashov, S.V., Arinbasarov, M.U., Adanin, V.M., and Boronin, A.M., Degradation of Benzenesulfonic and *p*-Toluenesulfonic Acids by *Pseudomonas putida* BS1331, *Prikl. Biokhim. Mikrobiol.*, 1998, vol. 32, no. 2, pp. 142–147.
18. Pham, M.-Q.K., Harvey, S.P., Weigand, W.A., and Bentley, W.E., Reactor Comparisons for the Biodegradation of Thiodiglycol, a Product of Mustard Gas Hydrolysis, *Appl. Biochem. Biotechnol.*, 1996, vol. 57/58, pp. 779–789.
19. McTavish, H. and Sun, Z., Methionine and Threonine Pathway Map, *The University of Minnesota Biocatalysis/Biodegradation Database: Microorganisms, Genomics, and Prediction*, 2000. (http://www.labmed.umn.edu/umbbd/met/met_map.html).