ISSN 0026-2617, Microbiology, 2014, Vol. 83, No. 5, pp. 577–584. © Pleiades Publishing, Ltd., 2014.

EXPERIMENTAL ARTICLES

Application of *Bacillus* sp. Strain VT-8 for Decontamination of TNT-polluted Sites

I. P. Solyanikova[a], I. V. Robota[a, b], D. M. Mazur[c], A. T. Lebedev[c], and L. A. Golovleva[a, b, 1]

[a] *Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia*

[b] *Pushchino State Institute of Natural Sciences, Pushchino, Russia*

[c] *Faculty of Chemistry, Lomonosov Moscow State University, Moscow, Russia*

Received January 21, 2014

Abstract— Bacterial strain *Bacillus* sp. VT-8 was shown to use trinitrotoluene (TNT) as the sole source of carbon, nitrogen, and energy, as well as to carry out its co-oxidation. Resistance of *Bacillus* sp. VT-8 to high TNT concentrations was shown. Efficient detoxification of TNT-contaminated soil and water samples was demonstrated. This strain may be recommended for TNT biodegradation at concentrations of up to 140 mg/L due to its high degradation activity and the absence of toxic effect of TNT on *Bacillus* sp. VT-8.

Keywords: TNT, degradation, *Bacillus* sp. VT-8, optimization of the detoxification process

DOI: 10.1134/S0026261714050257

The degradation potential of bacilli long remained ambiguous, and these bacteria were not considered promising for commercial applications in the biological treatment of polluted soils and wastewaters. Some authors reported the ability of members of this genus to utilize linear and polycyclic hydrocarbons (PAH) (at high temperatures) [1, 2] and cresol [3] as growth substrates and to transform halophenols [4], which was evidence of the high biodegradative potential of bacilli. The studies of recent years have shown that members of the genus *Bacillus* may be a source of biotechnologically valuable metabolites and enzymes [5] and are able to degrade various toxic and stable compounds, including a wide range of nitroaromatic compounds such as 3-, 4-nitrobenzoate, 2-nitrotoluene, nitrobenzene [6], chloronitrobenzene [7], 2,4-dinitrotoluene [8], 2,4,6-trinitrophenol [9], 4-chloro-2-nitrophenol [10], and 2,4-dinitroanisol, which was proposed to be used as an explosive instead of TNT [11]. TNT was extensively used for military and industrial purposes, and the peak of its production fell on the years of World War II. According to the published data, the annual TNT production was up to 1000000 kg, resulting in accumulation of this compound in soil and groundwater at the areas of its production and storage. In the United States, Germany, and Canada, pollution with this explosive remains a serious hazard; the sediments and soils of some industrial regions contain up to 10 g TNT/kg [12].

TNT has a considerable toxic effect on all ecosystems. The people exposed to TNT suffer from aplastic anemia, toxic hepatitis, cataracts, hepatomegaly, and liver tumors [13]. The US Environmental Protection Agency defines TNT as a class C potentially carcinogenic compound [14, 15]. TNT is more resistant to microbial degradation than mono- and dinitrotoluenes because of the symmetrical arrangement of three nitro groups in its aromatic ring, which excludes the efficiency of oxidation reactions at the first stages of TNT degradation: the widespread tactics for aerobic strains degrading other xenobiotics. The first stage of TNT metabolism under aerobic conditions is reduction of one of the two (*ortho-, para-*) nitro groups with formation of various isomers of TNT amino-nitro derivatives [16].

The goal of the present work was to study the ability of the strain *Bacillus* sp. VT-8 to carry out TNT biodegradation with assessment of its efficiency depending on the presence of additional substrates and initial concentration of the toxicant, to optimize the TNT biodegradation processes, and to test the ability of the strain *Bacillus* sp. VT-8 to degrade TNT in soil samples.

MATERIALS AND METHODS

The subject of research was the strain from the laboratory culture collection of degraders of aromatic compounds, which was identified as *Bacillus* sp. VT-8 by the results of 16S RNA–DNA analysis. The strain *Bacillus* sp. VT-8 was adapted to growth on TNT for half a year as described previously [17].

The ability of the strain *Bacillus* sp. VT-8 to utilize TNT with an additional growth source was studied by

[1] Corresponding author; e-mail: golovleva@ibpm.pushchino.ru

cultivating the strain on Petri dishes with solid nitrogen-free mineral medium containing 50 mg/L TNT and 10% (vol/vol) Luria Broth (LB) [17]. The grown cells were washed with liquid nitrogen-free mineral medium and used as inoculum (10%, vol/vol) to inoculate 750-mL flasks with 100 mL of the medium with 50 mg/L TNT and LB (5%, vol/vol) or succinate (500 mg/L). For the biological control, the cells were cultivated under the same conditions without TNT addition. For the chemical control, uninoculated flasks with TNT and LB/succinate in the mineral medium were incubated for the same time as in the experiment.

The ability of the strain *Bacillus* sp. VT-8 to utilize TNT as the sole carbon, nitrogen, and energy source was studied similarly but with addition of TNT (final concentration, 50 mg/L) when inoculating the culture into the liquid medium.

Dependence of TNT transformation by the strain *Bacillus* sp. VT-8 on TNT concentration was determined by cell cultivation in the flasks with a mineral medium containing 69, 104, or 139 mg/L TNT and 5% LB. The samples were taken on days 3 and 6 of the cultivation. The cells were harvested by centrifugation; TNT and its transformation products were extracted from the supernatant with a twofold volume of ethyl acetate. Residual TNT was assayed by the method of gas chromatography as described [17].

The intermediates of TNT transformation were determined as follows: the strain *Bacillus* sp. VT-8 was grown in flasks with 200 mL mineral medium, 50 mg/L TNT, and 5% LB. Samples (the whole culture) were taken at the zero moment and on days 2, 4, 6, 8, and 10 of growth. The supernatant was extracted with a twofold volume of ethyl acetate. The solvent was evaporated from the extract; the samples for the analysis were prepared as described [17], evaporated, and analyzed. The intermediates were identified by gas-chromatography/mass-spectrometry (GC/MS) in a LECO Pegasus 4D mass spectrometer (United States). Ionization energy was 70 eV; scanned masses were 29–500 Da.

Soil experiment was carried out by the microcosm method with the soil collected from an agricultural area, supplemented with TNT (100 mg/kg of soil), followed by thorough mixing and removal of large plant residues. The bacterial culture grown on the solid medium with 50 mg/L TNT and 10% LB was used as an introducent. Cell suspension was washed off the Petri dishes and added to the soil (50 mL/1.5 kg). Microbial succession was then initiated by moistening. Soil humidity was maintained by periodic moistening with 150 mL of tap water once every 6 days during the experiment; covering material was used to protect the soil from getting dry. Soil samples (100 g) were taken for analysis after 15, 30, 60, and 75 days. The soil sample treated with TNT without introducing the cells of the studied strain was used as a control. Soil sample (100 g) was incubated with ethyl acetate (200 mL of the solvent per flask) under stirring (200 rpm) for 12 h, and then TNT and its degradation products were extracted by the procedure described above.

The activity of TNT nitroreductase (TNT-NR) was assessed by cultivating the strain in a liquid mineral medium with 50 mg/L TNT and 200 mg/L succinate. The TNT-NR activity was measured beginning from day 4 of the cultivation. The cells were disrupted in an IBPM press. The supernatant (cell-free extract) was used to determine the enzyme activity as described [17].

TNT biodegradation was optimized by (1) addition of surfactants to enhance the permeability of microbial cells and (2) immobilization of the cells of degrader strain on polycaproamide fiber. The effect of surfactants on the ability of *Bacillus* sp. VT-8 to degrade TNT was determined using nonionic detergents Genapol ($OH(CH_2CH_2O)_n(CH_2)_mH$) and Tween-80. Tween-80 was used at concentrations of 0.25 and 1% in the variants with TNT (100 mg/L) and LB (5%, vol/vol) added to the medium. The culture grown in the medium with Tween-80 and LB was used as a biological control; uninoculated medium with TNT (100 mg/L), Tween-80 (0.25%) and LB (5%, vol/vol) was used as a chemical control. Genapol was used at a concentration of 0.1% in the variants with TNT introduced at a concentration of 50 mg/L in the absence of additional growth substrates. Samples were taken at the zero moment and on days 2, 4, 7, 10, 13, 17, and 20. The culture with TNT without Genapol was used as a control; samples were taken on days 2, 7, and 17. Sterile medium with TNT was used as a chemical control.

The cells of *Bacillus* sp. VT-8 were immobilized as follows: polycaproamide fiber (2 g per 200 mL of the mineral medium) was autoclaved for 30 min at 1 atm. The cells grown on the solid medium with TNT (100 mg/L) and LB (5%, vol/vol) were washed off ten plates with 100 mL of the medium, resuspended, and dispensed into a flask by 10 mL ($OD_{545} = 0.8$). After addition of LB (5%, vol/vol), they were incubated under stirring on a shaker (200 rpm) for 4 h, then TNT was introduced at concentrations of 67, 100, and 134 mg/L. Samples (the whole culture) were taken for analysis after 3 and 6 days of growth. TNT was extracted with ethyl acetate from the sample containing the culture liquid and the fiber with immobilized cells as described above. As a biological control, the cells were immobilized on the fiber and cultivated in the mineral medium with LB without TNT. The mineral medium with TNT, LB, and fiber not inoculated with *Bacillus* sp. VT-8 cells was used as a chemical control.

The experiments were performed in triplicate. The results of typical experiment are shown on the figures.

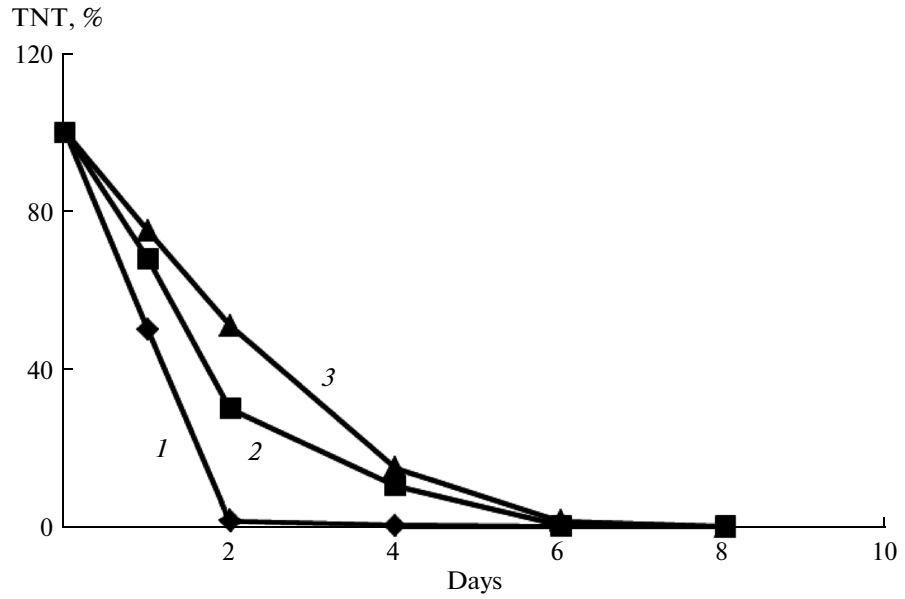

Fig. 1. Dynamics of TNT decrease in the cultures of *Bacillus* sp. VT-8 (*1*), *Kacuria palustris* RS32 (*2*) [17], and *Acinetobacter* sp. VT 11 (*3*) [17] during its transformation under conditions of cometabolism at a 5% (vol/vol) content of LB in the medium and initial TNT concentrations of 40.4, 70, and 62 mg/L, respectively.

RESULTS AND DISCUSSION

Growth of Bacillus sp. VT-8 on TNT in the Presence of Cosubstrate

Bacillus sp. VT-8 was able to transform TNT on addition of extra growth substrates (LB, 10%, vol/vol). The strain transformed over 98% TNT in the presence of LB during 2 days of growth (Fig. 1), with half of the introduced toxicant removed during the first 24 h. Comparison with the rates of TNT degradation under the influence of various previously studied bacteria showed that the strain VT-8 was one of the most efficient destructors in this respect, along with the cultures of *Kacuria palustris* RS32 and *Acinetobacter* sp. VT11 [17]. Three products of TNT metabolism were found in 2- to 4-day cultures by the method of TLC. One of these products disappeared after 48 h of cultivation. GC/MS analysis of the culture liquid extract made it possible to identify dinitro-amino and diamino-nitro TNT derivatives (with the masses analogous to those presented in [16, 17]). Identical compounds were also formed during TNT transformation by a *Bacillus cereus* strain [18]. However, the latter culture was less active than *Bacillus* sp. VT-8: under conditions of co-metabolism, this culture transformed only 68 and 77% of introduced toxicant at its initial concentrations of 50 and 75 mg/L, respectively [18].

Investigation of the dynamics of TNT transformation (55 mg/L) by the *Bacillus* sp. VT-8 culture in the presence of succinate as an additional carbon source showed that the amount of TNT decreased more smoothly and less rapidly than in the presence of LB (Figs. 1, 2): the residual amount of TNT after 12 days of cultivation was 69%. According to the TLC data, TNT decrease correlated with an increase in the number of metabolites up to eight on day 8 and their decrease to seven on day 12 of growth. This difference is most likely determined by the presence of an easily accessible additional nitrogen source for the strain when using LB and by the absence of such source when using succinate, which could be utilized only as an additional carbon source.

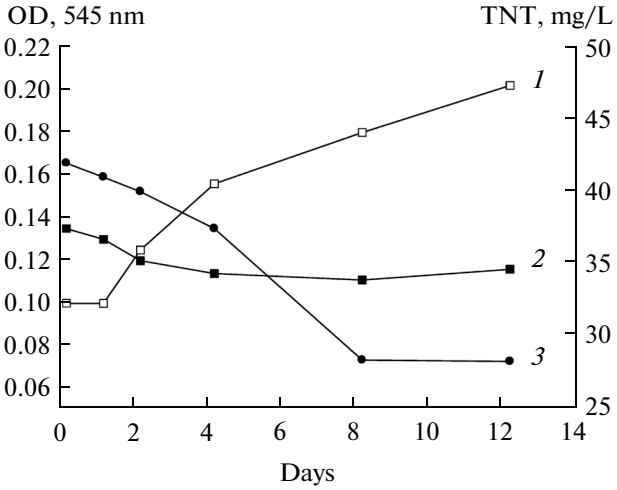

Fig. 2. TNT transformation in the *Bacillus* sp. VT-8 culture in the presence of succinate: OD of the culture in the presence of TNT and succinate (*1*); OD of the culture without TNT and succinate (*2*); and TNT content (*3*).

Concentration dependence of TNT transformation by the strain *Bacillus* sp. VT-8 (sample volume, 200 mL)

Day of cultivation	TNT, mg/L		
0	69	104	139
3	0.5	11.2	51
6	0.2	1.0	3.9

Ability of Bacillus sp. VT-8 to Transform TNT at Different Concentrations

The strain *Bacillus* sp. VT-8 could transform TNT at a concentration of 40 mg/L. This substrate concentration had no toxic effect on the cells since there were no differences in the increase in optical density of the culture growing on LB with or without TNT. The three concentrations (69, 104, and 139 mg/L) that exceeded the subinhibitory concentration approximately 1.5-, 2.5-, and 3.5-fold, respectively, were chosen to determine the amount of TNT toxic for the strain *Bacillus* sp. VT-8. Our experiments showed that the strain *Bacillus* sp. VT-8 degraded in three days nearly 100% of TNT taken at a concentration of 69 mg/L. Thus, the 1.7-fold increase in TNT concentration had no negative effect on biotransformation processes. Moreover, the cultures remained active when TNT concentration was increased from 69 to 139 mg/L. At the same time, the substrate at the initial concentration of 104 mg/L decreased by 89% after 3 days, and less than 1% of the initially introduced TNT was left by day 6 of cultivation (table).

Previously, when studying the toxic effect of TNT on the growth of *Bacillus subtilis* SK1, Kurinenko et al. showed that the culture was able to transform this compound at concentrations not exceeding 50 mg/L; at 100–200 mg/L of TNT, the total amount of utilized TNT did not depend on the initial concentration and was about 40 mg/L in the first 24 h of growth, with no decrease in TNT level during the next 24 h [19].

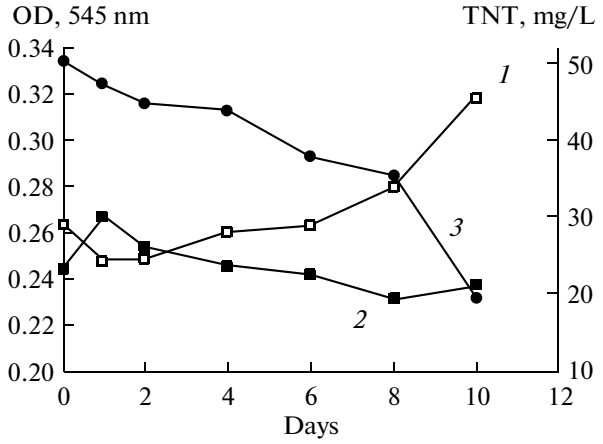

Fig. 3. Growth of the strain *Bacillus* sp. VT-8 in the medium with TNT (50.3 mg/L) as the only carbon, nitrogen, and energy source: OD of the culture in the presence of TNT (*1*); OD of the culture without TNT (*2*); and TNT content (*3*).

The increase in the initial TNT concentration during the cultivation of *Bacillus* sp. VT-8 cells naturally resulted in a lower rate transformation, as can be seen from the comparison of residual amounts of TNT at concentrations of 104 and 139 mg/L in dynamics. Nevertheless, even at the highest TNT concentration in this experiment (139 mg/L), the amount of the toxicant decreased by 63% during the first three days and by 97% by the end of cultivation (table).

Comparison of the absolute values of TNT decrease (in mg) in each experiment showed that the strain transformed 13.8 mg TNT during the entire period of cultivation at the initial concentration of 69 mg/L (sample volume, 200 mL) and 27.0 mg TNT in the variant with the initial concentration of 139 mg/L (sample volume, 200 mL), which indicated considerable resistance of this strain to the negative effects of TNT.

According to the results of GC/MS, the intermediates of TNT transformation under the given conditions were 3,5-dinitro-*p*-toluidine and 2-methyl-3,5-dinitroaniline, as well as two dinitrotoluene isomers in small amounts (the substances were identified by the masses given in [16, 17]). This fact indicates that the next stage of transformation is deamination of the reduced nitro group, which is the evidence of deep transformations of the TNT molecule. Dinitrotoluene was detected only in the first two days; then it disappeared during further substrate transformation. This process was accompanied by a tendency toward the increase in the concentration of formed dinitrotoluene as the initial concentration of TNT increased from 69 to 139 mg/L, with the maximum intensity of the metabolite peak at 139 mg/L.

Thus, the bacterium *Bacillus* sp. VT-8 proved to be able to transform TNT at substrate concentrations considered toxic for many microorganisms, including some members of this genus [18, 19]. The increase in the initial TNT concentration to 139 mg/L had no inhibitory effect on the strain under investigation.

Growth of the Strain Bacillus sp. VT-8 in the Medium with TNT as the Sole Carbon, Nitrogen, and Energy Source

Investigation of the ability of the strain *Bacillus* sp. VT-8 to utilize TNT as the only carbon, nitrogen, and energy source demonstrated a slight decrease in OD in the first day of cultivation with TNT, followed by a uniform increase in cell density (Fig. 3). The maximum increase in OD was observed on days 8–10 of cultivation. These data correlated with the rate of TNT decrease in the culture medium, which also increased by days 8–10. In the control variant (cultivation without TNT), OD gradually decreased as the cells died off due to the absence of a nutrient source. The only

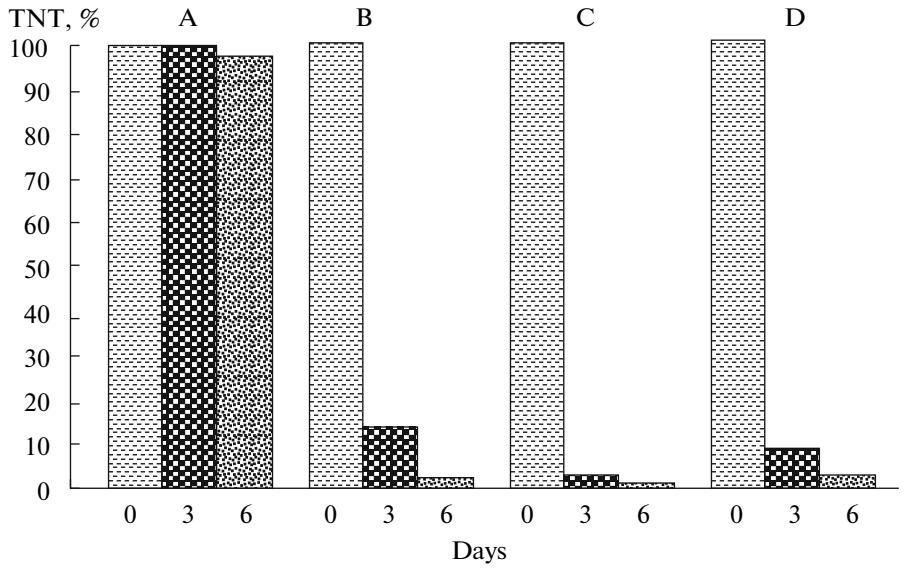

Fig. 4. Residual amount (%) of TNT (88 mg/L = 100%) in the culture of *Bacillus* sp. VT-8 in the presence of LB (5%, vol/vol) with 0.25% (C) and 1% (D) Tween-80. The controls: the medium with TNT, LB, and Tween-80 (0.25%) (A); the cells of the strain in the medium with TNT and LB without Tween-80 (B).

metabolite found in all experimental samples was identified as 2,6-dinitro-4-aminotoluene; its amount was insignificant compared to TNT concentration. No other metabolites were found in the extracts of the strain at different stages of its cultivation.

Optimization of TNT Biodegradation by the Strain Bacillus sp. VT-8

Surfactant application. Though the solubility of TNT is higher than the concentrations used in this work, one of potential approaches to enhancement of its biodegradation efficiency was addition of surface-active agents (surfactants) to the medium, since some works have shown that the introduction of Tween into biological systems increases the reaction rate several-fold [20]. This effect can be accounted for by the action of surfactants as chemical chaperones or by enhanced permeability of the cell membrane. However, surfactants at high concentrations may have a negative effect on living cells, causing cell membrane damage that may lead to cell death. In the present work we used two types of surfactants: Tween-80 and Genapol. The choice of the second compound was determined by its low cost, which is an advantage for further development of biotechnological processes, where it would be important to reduce the total cost of microbial preparations.

The measurement of residual amount of TNT after Tween-80 addition to the medium showed a considerable acceleration of the degradation processes in the presence of this surfactant (Figs. 3, 4). The introduction of Tween at a concentration of 1% resulted in TNT decrease by 92% after 3 days and by 98% after 6 days (Fig. 4D). Fourfold decrease in the Tween concentration accelerated TNT degradation: the level of TNT decreased by 99% after 3 days and by 99.8% after 6 days (Fig. 4C).

Thus, it was shown that the addition of Tween-80 had a positive effect on TNT biodegradation by the strain *Bacillus* sp. VT-8, with an insignificant advantage noted for the lower surfactant concentration. It was probably associated with a weaker influence of the surfactant on a living cell, though there were no substantial differences in the rates of TNT decrease. At the same time, the possibility of using lower surfactant concentrations makes this process more biotechnologically advantageous.

When Tween was replaced by less expensive Genapol, the same tendency toward more intense decrease in TNT concentration in the culture was observed in the presence of this detergent compared to the control (without the surfactant). Since it was shown previously that the bacterium *Bacillus* sp. VT-8 was able to utilize TNT as the only substrate, this series of experiments was performed without additional growth substrates. After 20-day cultivation, the level of TNT in the samples decreased by 40%. Comparison with the control variant where the culture grew in the medium with TNT without surfactant showed that the substrate decrease in the presence of the surfactant was greater than without Genapol. Thus, these findings demonstrate the positive effect of surfactants on the rate of microbial TNT degradation, which is due to higher availability of the substrate to the cells.

Immobilization of bacterial cells on polycaproamide fiber. The potential effect of bacterial cell immobilization on the rate of TNT transformation was analyzed

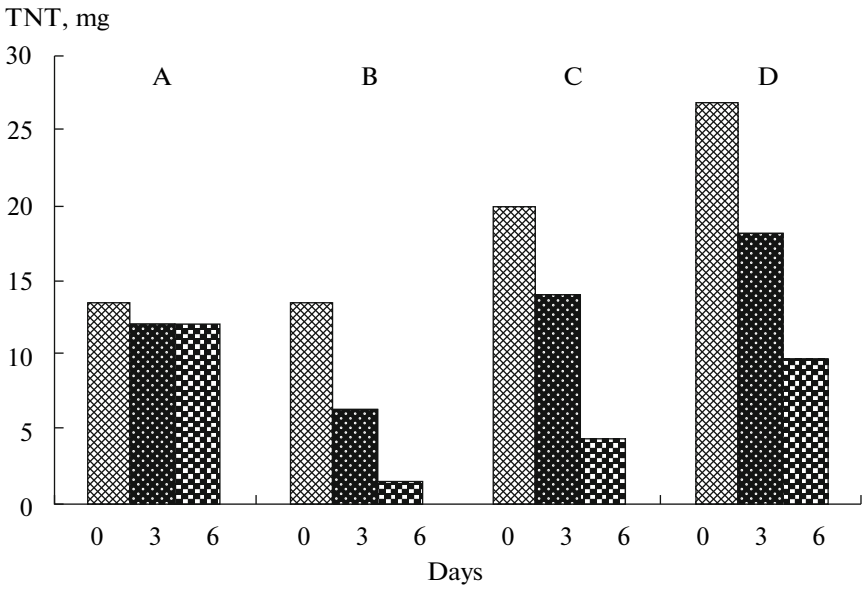

Fig. 5. Residual amount (mg) of TNT in the medium after transformation by the *Bacillus* sp. VT-8 cells immobilized on polycaproamide fiber at initial concentrations of the toxicant of 67 mg/L (B), 100 mg/L (C), and 134 mg/L (D) in the presence of LB (5%, vol/vol). Chemical control: 67 mg/L TNT (A) with LB (5%, vol/vol) and fiber without inoculation. TNT content (mg) was assayed by the method of GC for the sample volume of 200 mL.

(Fig. 5). Cell immobilization is known to play a positive role in pollutant degradation processes, primarily because immobilized cells are less susceptible to the negative effect of toxicants. Immobilization of the cells of *Bacillus amyloliquefaciens* strain WJDB-1 in alginate–chitosan microcapsules enhanced the efficiency of phenol degradation at concentrations up to 379 mg/L, while unimmobilized cells could incompletely degrade 200 mg/L of phenol during 36 h (residual phenol concentration was 30 mg/L) [21]. Immobilization of the *Bacillus* (NCIM 5220) culture also had a positive effect on utilization of di-*n*-butyl phthalate (a component of plasticizers, repellents, and artificial fibers) [22].

Polycaproamide fiber was chosen as a carrier for immobilization, because it allows immobilization of a great number of cells with uniform filling of both the carrier and the culture liquid.

The measurement of the rate of TNT decrease during its degradation by immobilized cells in the cultures with different substrate concentrations showed that this rate was maximal at the lowest concentration (67 mg/L), which corresponded to the patterns of TNT transformation by this strain presented above (Fig. 5). At the same time, the final substrate concentration was 7.4 mg/L, i.e., TNT concentration decreased tenfold after 6 days of cultivation. The minimal TNT decrease was observed in the variant with the concentration of 134 mg/L: after 6 days of cultivation, the loss of the substrate was 64%.

At the same time, analysis of the dynamics of the absolute amount of TNT in each sample showed that TNT decrease was in general more intense in the variant with its higher concentration (Fig. 5). The amount of TNT at the initial concentrations of 67, 100, and 134 mg/L (in the culture liquid volume of 200 mL) decreased by 7.4, 6.3, and 8.76 mg after 3 days and by 12, 15.8, and 17.3 mg after 6 days, respectively.

Comparison of the data on TNT degradation in the presence of surfactants and by immobilized cells showed lower rates of this process in the latter case. However, cell immobilization is sometimes necessary for water sample purification as it prolongs the application of microbial degraders and maintains the biodegradation processes at a high level for a long time.

TNT Nitroreductase Activity Assay

It is known that the enzyme initiating detoxification of the environment from TNT contamination is TNT nitroreductase (TNT-NR), which has been found in a number of microorganisms [23]. We made an attempt to assess the TNT-NR activity in the strain *Bacillus* sp. VT-8 grown in the presence of TNT under different cultivation conditions. The main methodological difficulties were (1) the low cell yield during cultivation of the strain in the medium with TNT as the only growth source and (2) high rates of the initial stages of TNT transformation during *Bacillus* sp. VT-8 cultivation in the presence of an additional co-oxidized substrate. Nevertheless, assessment of TNT-NR activity in the samples obtained during cultivation of the strain *Bacillus* sp. VT-8 on TNT in the variant with LB co-oxidation showed that TNT-NR activity was present in the cell-free extract of bacteria even in the samples with extremely low residual con-

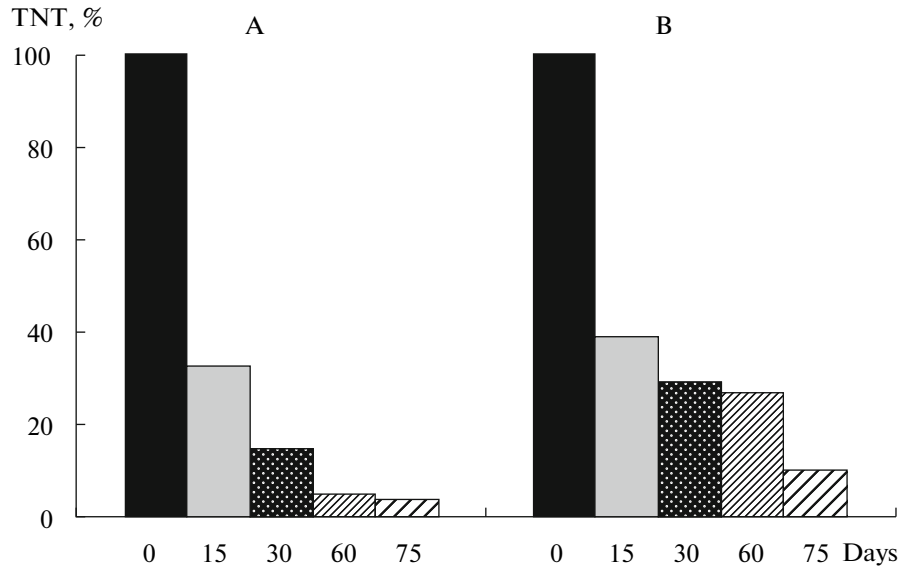

Fig. 6. Residual amount (%) of TNT (100 mg/kg soil) in soil samples inoculated with bacterial cultures of *Bacillus* sp. VT-8 (A) and *R. opacus* 1G (B) [17] after 0, 15, 30, 60, and 75 days of cultivation (the amount of TNT assayed on day 0 in 100 g of soil, equal to 7.17 mg, was taken as 100%).

tents of TNT, though its level was not very high: 0.007–0.008 U/mg protein. TNT-NR activity was higher by an order of magnitude (0.013–0.027 U/mg protein) in the samples obtained in the variants with sufficient amounts of TNT in the growth medium. The measurement of TNT-NR activity in cell-free extracts of the strain *Bacillus* sp. VT-8 obtained from the biomass grown at different substrate concentrations showed that its levels in the extracts with TNT concentrations of 69 and 104 mg/L on day 3 of growth differed twofold: 0.013 and 0.028 U/mg protein, respectively. The low enzyme activity in the first variant correlated with nearly complete elimination of TNT from the reaction medium. On day 6 of cultivation, TNT-NR activity decreased by an order of magnitude in both cases, reflecting the complete loss of TNT.

Identification of the type of cofactor needed by this enzyme to manifest its activity showed that TNT-NR was active in the presence of NADH. In case of its replacement by NADPH, TNT-NR activity was absent. Its level in the extracts under study was different but, on average, comparable with the levels of activity of TNT-NR from the previously described bacterial strains [17] and of the samples of purified TNT-NR from the strain *Pseudomonas putida* JLR11 [23].

TNT Degradation by the Strain Bacillus sp. VT-8 in Soil

Since isolation of the strains and investigation of their degradative activity against toxic substrates are determined by the need to obtain biopreparations for decreasing the level of pollution at contaminated sites, one of the most important problems is to study the ability of the strains to degrade TNT in soil. The difficulty of this investigation results from high reactivity of soil components to the products of TNT transformation. The experiments with different bacterial and fungal strains have shown that TNT degradation in soil under the influence of introduced and indigenous autochthonous organisms resulted in the interaction of hydroxyamino and amino groups of the nitroaromatic ring formed during the transformation with the quinone and carbonyl functional groups of the soil humic fraction, which determines the formation of covalently bound TNT derivatives that become inaccessible for further transformation and thereby pass into the category of low-toxic compounds [24].

The model experiment with the strain *Bacillus* sp. VT-8 introduced into TNT-contaminated soil was performed. Figure 6 shows the results of determination of residual amounts of the toxicant. In the control variant, with TNT introduced into nonsterile soil, its amount remained constant throughout the experiment (data not shown). The data on TNT decrease in case of introduction into soil of the strain *Rhodococcus opacus* 1G, which previously demonstrated the best results among all of the tested laboratory strains [17], are presented for comparison. With the strain *Bacillus* sp. VT-8 as an introducent, the residual amount of the toxicant in soil was already 15% after 1 month, and only 5% of its initial amount was detected after 2 months. At the same time, 94 and 92% TNT was detected in the control variant (nonsterile soil of moderate humidity) after 1 and 2 months of cultivation, respectively, compared to TNT content in the beginning of the experiment.

Thus, our study showed that the strain *Bacillus* sp. VT-8, in contrast to other bacteria of this genus, is highly resistant to the toxic effect of TNT, can perform detoxification processes in aqueous and soil samples under conditions of co-metabolism, and shows a substantial decrease in TNT when this compound is used as the only growth substrate, which makes the strain *Bacillus* sp. VT-8 promising for bioremediation of TNT-polluted sites.

ACKNOWLEDGMENTS

The work was supported by the International Science and Technology Centre (project G-1408) and the Russian Foundation for Basic Research (project 11-04-96028-p_ural_a).

REFERENCES

1. Sorkhoh, N.A., Ibrahim, A.S., Ghannoum, M.A., and Radwan, S.S., High-temperature hydrocarbon degradation by *Bacillus stearothermophilus* from oil-polluted Kuwaiti desert, *Appl. Microbiol. Biotechnol.*, 1993, vol. 39, pp. 123–126.
2. Feitkenhauer, H., Müller, R., and Märkl, H. Degradation of polycyclic aromatic hydrocarbons and long chain alkanes at 60–70°C by *Thermus* and *Bacillus* spp., *Biodegradation*, 2003, vol. 14, pp. 367–372.
3. Tallur, P.N., Megadi, V.B., Kamanavalli, C.M., and Ninnekar, H.Z., Biodegradation of *p*-cresol by *Bacillus* sp. strain PHN, *Cur. Microbiol.*, 2006, vol. 53, pp. 529–533.
4. Reinscheid, U.M., Bauer, M.P., and Müller, R., Biotransformation of halophenols by a thermophilic *Bacillus* sp., *Biodegradation*, 1996, vol. 7, pp. 455–461.
5. Liu, L., Liu, Y., Shin, H., Chen, R.R., Wang, N.S., Li, J., Du, G., and Chen, J., Developing *Bacillus* spp. as a cell factory for production of microbial enzymes and industrially important biochemicals in the context of systems and synthetic biology, *Appl. Microbiol. Biotechnol.*, 2013, vol. 97, pp. 6113–6127.
6. Mulla, S.I., Manjunatha, T.P., Hoskeri, R.S., Tallur, P.N., and Ninnekar, H.Z., Biodegradation of 3-nitrobenzoate by *Bacillus flexus* strain XJU-4, *J. Microbiol. Biotechnol.*, 2011, vol. 27, pp. 1587–1592.
7. Ji Y., Qu Z., Li W., Xu C., Zhu X. Study on the screening for chloronitrobenzene: degrading bacteria and degradation of chloronitrobenzene, in *Information Technology and Agricultural Engineering*, Zhu, E. and Sambath, S., Eds., Berlin: Springer, 2012. AISC 134, pp. 23-29.
8. Wen, D., Li, G., Xing, R., Park, S., and Rittmann, B.E., 2,4-DNT removal in intimately coupled hotobiocatalysis: the roles of adsorption, photolysis, photocatalysis, and biotransformation, *Appl. Microbiol. Biotechnol.*, 2012, vol. 95, pp. 263–272.
9. Singh, B., Kaur, J., and Singh, K., 2,4,6-Trinitrophenol degradation by *Bacillus cereus* isolated from a firing range, *Biotechnol. Lett.*, 2011, vol. 33, pp. 2411–2415.
10. Arora, P.K. and Jain, R.K., Biotransformation of 4-chloro-2-nitrophenol into 5-chloro-2-methylbenzoxazole by a marine *Bacillus* sp. strain MW-1, *Biodegradation*, 2012, vol. 23, pp. 325–331.
11. Perreault, N.N., Manno, D., Halasz, A., Thiboutot, S., Ampleman, G., and Hawari, J., Aerobic biotransformation of 2,4-dinitroanisole in soil and soil *Bacillus* sp., *Biodegradation*, 2012, vol. 23, pp. 287–295.
12. Maeda, T. and Ogawa Hiroaki, I., Microbial degradation of 2,4,6-trinitrotoluene: application to explosives sensor, in *Microbial Degradation of Xenobiotics, Environmental Science and Engineering*, Singh, S.N., Ed., Berlin: Springer, 2012, pp. 213–233. DOI: 10.1007/978-3-642-23789-8_8
13. Sabbioni, G., Sepai, O., Norppa, H., Yan, H., Hirvonen, A., and Zheng, Y., Comparison of biomarkers in workers exposed to 2,4,6-trinitrotoluene, *Biomarkers*, 2007, vol. 12, pp. 21–37.
14. Leung, K.H., Yao, M., Stearns, R., and Chiu, S.-H.L., Mechanism of bioactivation and covalent binding of 2,4,6-trinitrotoluene, *Chem. Biol. Interact.,* 1995, vol. 97, pp. 37–51.
15. Sarlauskas, J., Nemeikaite-Ceniene, A., Anusevicius, Z., Miseviciene, L., Julvez, M.M., Medina, M., Gomez-Moreno, C., and Cenas, N., Flavoenzyme-catalyzed redox cycling of hydroxylamino- and amino metabolites of 2,4,6-trinitrotoluene: implications for their cytotoxicity, *Arch. Biochem. Biophys.*, 2004, vol. 425, pp. 184–192.
16. Esteve-Nuñez, A., Caballero, A., and Ramos, J.L., Biological degradation of 2,4,6-trinitrotoluene, *Microbiol. Mol. Biol. Rev.*, 2001, vol. 65, pp. 335–352.
17. Solyanikova, I.P., Baskunov, B.P., Baboshin, M.A., Saralov, A.I., and Golovleva, L.A., Detoxification of high TNT concentrations by bacteria, *Appl. Biochem. Microbiol.*, 2012, vol. 48, no. 1, pp. 21–27.
18. Mercimek, H.A., Dincer, S., Guzeldag, G., Ozsavli, A., and Matyar, F., Aerobic biodegradation of 2,4,6-trinitrotoluene (TNT) by *Bacillus cereus* isolated from contaminated soil, *Microb. Ecol.*, 2013, vol. 66, pp. 512–521.
19. Kurinenko, B.M., Yakovleva, G.Yu., Danivarova, N.A., and Abreimova, Yu.V., Effect of toxic concentrations of 2,4,6-trinitrotoluene on the physical properties and morphology of *Bacillus subtilis* SK1 cells, *Appl. Biochem. Microbiol.,* 2003, vol. 39, no. 3, pp. 194–198.
20. *Detergents. A Guide to the Properties and Uses of Detergents in Biological Systems*, Bhairi, S.M. and Mohan, C., Eds., San Diego: EMD Biosciences, 2007.
21. Lu, D., Zhang, Y., Niu, S., Wang, L., Lin, S., Wang, C., Ye, W., and Yan, C., Study of phenol biodegradation using *Bacillus amyloliquefaciens* strain WJDB-1 immobilized in alginate-chitosan-alginate (ACA) microcapsules by electrochemical method, *Biodegradation*, 2012, vol. 23, pp. 209–219.
22. Patil, N.K. and Karegoudar, T.B., Parametric studies on batch degradation of a plasticizer di-*n*-butylphthalate by immobilized bacillus sp., *J. Microbiol. Biotechnol.*, 2005, vol. 21, pp. 1493–1498.
23. Lee, B.U., Park, S.C., Cho, Y.S., Kahng, H.Y., and Oh, K.H., Expression and characterization of the TNT nitroreductase of *Pseudomonas* sp. HK-6 in *Escherichia coli, Curr. Microbiol.,* 2008, vol. 56, no. 4, pp. 386–390.
24. Smets, B.F., Yin, H., and Esteve-Nuñez, A., TNT biotransformation: when chemistry confronts mineralization, *Appl. Microbiol. Biotechnol.*, 2007, vol. 76, pp. 267–277.

Translated by E. Makeeva