ISSN 0026-2617, Microbiology, 2012, Vol. 81, No. 1, pp. 44–50. © Pleiades Publishing, Ltd., 2012.
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EXPERIMENTAL ARTICLES

# Biodegradation of Glyphosate by Soil Bacteria: Optimization of Cultivation and the Method for Active Biomass Storage

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Received April 21, 2011

**Abstract**—Conditions for obtaining the active biomass of *Ochrobactrum anthropi* GPK 3 and *Achromobacter* sp. Kg 16, bacteria which are able to degrade the herbicide glyphosate (N-phosphonomethylglycine), were investigated. In the batch culture, degradation was most effective in the medium with pH 6.0–7.0 and aeration at 10–60% of air saturation supplemented with glutamate and ammonium chloride as sources of carbon and nitrogen, respectively. Due to the adaptation of the cells and induction of the relevant enzymatic systems, the inoculum grown in the presence of glyphosate exhibited 1.5–2-fold higher efficiency of xenobiotic degradation than that grown with other sources of phosphorus (orthophosphate and methylphosphonic acid). The efficiency of the toxicant decomposition increased with an increase in a specific load of glyphosate, which the cells were subjected to during the initial stage of growth. The specific load was regulated both by the initial cell concentration and the concentration of the phosphorus source, and the effect was probably determined by its availability to microorganisms. Storage of the liquid biopreparation as a paste with stabilizers (ascorbate, thiourea, and glutamate) at room temperature for 50 days resulted in high level of bacteria viability and a degrading activity approximately equal to that obtained when the bacteria were maintained on the agar medium containing glyphosate at 4°C with monthly transfers to the fresh culture medium.

*Keywords*: glyphosate, glyphosate-degrading microorganisms, biodegradation, cultivation conditions, optimization, storage of biopreparations.

**DOI:** 10.1134/S0026261712010134

An interest in the biodegradation of organophosphonates—the compounds containing a carbon-phosphorus (C–P) bond in their structure—has increased in recent decades as a result of environmental problems [1]. One of the most widespread compounds of this class is glyphosate (N-phosphonomethylglycine, GP). Since it has herbicidal properties, the preparations on the basis of glyphosate are applied to eliminate annual and perennial weeds after their emergence. The recent appearance of glyphosate-resistant recombinant plants resulted in the escalated application of glyphosate-containing herbicides and, subsequently, to the extension of the pollution sites [2]. Unlike the easily degradable C–O–P ester bond in phosphates, the C–P bond in the phosphonates is resistant to physicochemical factors [3, 4]. However, under certain conditions, it may be cleaved by the microbial enzymatic systems [5, 6].

The most active GP-degrading strains were usually isolated from the soils contaminated with this herbicide [7, 8]. The examples are *Ochrobactrum anthropi* GPK 3 and *Achromobacter* sp. Kg 16 [9], which possess a high degradative potential under environmental conditions, survive well in the soil, and are not pathogenic for mammals. These organisms therefore may be used in the production of biopreparations for remediation of the GP-contaminated territories with acceptable effectiveness and ecological benefit. Practical application of microorganisms requires a detailed study of the regulation of their degradative activity. Important factors affecting the metabolism of these microorganisms are the type of nutrients (especially the carbon and nitrogen sources), and the cultivation conditions resulting in enhanced efficiency of the toxicant degradation and accumulation of an active biomass of the degrading strains. Identification of these factors will make it possible to know which compounds can be used as nutrients for microorganisms in soil during bioremediation. Previously, the attention of scientists was mainly focused on the screening of microorganisms for their ability to degrade organophosphonates [5, 10, 11]. However, the comprehensive studies of the physiological regulation of this process in bacterial cells are rather few [12].

The goal of the present work was to select optimal conditions for the cultivation of *Ochrobactrum anthropi* GPK 3 and *Achromobacter* sp. Kg 16, providing maximal effectiveness of the herbicide degradation, as well as to work out the storage conditions for the biomass intended for introduction into the soil.

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## MATERIALS AND METHODS

**Microorganisms.** Two strains of GP-degrading bacteria were used for the study: *O. anthropi* GPK 3 (VKM B-2554 D) and *Achromobacter* sp. Kg 16 (VKM B-2534 D). The strains were isolated from the samples of GP-contaminated soils by the enrichment culture technique under the selective pressure of the toxicant [7]. The strains were maintained in the laboratory collection on MS1 agar medium supplemented with 500 mg/L of glyphosate and 10 g/L of glutamate. The taxonomy of the strains were identified by the analysis of their 16S rRNA gene sequences using the BLAST software [13].

**Culture medium and cultivation conditions.** The strains were cultivated in MS1 mineral salt culture medium [7]. Glyphosate (500 mg/L) as the herbicide GroundBio (36% aqueous solution of the isopropylamine salt, Technoexport, Russia), analogous to the herbicide Roundup (Monsanto, United States), was used as the phosphorus source, and glutamate (10 g/L) was the source of carbon.

Investigation of the effect of other carbon sources on bacterial growth and destructive activity was carried out using the same culture medium containing carbohydrates (glucose, sucrose, maltose, arabinose, xylose, lactose, and trehalose), organic acids (pyruvate, succinate, fumarate, citrate, malate, and acetate), alcohols (ethanol and glycerol), and an amino acid glutamate. All substrates were added into the medium in the concentration of 1%.

The nitrogen-containing organic substances (glutamate, glyphosate, and urea) and mineral salts ($NH_4NO_3$, $(NH_4)_2SO_4$, and $KNO_3$) were added to MS1 medium instead of $NH_4Cl$ as the sources of nitrogen. The concentration of these substances was adjusted to 1 g/L of nitrogen, which corresponds to its content in 10 g/L of glutamic acid. Glutamate, succinate, glycerol, and glucose were used as the sources of carbon in these experiments.

Microorganisms were cultivated at 28–30°C in 750-mL flasks with 100 mL of the medium on a rotary shaker at 180–200 rpm. The pH of the medium was maintained at 6.0–7.5 by the addition of the sterile 20% $H_2SO_4$ solution.

The effect of pH and aeration on bacterial growth and effectiveness of glyphosate degradation was studied in a 10-l bioreactor (type of ANKUM-2M, Russia) with the fill factor of 0.6, at 28°C. To maintain pH value, 20% solution of $H_2SO_4$ was used. Aeration intensity was regulated by changing the agitation rate and the volume of air supply. Experiments were carried out at pH 6.0, 7.0, and 8.0, and aeration at 50–60% of air saturation (intense aeration) or 10–20% air saturation (low aeration).

The inoculum was grown for 3 days on the MS1 agar medium containing glyphosate and glutamate. Then the cells were washed into the sterile flask with the liquid culture medium of the same composition, containing the carbon source but no source of phosphorus, and incubated for 48 hours on a rotary shaker to achieve phosphorus starvation. The microbial suspension was then added to the medium, with the initial optical density of 0.1–0.2 $OD_{560}$ (optical density at 560 nm).

To detect the effect of the source of phosphorus, the inoculum was cultivated on the MS1 agar medium containing 1 g/L of orthophosphate, 500 mg/L of glyphosate, or 300 mg/L of methylphosphonate, subjected to the starvation as described above, and cultivated in the liquid culture medium with the same substrates. At the beginning of the stationary phase, the cells were washed with the medium without phosphorus sources and used for inoculation of the experimental flasks containing glyphosate.

**Control of microbial growth and degrading activity.** The growth of bacterial cultures was controlled by the change $OD_{560}$ using a Specol 221 spectrophotometer (Germany), and was then expressed as dry biomass weight (g/L) with a coefficient of 0.5 (g/U $OD_{560}$). Degradation efficiency ($Q_{GP}$) was calculated as the ratio of glyphosate uptake to the quantity of the dry biomass (mg/g).

**Study of the conditions for long-term storage of the biomass.** To investigate the conditions providing preservation of viability and degrading activity of the cells, bacteria were grown in an ANKUM-2M bioreactor under selected optimal conditions. The cells were collected at the end of the retardation growth phase, concentrated by centrifugation (5200 *g*, 30 min, 4°C), and 0.5 g of the biomass was resuspended in 0.5 mL solution of stabilizer (composition and concentration of the stabilizers are presented in the Results and Discussion section). The obtained suspension was a liquid form of the preparation with a titer of $10^{10}$–$10^{12}$ CFU/mL. The suspensions were stored in closed test tubes at 4, 20–22, and 28°C. During storage, the samples were regularly taken and the colony-forming units (CFU) titer was determined. Cell viability was calculated as the change in the number of CFU on the Luria-Bertani medium (LB). Finally, $Q_{GP}$ of the stored biomass was checked in batch culture with the medium containing glyphosate, according to the method described above.

**Analytical methods.** Concentration of inorganic phosphorus was monitored spectrophotometrically by detecting the formation of the phosphomolybdate–Malachite Green complex under acidic conditions [14]. Total phosphorus amount in the culture liquid was determined by the same method after hydrolysis with ammonium persulfate (400 g/L) for 1 h [5]. The ratio of the culture liquid and ammonium persulfate was 1 : 1. The content of glyphosate in the sample was calculated from the difference between the concentrations of total and inorganic phosphorus, with a coefficient of 5.45. Total phosphorus concentration in pre-

**Table 1.** Growth of *O. anthropi* GPK 3 and efficiency of glyphosate degradation for the inocula grown on the medium with various sources of phosphorus

| Parameters | Phosphorus source in the inoculum | | |
|---|---|---|---|
| | Glyphosate | Methylphosphonate | Orthophosphate |
| | Phosphorus source in the experiment | | |
| | Glyphosate | Glyphosate | Glyphosate |
| Biomass, $OD_{560}$ | 4.8 | 3.0 | 2.4 |
| Glyphosate consumption, mg/L | 120 | 79 | 57 |
| $Q_{GP}$, mg/g | 50 | 53 | 47.5 |

liminarily diluted culture liquid did not exceed 20 mg/L.

In the tables, the mean values of experiments performed in triplicate are shown.

## RESULTS AND DISCUSSION

**Phosphorus source in the inoculum.** Evidence exists in the literature that the cells have to adapt themselves to alternative nutrient sources when certain nutrients are deficient in the medium [6, 10, 15]. We have shown previously [7] that preliminary phosphorus starvation of *O. anthropi* GPK 3 grown in the medium containing glyphosate caused a 30% increase in the efficiency of glyphosate degradation. However, it was not clear whether this parameter would change in the case of preliminary cultivation of bacteria with other sources of phosphorus: methylphosphonate or orthophosphate.

Our results demonstrated that the change of the type of phosphorus source during the preparation of the inoculum affected both the growth of the culture and the decrease in glyphosate concentration during subsequent cultivation in the medium containing this substrate (Table 1). Both the highest level of biomass and the maximal amount of the utilized glyphosate were observed in the variant when the same source of phosphorus was used (glyphosate–glyphosate). These parameters decreased 1.5-fold when methylphosphonate was added as the source of phosphorus, and 2-fold when inorganic phosphate was used. The adapted cells utilized twice as much glyphosate as the nonadapted ones. This was probably due to the induction of the enzyme systems of glyphosphate metabolism specific for this phosphonate. The capacity of adapted cells for glyphosate degradation was retained after transfers on selective agar media. Therefore, the necessity for preliminary adaptation of GP-degrading bacteria to this substrate is evident.

**Carbon and nitrogen sources.** The presence of accessible carbon sources is an important factor determining the viability of degrading microorganisms in soil. Members of the genus *Ochrobactrum* are known to be able to assimilate a wide range of carbohydrates, organic acids, and amino acids [16], while bacteria of the genus *Achromobacter* utilize organic acids and amino acids, but not carbohydrates or alcohols [17].

Thus, it seemed expedient to determine the ability of the studied bacteria to utilize various classes of organic substances as carbon sources in the presence of glyphosate as the source of phosphorus. *O. anthropi* GPK 3 strain was found to assimilate most of the tested carbohydrates (except lactose and trehalose), organic acids, glutamate and glycerol. For this strain, the stationary-phase biomass in the media containing these carbon substrates was 1.0–2.5 g/L. *Achromobacter* sp. Kg 16 efficiently utilized only organic acids and glutamate (biomass 0.5–1.0 g/L), while no growth was observed in the media containing carbohydrates and alcohols.

The values of biomass and $Q_{GP}$ were determined for both strains grown in the media containing various nitrogen sources (Table 2). The highest values were obtained in the media with glutamate and glutamate + $NH_4Cl$ as the carbon and nitrogen sources. When succinate was used instead of glutamate as the carbon source, a certain decrease in the efficiency of glyphosate biodestruction resulted. Both strains utilized ammonium nitrogen for the synthesis of cell compounds, while nitrate nitrogen and urea nitrogen were assimilated by *Achromobacter* sp. Kg 16 but not by *O. anthropi* GPK 3.

Importantly, none of the strains utilized glyphosate as the sole nitrogen or carbon source, although the ability of microorganisms (mutant strain of *Arthrobacter* sp.) to utilize glyphosate as the source of both nitrogen and phosphorus was reported previously [18].

Based on the results obtained, glutamate was chosen as the most suitable carbon source for the cultivation, since it could be consumed both as the source of carbon and nitrogen. To prevent the limitation of growth by nitrogen, ammonium chloride was not excluded from the MS1 medium.

The obtained data demonstrated that assimilation of a wide range of carbon and nitrogen sources by the strains used in the study may enable them to adapt quickly after their introduction into the soil, and to provide for cell growth and glyphosate-degrading

**Table 2.** Effect of a nitrogen source on the growth and glyphosate degradation efficiency by *O. anthropi* GPK 3 and *Achromobacter* sp. Kg 16

| Carbon source | Nitrogen source | *O. anthropi* GPK 3 | | *Achromobacter* sp. Kg16 | |
|---|---|---|---|---|---|
| | | Biomass, $OD_{560}$ | $Q_{GP}$, mg/g | Biomass, $OD_{560}$ | $Q_{GP}$, mg/g |
| Glutamate | Glutamate | 5.6 | 81 | 2.4 | 43.5 |
| | Glutamate + $NH_4Cl$ | 6.0 | 84 | 2.0 | 45 |
| Succinate | $NH_4Cl$ | 5.2 | 64 | 2.0 | 33.5 |
| | $KNO_3$ | 0.1 | 0 | 1.7 | 32 |
| | $NH_4NO_3$ | 5.2 | 40 | n/d | n/d |
| | $(NH_4)_2SO_4$ | 5.2 | 33 | n/d | n/d |
| | Urea | 0.1 | 0 | 1.8 | 23 |
| | Glyphosate | 0.1 | 0 | 0.1 | 0 |
| Glucose | $NH_4Cl$ | 5.0 | 71 | 0.1 | 0 |
| Glycerol | $NH_4Cl$ | 4.0 | 38 | 0.1 | 0 |

Note: n/d; stands for "not determined".

**Table 3.** Growth parameters and glyphosate destruction efficiency, depending on the initial concentration of *O. anthropi* GPK 3 and *Achromobacter* sp. Kg 16 cells in the medium

| Strain | Initial biomass, g/L | 0.025–0.125 | 0.125–0.25 | 0.35–0.5 |
|---|---|---|---|---|
| | Specific load of glyphosate, g/g of biomass | 20–4 | 4–2 | 1.4–1 |
| *O. anthropi* GPK 3 | Biomass at the stationary phase, g/L | 3.1 | 3.45 | 3.35 |
| | Lag-phase, h | 15 | 22 | 36 |
| | $\mu$, $h^{-1}$ | 0.13 | 0.07 | 0.04 |
| | $Q_{GP}$, mg/g | 79 | 72 | 64 |
| *Achromobacter* sp. Kg 16 | Biomass at the stationary phase, g/L | 1.0 | 0.85 | 1.2 |
| | Lag-phase, h | 16 | 20 | 40 |
| | $\mu$, $h^{-1}$ | 0.075 | 0.05 | 0.02 |
| | $Q_{GP}$, mg/g | 43 | 38 | 14 |

activity. Nevertheless, the individual peculiarities of the strains resulting from their taxonomy should be considered.

**Specific load of the phosphorus source.** Cell concentration and concentration-dependent intercellular interactions are important factors affecting the biochemical and physiological processes. While it mainly defines the specific load of the carbon source on cell metabolism [19, 20], it may also act as a regulatory factor influencing the effect of other nutrient components. When methylphosphonate was utilized by *E. coli* as the source of phosphorus in a dense suspension (OD = 1.0 U), its degrading activity decreased two- to threefold [21].

Specific load is calculated as the ratio of the initial substrate concentration in the medium and the amount of biomass at the moment of inoculation (g of glyphosate/g of the cells). For *O. anthropi* GPK 3 and *Achromobacter* sp. Kg 16, the effect of specific load of the phosphorus source was studied while the following parameters varied: (1) initial concentration of the cells changing from 0.025 to 0.5 g/L, with glyphosate concentration of 0.5 g/L (specific load from 20 to 1) and (2) initial concentration of glyphosate changing from 1 to 0.1 g/L, with the cell concentration of 0.1 g/L (specific load from 10 to 1). An increase in cell concentration and decrease in specific load of the substrate led to the extension of the lag phase and to lower specific growth rate, resulting in a prolongation of cultivation (Table 3). The efficiency of glyphosate destruction also decreased. This may probably be explained by lower accessibility of the substrate and insufficient oxygen supply due to the high density of inoculation.

When the specific load changed from 10 to 1 due to the changes in the initial glyphosate concentration,

**Table 4.** Effect of pH and aeration on the growth and glyphosate degradation efficiency during cultivation of *O. anthropi* GPK 3 in a bioreactor

| pH; **$pO_2$,%** | Lag-phase, h | μ, $h^{-1}$ | Biomass, $OD_{560}$ | Glyphosate consumption, mg/L | $Q_{GP}$, mg/g |
|---|---|---|---|---|---|
| 6.0; **50–60** | 16 | 0.12 | 7.2 | 444 | 123 |
| 7.0; **50–60** | 22 | 0.14 | 7.0 | 461 | 132 |
| 8.0; **50–60** | 37 | 0.09 | 3.6 | 200 | 111 |
| 7.0; **10–20** | 20 | 0.12 | 6.8 | 415 | 122 |

the same pattern was observed: $Q_{GP}$ decreased from 84 to 53 for *O. anthropi* GPK 3 and from 45 to 30 for *Achromobacter* sp. Kg 16.

**Cultivation conditions.** Determination of the optimal values of pH and aeration is a prerequisite for the cultivation of microorganisms.

Preliminary studies of *O. anthropi* GPK 3 grown in flasks indicated that a pH value of 5.0 was growth-inhibiting, while at pH 9.0 the amount of biomass decreased drastically. Glyphosate was not utilized or was utilized insufficiently (data not shown).

When this strain was grown in a bioreactor (Table 4) with variations in pH values from 6.0 to 8.0, the lag phase duration increased, and the specific growth rate decreased. The pH values of 6.0–7.0 were optimal for bacterial growth. Degradation characteristics (the amount of utilized glyphosate and $Q_{GP}$) were highest at these pH values as well.

Bacteria grew well and retained high degrading potential independently from the oxygen supply in the medium. In the range of 10–60% air saturation and optimal pH values, both the biomass of bacteria and $Q_{GP}$ varied insignificantly (Table 4).

The experiments with cultivation of bacteria in the flasks (Tables 2, 3) and in a bioreactor (Table 4) showed the efficiency of herbicide destruction to increase significantly when the cells were grown under controlled conditions of temperature, pH, and aeration.

**Accumulation and storage of the liquid biopreparation.** As a result of these experiments, optimal conditions of cultivation providing accumulation of an active biomass of bacterial degraders were identified: cultivation of the inoculum on solid MS1 mineral medium supplemented with 500 mg/L of glyphosate and 10 g/L of glutamate with subsequent phosphorus starvation for 48 h; inoculation of the medium in a bioreactor with the initial $OD_{560}$ not exceeding 0.2 $OD_{560}$; cultivation at pH 7.0, 50% air saturation, and 28°C.

In remediation biotechnology of contaminated soils, one of the major problems is developing a method for conservation of the active biomass of degrading bacteria. For this purpose, the liquid form of the preparation is used (as a paste), containing various stabilizers. The advantage of the liquid preparations is their cheap production and application with the maximal preservation of degrading properties of the microorganisms.

In this work, thiourea, glycerol, ascorbic acid, and sodium glutamate were used as stabilizers for deceleration of the oxidation-reduction processes, NaCl was used for the preservation of the necessary osmotic pressure, and EDTA, as an inhibitor of proteases and nucleases. For quick evaluation of their effectiveness, the changes of CFU numbers in the biopreparation were determined during its storage at 28°C.

The results demonstrated (Table 5) that the viability of the strains and effectiveness of stabilizers differed significantly depending on the taxonomical position of bacteria, and were in accordance with the experimental data of other researchers [22]. *O. anthropi* GPK 3 retained high titer after three weeks of storage at 28°C; *Achromobacter* sp. Kg 16 retained high titer during one week. The best results for the former strain were obtained using ascorbate, thiourea, and glutamate, for the latter—using glutamate.

Viability of the cells and the preservation of destructive activity of the biopreparation for both strains were determined after 50 days of storage with selected stabilizers at 4 and 20–22°C. At low temperature the titer of microorganisms did not change, while at room temperature it decreased by two orders of magnitude, but still remained sufficiently high. The effectiveness of glyphosate degradation under these conditions was comparable to that for the bacteria in the control variants where the cultures were maintained on the agar medium with glyphosate at 4°C with monthly transfers to the fresh medium (Table 6).

Thus, optimal conditions were selected for batch cultivation of the glyphosate-degrading strains, providing maximal efficiency of degradation. Selection of

**Table 5.** CFU variations during storage of *O. anthropi* GPK 3 and *Achromobacter* sp. Kg 16 biomass at 28°C

| Stabilizers, concentration | *O. anthropi* GPK 3* | | *Achromobacter* sp. Kg 16* | |
|---|---|---|---|---|
| | Titer after storage, CFU/mL | | Titer after storage, CFU/mL | |
| | 1 week | 3 week | 1 week | 3 week |
| NaCl, 0.9% | $2.7 \times 10^{11}$ | $8.0 \times 10^{7}$ | $1.3 \times 10^{10}$ | ** |
| Ascorbate, 0.3% | $3.5 \times 10^{11}$ | $3.0 \times 10^{9}$ | $1.7 \times 10^{10}$ | ** |
| Thiourea, 1% | $4.0 \times 10^{11}$ | $2.5 \times 10^{9}$ | $7.6 \times 10^{8}$ | $4.0 \times 10^{4}$ |
| Sucrose, 10% | $3.0 \times 10^{11}$ | $1.4 \times 10^{8}$ | $6.8 \times 10^{9}$ | ** |
| Glycerol, 10% | $1.5 \times 10^{11}$ | $4.0 \times 10^{6}$ | $5.7 \times 10^{8}$ | ** |
| EDTA, 5 mM | $8.0 \times 10^{10}$ | $1.4 \times 10^{8}$ | $1.0 \times 10^{4}$ | ** |
| Glutamate, 2% | $5.3 \times 10^{11}$ | $1.2 \times 10^{9}$ | $1.5 \times 10^{10}$ | $3.0 \times 10^{6}$ |
| Tap water | $6.6 \times 10^{8}$ | $1.0 \times 10^{8}$ | $5.0 \times 10^{7}$ | ** |
| Distilled water | $8.0 \times 10^{7}$ | $6.0 \times 10^{7}$ | $5.3 \times 10^{7}$ | ** |

Notes:*Initial cell titer for *O. anthropi* GPK 3 was $3–5.4 \times 10^{11}$, for *Achromobacter* sp. Kg 16, $2.4 \times 10^{10}$ CFU/mL. Titer below $10^{3}$ CFU/mL.

**Table 6.** Biomass and degradation activity of *O. anthropi* GPK 3 and *Achromobacter* sp. Kg 16 after storage of liquid biopreparations for 50 days at various temperatures with stabilizers

| Stabilizers | Storage conditions | | | | |
|---|---|---|---|---|---|
| | 4°C | | 20–22°C | | |
| | Biomass, $OD_{560}$ | $Q_{GP}$, mg/g | Cell titer, CFU/mL** | Biomass, $OD_{560}$ | $Q_{GP}$, mg/g |
| *O. anthropi* GPK 3 | | | | | |
| NaCl, 0.8% | 6.0 | 69 | $9.9 \times 10^{7}$ | 6.1 | 72 |
| Ascorbate, 0.3% | 6.0 | 69 | $1.3 \times 10^{9}$ | 5.7 | 74 |
| Thiourea, 1% | 6.7 | 68 | $8.5 \times 10^{8}$ | 6.4 | 73 |
| Glutamate, 2 % | 6.6 | 53 | $1.7 \times 10^{9}$ | 6.1 | 57 |
| Control* | 6.5 | 73 | | | |
| *Achromobacter* sp. Kg 16 | | | | | |
| Glutamate, 2% | n/d | n/d | $1.4 \times 10^{8}$ | 1.6 | 36 |
| Control* | 1.7 | 39 | | | |

Notes: * Monthly transfers of the culture to the fresh agar medium with GP, storage at 4°C.
** An initial cell titer for *O. anthropi* GPK 3 was $3–5.4 \times 10^{11}$, for *Achromobacter* sp. Kg 16, $2.4 \times 10^{10}$ CFU/mL. n/d, stands for "not determined."

the carbon and nitrogen sources for the growth of bacteria was found to depend strongly on the taxonomical position of these strains. *O. anthropi* GPK 3 utilized a wide range of the carbon-containing substrates (carbohydrates, organic acids, alcohols, and glutamate), while *Achromobacter* sp. Kg 16 did not assimilate carbohydrates and alcohols. Both strains utilized nitrogen of the glutamate, and ammonium nitrogen of the mineral salts. Nitrate nitrogen and urea nitrogen were used by strain Kg 16 but not by strain GPK 3. For both strains, glyphosate acted only as a source of phosphorus but not as a carbon or nitrogen source. Preliminary adaptation of the cells to this phosphorus source in the inoculum was required for their maximal degradative activity. The relation between an initial specific load of the substrate and the characteristics of growth and substrate degradation during cultivation was established. When the substrate-specific load was high, the maximal specific growth rate increased and the lag phase duration decreased. Duration of the cultivation decreased in general, and the efficiency of the toxicant decomposition increased. For each strain, a liquid form of the preparation containing stabilizers was developed. The stabilizers facilitated the preservation of viability and functional activity of the degraders during long-term storage of the biomass within a wide range of temperatures. These data represent an important stage of study and must be taken into account

when developing the technology concerning remediation of the soil polluted with glyphosate.

## ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, project no. 09-04-00320.

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