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SOIL BIOLOGY

The Impact of Hydrogen Emission on the Structure of Soil Microbial Biomass

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Abstract—Population density and biomass of microorganisms were studied in background soils and in soils affected by hydrogen degassing. These parameters were lower in the latter soils. Actinomycetes and fungal mycelium could not be isolated from the soils treated with hydrogen already on the fourteenth day of the experiment; the number and biomass of fungal spores decreased to zero levels even earlier, on the seventh day. Fungi represent a specific physiological group, and their capacity for environmental adaptation is much lower than that of bacteria.

Keywords: soil, hydrogen emission, bacteria, fungal and actinomycetic mycelium, population density and biomass

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INTRODUCTION

Geologists recorded the increasing hydrogen emission from the Earth's interior at the end of the 20th century. The problem of the genesis of deep-earth gases can only be solved on the basis of cosmogonic concept of the origin of the solar system [8]. The idea of deep origin of endogenous gases is shared by most of modern researchers; the discussions concern the intensity of their emission and the core or mantle source of these gases. Rift zones—huge splits of the lithosphere forming a single system—serve as the major channels of the Earth degassing, via which the gases dissolved in the outer core of the planet come to the surface on the continents and in the oceans. A remarkable feature of the deep degassing of the plant is its temporal and spatial instability [8].

Hydrogen degassing is believed to be related to many planetary phenomena, including the tectonic and magmatic activity, the depletion of the ozone layer [23], and degradation of the soil layer. In the zones of discharge of deep hydrogen to the surface, depressions related to surface subsidence have been discovered. They are marked by light-colored rings in places of the direct emission of hydrogen fluxes. According to Sukhanova, hydrogen exerts the destructive effect on the soil humus, and the upper soil horizon has a lighter (light gray) color in such places [22].

Soil microorganisms with their capacity for self-regulation can serve as sensitive indicators of various natural and anthropogenic impacts on the soil cover. Data on the quantity, composition, and potential and actual activities of soil microflora are necessary for adequate understanding of the mechanisms regulating the composition and functioning of the microbial community and for controlling the growth and activity of microorganisms participating in the decomposition of various hazardous substances in soils [6, 26].

Temporal soil waterlogging is often observed in the local depressions in places of hydrogen efflux [3, 21]. According to available data, the activity of microbial communities decreases in seasonally waterlogged soils (chernozems, meadow-chernozemic soils, and chernozemic-meadow soils) of the Kamennaya Steppe (chernozems, meadow chernozem soils, and chernozem meadow soils) with an increase in the degree of hydromorphism; at the same time, the number of colonies of nitrogen-fixing bacteria from the genus *Azotobacter* increases; these bacteria can fix mineral nitrogen and synthesize biologically active substances. The total number of microorganisms in the A horizon (0–20 cm) in the chernozemic-meadow soil of the depression reached 69.7 million CFU per 1 g of absolutely dry soil and was somewhat higher than that in the ordinary chernozem (61.3 million CFU per 1 g of absolutely dry soil) [24].

In connection with this, data on the structure of soil microbial communities in the samples taken from such zones do not allow us to argue that it is hydrogen that affects microorganisms; we should consider the combined action of both factors: temporal waterlogging and hydrogen degassing. In such soils, the total microbial biomass sharply decreases, and the contribution of bacteria to the total microbial biomass becomes greater [18]. To estimate the impact of hydrogen, we performed

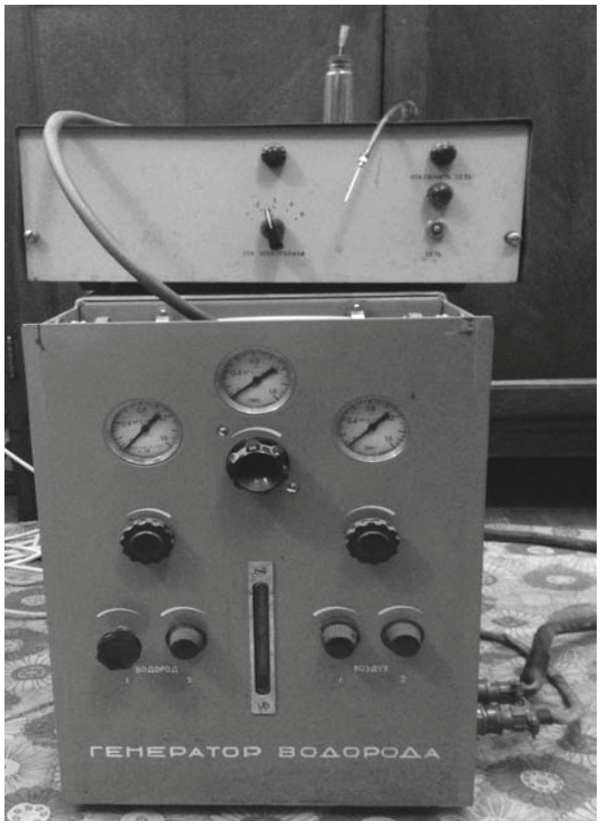

Fig. 1. Hydrogen generator SGS-2.

a laboratory experiment: hydrogen was passed through the soil, in which the numbers, structure, and biomass of soil microorganisms were determined.

In this paper, we analyze the impact of hydrogen on the numbers and biomass of soil microorganisms (bacteria, actinomycetes, and fungal mycelium and spores) and on the size of bacterial cells in an ordinary chernozem.

OBJECTS AND METHODS

We studied an ordinary medium-humus silt loamy chernozem developed from loesslike calcareous loam on the territory of the Dokuchaev Research Institute of Agriculture in the Central Chernozemic Region (Voronezh oblast). The samples were taken under a shelterbelt from the depth of 0–50 cm (A horizon) [4].

Hydrogen was obtained with the help of hydrogen generator SGS-2 (Fig. 1). Soil samples (5 g) were placed into 15-mL penicillin flasks; the flasks were hermetically sealed with rubber plugs, and the gas phase above the soil was replaced by molecular hydrogen. Hydrogen flows (100 mL/min) were passed through the soil for 5 min each two days (48 h). The samples were incubated in a thermostat at 28°C for 30 days; the samples for the analysis were taken on at the beginning of the incubation and on the 3rd, 7th, 14th, 21st, and 30th day. The samples were taken in triplicate to provide data reliability.

Ultrasonic dispersion in a low-frequency disperser UZDN-1 (22 kHz, 0.44 A, 2 min) was used as the main procedure of preliminary treatment of the samples for the microbiological analysis [5].

Methods of counting microorganisms. The total number of microorganisms was determined with the help of luminescent microscopy. The specimens were prepared by conventional method [10]. Suspensions of soil samples were placed on carefully degreased specimen slides (0.02 mL for bacterial specimen, 0.04 mL for fungal specimen) and uniformly spread with a loop on the area 4 cm^2. The specimens were fixed in the burner flame after complete drying. Twelve specimens were prepared for every sample. The specimens for bacteria counting were stained with acridine orange solution (1 : 10000, during 2–3 min); the specimens for counting fungal spores and mycelium were stained with calcofluor white during 15 min [12].

The number of cells (mycelium) per 1 g soil was calculated according to the following equation: $N = S_1 \times a \times n/v \times S_2 \times c$, where N is the number of cells (mycelium length, μm) per 1 g soil; S_1 is the specimen area (μm^2); a is the number of cells (mycelium length, μμm) per visual field (with data averaging for all the specimens); n is the dilution of the soil suspension (mL); v is the volume of a drop placed on a slide (mL); S_2 is the area of visual field μm^2); and c is the weighed soil portion (g).

Taking into account the measured diameter of spores and fungal mycelium, actual biomass was calculated according to the following formulae: for spores, $0.0836\ r^3 \times 10^{-11}$ g, for mycelium, $0.628\ r^2 \times 10^{-6}$ g) [11]. Bacterial biomass was calculated taking into account the fact that the dry biomass of one bacterial cell of 0.1 μm^3 in volume is 2×10^{-14} g [7].

Standard deviation (σ_{n-1}) did not exceed 10% for the estimated number of bacteria and 15% for the estimated fungal mycelium and spores and for actinomycetic mycelium.

The method of cascade filtration of soil suspension was used to determine bacterial size. We used nuclear filters with pore diameters 1.85; 1.45; 0.45; 0.38; 0.23 and 0.2 μm [14]. Intrinsic luminescence of the filters was quenched by staining with Sudan black saturated alcohol solution (Germany) [25]. Nuclear filters were placed into this solution for indefinite time. Filters were taken out, rinsed in boiled water, dried, and used for filtering. Filtering was carried out using Bunsen flask connected to water-jet pump. Four layers of filtering paper were placed on the surface of filter holder, nuclear filter was placed above and pressed down to the device surface with a metal ring; 1 mL of soil suspension and 9 mL of sterile water were added. Then, the solution was filtered successively filtered the filters with different mesh sizes (from larger sizes to smaller sizes).

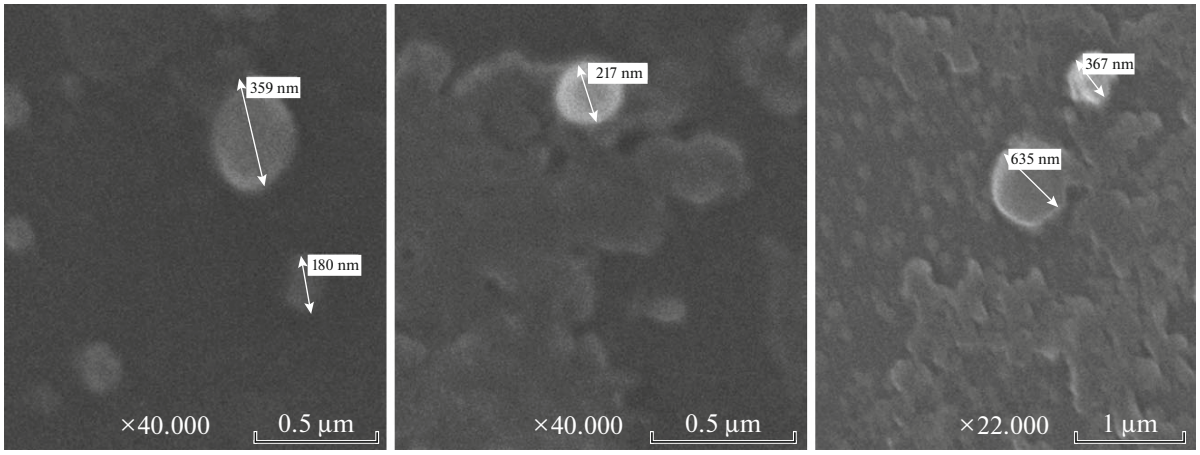

Fig. 2. Cells from the filtrates of typical chernozem (cropland) determined by the method of scanning electron microscopy (according to [13]).

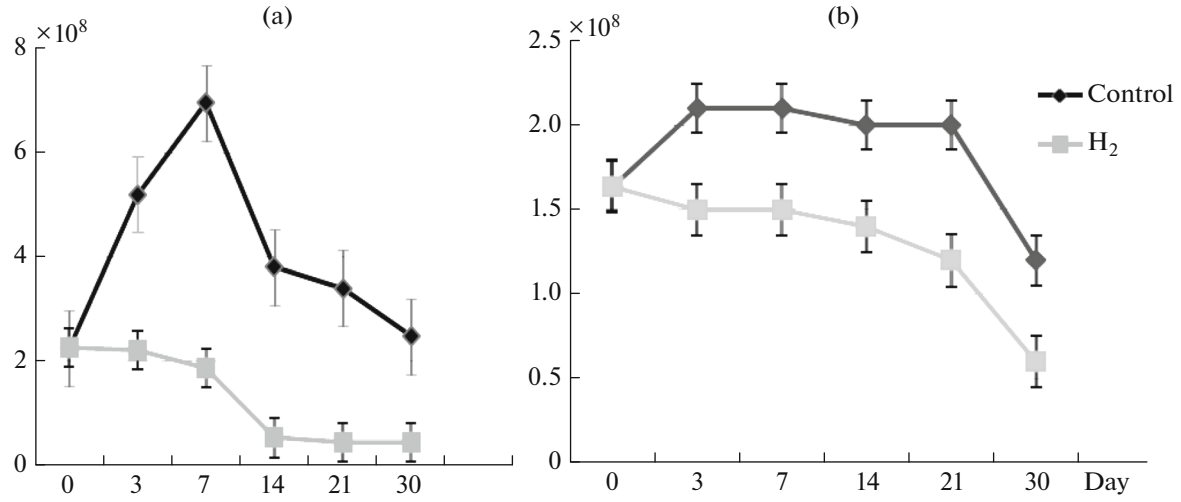

Fig. 3. Numbers of bacteria in the control and hydrogen-treated samples determined by the methods of (a) luminescent microscopy (on glass slides and (b) cascade filtration (on filters).

We used two filters with mesh size 1.85 μm. The first filter was used to separate large soil particles from the suspension, and bacterial cells were counted on the second filter.

It was accepted for further calculations that the cells have a spherical shape (Fig. 2), because most cells have precisely this shape; the portion of rod-shaped cells was insignificant and did not affect further calculations.

The following equation was used to count the numbers of bacteria according to the method of cascade filtration with due account for the areas of the filters and the visual field of the microscope:

$$N_b = a \times 1.13 \times 10^7.$$

Taking into account the dry weight of a bacterial cell equal to 2×10^{-14} g, the total biomass of the cells was calculated as follows:

$$B_b = 3/4\pi r^3 \times 2 \times 10^{-14}/(0.1 \times N_b),$$

where b is the size of each fraction, r is the cell radius (μm), N is the number of cells (cells/g), and B is the biomass (μg).

Average volume of one cell was calculated by the formula: $V = B_{tot}/(N_{tot} \times a)$,where B_{tot} and N_{tot} are the biomass and total number of the cells in all the fractions, a is the density of one cell (1×10^{-12} g/cm^3), and V is the average volume of one cell (μm^3) [17].

RESULTS AND DISCUSSION

The results obtained by the methods of luminescent microscopy and cascade filtration demonstrated a decrease in the population density of bacteria in the soils treated with hydrogen in comparison with the control soils (Fig. 3). The maximum difference was observed on the 21st day with the use of the method of luminescent microscopy. On the 30th day, the number of bacterial cells in the background (control) soil

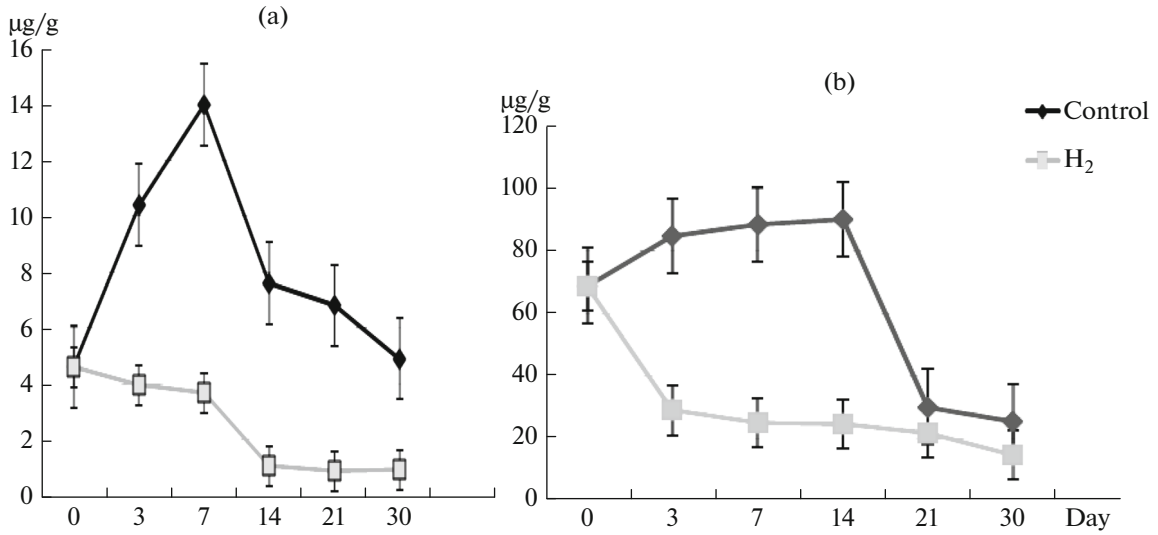

Fig. 4. Bacterial biomass calculated on the basis of data of bacterial accounting on (a) glass slides and (b) filters in the control and hydrogen-treated samples.

exceeded that in the soil treated with hydrogen by eight times. The maximum number of cells in the control soil (6.9×10^8) was observed on the 7th day. Then, the number of cells as determined by counting on glass slides decreased significantly and comprised no more than 1×10^8 cells in the soil treated with hydrogen and about 3×10^8 cells in the control soil.

The biomass of bacteria determined by the method of cascade filtration method was higher than the biomass of bacteria determined by the method of luminescent microscopy. This can be explained by the fact that the volume of one bacterial cell in the second case is traditionally accepted to be 0.1 μm^3, whereas in the first case counted bacteria with different volumes, and the total bacterial biomass is mainly determined by large cells with diameters of 1.85 and 1.43 μm (Fig. 4). A small average diameter of the cells (table) can be explained by the following factors: first, cultivation temperature 28°C acted as a stress factor suppressing the development and growth of the cells; second, anaerobic environment could exert a negative influence on the size of the cells [2]. As follows from published data, the deficit of nutrients may also affect the size of the cells [1, 9]. On the 14th day, the bacterial biomass determined by the method of luminescent microscopy in the control soil was equal to 5.4 μg/g (taking into account the cell volume of 0.1 μm^3 and the cell number of 7×10^8). Taking into account the calculated average cell volume of 0.041 μm^3 (table), it was equal to 2.25 μg/g. As determined by the method of cascade filtration, it amounted to 90.1 μg/g (Fig. 4). Even if we calculate the biomass on the basis of data on the calculated cell volume presented in the table, it will remain lower in the soil treated with hydrogen.

Average volume of bacterial cells (μm^3) in the control and hydrogen-treated soil samples

Soil	Day of incubation					
	0	3	7	14	21	30
Control	0.044	0.052	0.032	0.041	0.026	0.028
Hydrogen-treated	0.044	0.027	0.025	0.034	0.025	0.023

As seen from Fig. 5, both the length and the biomass of fungal mycelium in the control soil increased on the third day of incubation up to 825 m/g and 3.1 mg/g, respectively. Then, they sharply decreased and reached zero values in the soil treated with hydrogen on the 14th day. The length of fungal mycelium remained at the level of 200 m/g in the control soil, and the mycelial biomass decreased by seven times in comparison with the beginning of the experiment.

The number and biomass of fungal spores (Fig. 6) in the samples reached zero values already on the seventh day. In general, the number and biomass of fungal spores and the length and biomass of mycelium in the chernozem treated with hydrogen were smaller than those in the control on the 3rd–7th days of the experiment and could not be determined later. The capacity of prokaryotes (with a much higher diversity of their physiological characteristics for developing specific groups tolerant to the influence of hydrogen proved to be higher than that of fungi. This was the reason for a higher suppression of fungi in comparison with bacteria under the impact of hydrogen. Fungi represent a very "compact" physiological group, and their capacity for adaptation to the environment is much lower than that of bacteria. This is also true for other kinds of impact on the soils [15, 16, 19, 20].

The presence of actinomycetic mycelium (Fig. 7) in the studied samples was insignificant; it reached

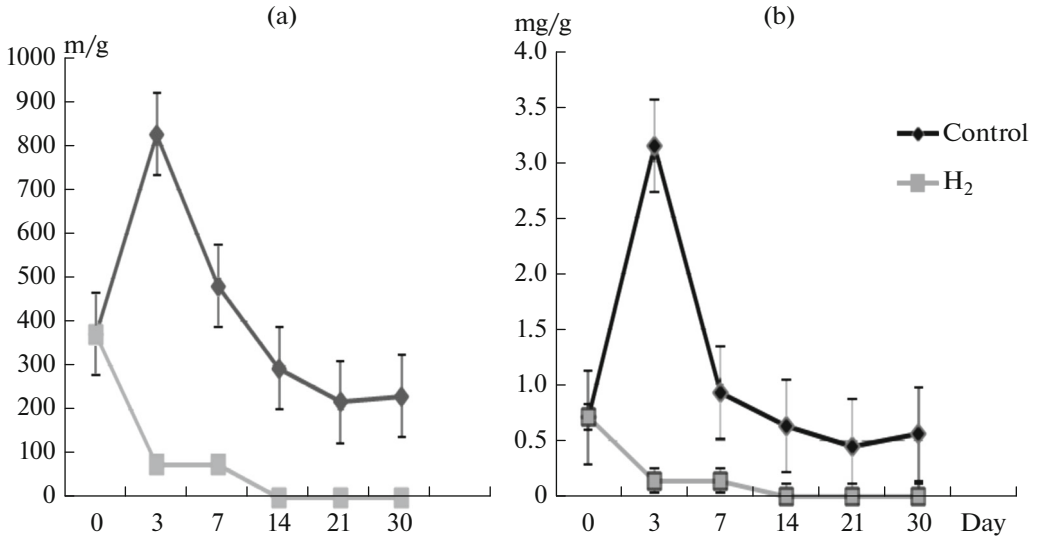

Fig. 5. The (a) length and (b) biomass of fungal mycelium in the control and hydrogen-treated soil samples.

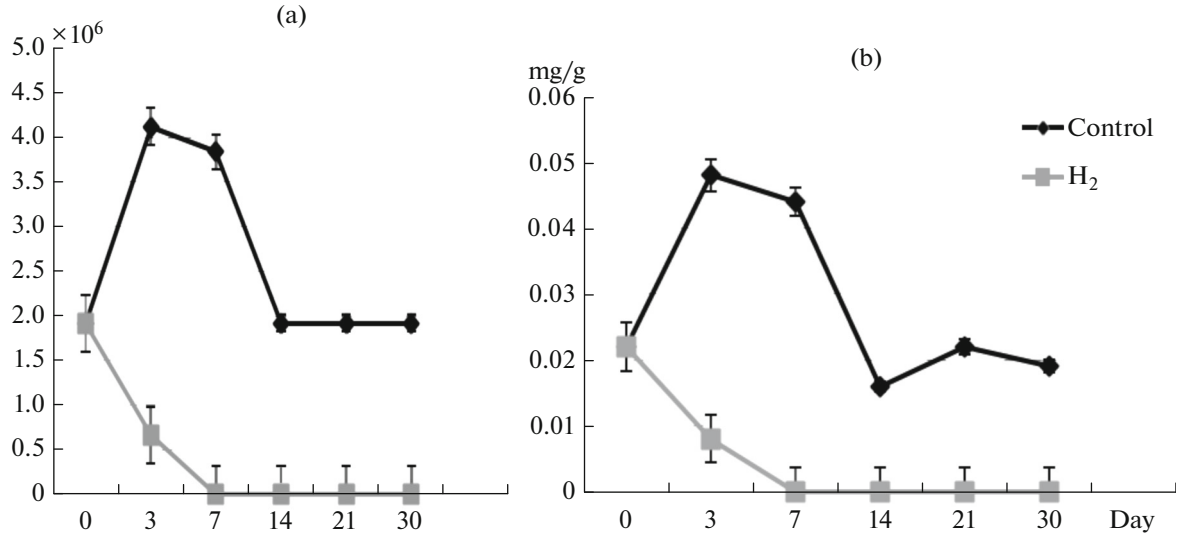

Fig. 6. The (a) number and (b) biomass of fungal spores (counted on slides) in the control and hydrogen-treated soil samples.

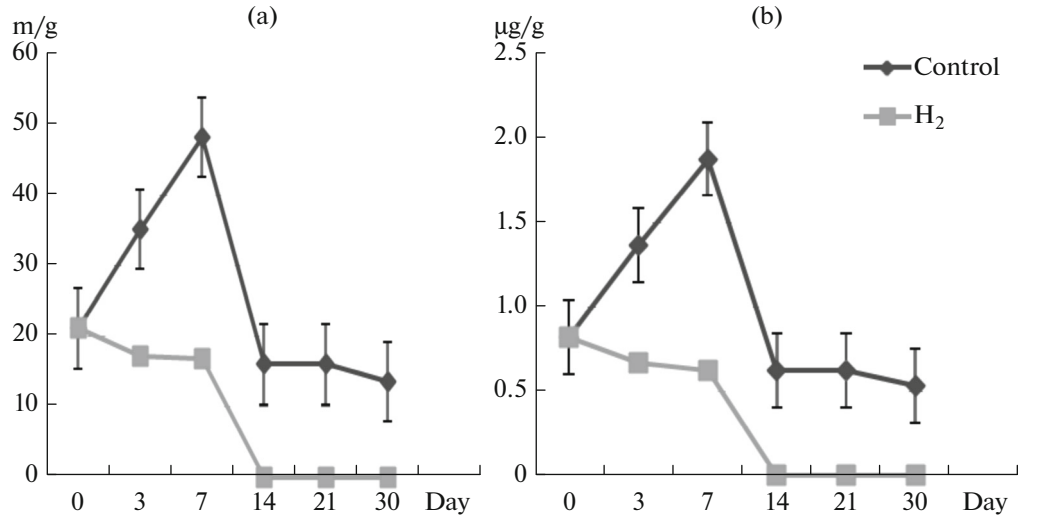

Fig. 7. The (a) length and (b) biomass of actinomycetic mycelium in the control and hydrogen-treated soil samples.

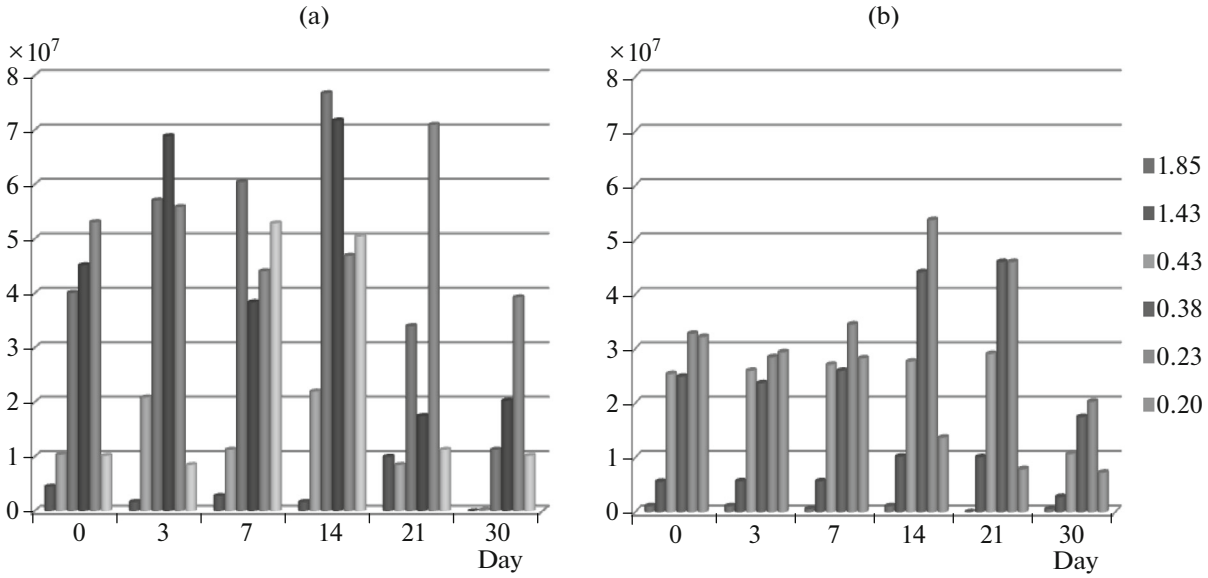

Fig. 8. Distribution of bacterial cells by size (μm) in the (a) control and (b) hydrogen-treated soil samples.

50 m/g on the seventh day of incubation in the control soil and disappeared from the soil treated with hydrogen on the 14th day. The biomass of actinomycetes was also very small. However, the inhibiting activity of hydrogen on the number and biomass of actinomycetes (as well as on the number and biomass of bacteria and fungi) could be clearly seen in our experiment.

The diameter and volume of bacterial cells in the chernozem treated with hydrogen were slightly lower than those in the control soil. However, on the 21st day of incubation, these parameters in the hydrogen-treated soil were even higher than those in the control soil.

Cell-size distribution obtained by the method of cascade filtration (Fig. 8) demonstrated that the group of small bacteria (with sizes of 0.20 and 0.23 μm) predominated in the soil treated with hydrogen; in the control soil, bacterial cells of 0.38 and 0.43 μm in size predominated. The maximum numbers of bacterial cells in both soils were reached on the 14th day of incubation. In the control soil, they were 1.5–3 times higher than those in the soil treated with hydrogen.

CONCLUSIONS

Hydrogen inhibited the development of soil microorganisms in an ordinary chernozem: the numbers and biomass of bacteria, fungal spores and mycelium, and the length of actinomycetic mycelium decreased in the hydrogen-treated soil samples in comparison with those in the control samples. Our results suggest that hydrogen surely affects the microbial community of the soils of depressions in places of hydrogen emission from the Earth's interior and with temporary soil waterlogging. However, the negative role of soil waterlogging on the state of soil microbial communities in such places cannot be excluded.

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