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EXPERIMENTAL ARTICLES

Thiocyanate Decomposition under Aerobic and Oxygen-Free Conditions by the Aboriginal Bacterial Community Isolated from the Waste Water of a Metallurgical Works

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Abstract—A mesophilic alkalitolerant aboriginal bacterial community capable of autotrophic thiocyanate decomposition under aerobic and oxygen-free conditions was isolated from reused water of a metallurgical works. The growth of the aboriginal bacterial community was optimal at pH 9.0. Ammonium and sulfate were the end products of thiocyanate decomposition under both aerobic and oxygen-free conditions. Under oxygen-free conditions, thiocyanate decomposition occurred in the presence of nitrate. Nitrite was accumulated as an intermediate product in the course of denitrification, and was subsequently used as an electron acceptor for thiocyanate oxidation. Dinitrogen was the end product of denitrification.

Key words: aboriginal community, industrial waste, oxygen-free thiocyanate oxidation.

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Among the components of the waste water of metallurgical works, thiocyanate is the hardest to remove; while its admissible concentration limit is the same as that of cyanide (0.05 mg/l), thiocyanate concentrations in the waste water are hundreds of times higher than the cyanide concentrations. Microbiological methods for decomposition of toxic substances are presently the most economical and environmentally friendly. Therefore, the search for aboriginal microorganisms capable of decomposing toxic substances in industrial waste remains topical.

A number of heterotrophic bacteria utilize thiocyanate as a source of nitrogen and sulfur [1–3]. The number of bacteria known to be able to oxidize thiocyanate to obtain energy is scarce. Apart from several *Thiobacillus thioparus* strains, these are *Thioalkalivibrio paradoxus* and *Tv. thiocyanoxidans* [4–7].

Although a considerable amount of information on thiocyanate decomposition by bacteria is presently available, little is known about the possibility of its anaerobic oxidation. In early publications of De Kruyff (1957) [8], *Thiobacillus denitrificans* was reported to grow with thiocyanate either aerobically or anaerobically, in the presence of nitrate as an electron acceptor; in the latter case, nitrate was reduced completely to N_2. *Th. thioparus* was only able to reduce nitrate to nitrite in the presence of thiocyanate. In subsequent works, *Th. denitrificans* was demonstrated to grow under anaerobic conditions by oxidizing sulfur compounds in the presence of nitrates [9].

Sorokin isolated two strains of obligate chemolithoautotrophic sulfur-oxidizing bacteria (ARhD1^T and ARhD2) from the sediments of soda lakes in the presence of thiocyanate and nitrate at pH 9.9 [10]. The isolates could grow aerobically or anaerobically with thiocyanate or thiosulfate as electron donors and nitrate or nitrite as electron acceptors. Cyanate was revealed as an intermediate product of thiocyanate oxidation; sulfate, ammonium, and gaseous dinitrogen were the end products:

$$5SCN^- + 8NO_3^- + H_2O \longrightarrow 5SO_4^{2-} + 4N_2 + 5CNO^- + 2H^+$$

$$5CNO^- + 5HCO_3^- + 10H^+ \longrightarrow 5NH_3 + 10CO_2$$

$$5SCN^- + 8NO_3 + H_2O + 8H^+ + 5HCO_3 \longrightarrow 5SO_4^{2-} + 5NH_3 + 10CO_2 + 4N_2.$$

The strains ARhD1^T and ARhD2 were described as new strains of the genus *Thioalkalivibrio* with a high similarity to *Tv. denitrificans*. The name *Thioalkalivibrio thiocyanodenitrificans* was proposed for these strains [10].

The goal of the present work was to isolate from reused water of a metallurgical works a bacterial community capable of thiocyanate destruction under aerobic and anaerobic conditions in the absence of sources of organic carbon.

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MATERIALS AND METHODS

Subjects of the study. Enrichment cultures from reused water after wet purification of the gases of a blast furnace of the Kosogorsk metallurgical works (Tula, Russia) were used as the subjects. The average composition of the waste of the reused water is shown in the table.

Cultivation media and conditions. For obtaining enrichment cultures and determination of their kinetic growth parameters and optimal cultivation conditions under oxygen-free and aerobic conditions, the liquid synthetic waste was used containing the following (g/l): $K_2PO_4 \cdot 3\ H_2O$, 1.0; $NaHCO_3$, 6.0; Na_2CO_3, 1.0; NaCl, 2.0; $NaNO_3$, 1.3 (under oxygen-free conditions); $MgCl_2 \cdot 6\ H_2O$, 0.5; KSCN, 0.7; $Ca(NO_3)_2 \cdot 4\ H_2O$, 0.05; pH 8.9–9.0.

Under oxygen-free conditions, the bacteria were cultivated in 500-ml vials sealed with rubber stoppers. The medium was poured to form a high layer (the medium volume was 470 ml). Under aerobic conditions, the bacteria were cultivated in 250-ml Erlenmeyer flasks with 100 ml of the medium on a temperature-controlled Heidolph unimax 1010 shaker (Germany) at 200 rpm. In order to avoid inhibition by the intermediate products of decomposition (nitrite) and excessive gas formation, thiocyanate and nitrate were added to the medium in small amounts after their consumption.

For determination of the optimal pH values for the growth of the bacterial community within the 6.0–8.0 range, a phosphate buffer was used (supplemented with 0.08 M Na^+ = 4.7 g/l NaCl).

Analytical methods. Bacterial growth was assessed by the method of direct cell count under a LYUMAM I-1 microscope at 1440× magnification (×90×1.6×10). Thiocyanate as ferrocyanate [11], nitrate [12], nitrite [13], and ammonium with the Nessler reagent [13] were determined colorimetrically (a KFK-3 photometer, Russia). Sulfate formation in the final samples was determined qualitatively using a Biotronik LC 500 liquid chromatograph. The content of gaseous nitrogen and oxygen was determined on an M 3700 gas chromatograph. The pH values were determined using a pH-150m device (Belarus).

RESULTS

Thiocyanate decomposition by the aboriginal bacterial community isolated from reused water under oxygen-free conditions. The enrichment culture of autotrophic thiocyanate-decomposing bacteria was obtained from the waste (the composition is shown in the table).

The sample of reused water was incubated under oxygen-free conditions in the presence of nitrate at 28°C for 30 days. On the 14th day, medium turbidity and gas production were observed. Microscopy revealed a bacterial community consisting of small- and medium-sized motile rods.

Composition of reused water after wet purification of the gases of the blast furnace

Ingredients	Results of analysis
Cyanide	9.0 mg/l
Thiocyanate	1.0 g/l
Ammonia	130 mg/l
Suspended matter	0.15 g/l
pH	8.9
Total alkalinity	430 mg-equiv/l
Mineral residue	25.0 g/l
Chlorides	2.0 g/l
Sulfates	0.8 g/l
Nitrates	10 mg/l
Iron	4.9 mg/l

As follows from the results shown in Fig. 1, pH 9.0 is the optimum for the growth of the aboriginal bacterial community cultivated on synthetic waste and in the sample of reused water.

The kinetic parameters of growth on SCN^- and its decomposition by the aboriginal community were studied at 28°C and pH 9.0 on synthetic waste and in a sample of reused water with thiocyanate under oxygen-free conditions upon denitrification (Figs. 2, 3).

The results obtained demonstrate that the bacterial community consumed thiocyanate to produce ammonium. Nitrate was reduced to nitrite, which was also utilized by the community in the process of oxygen-free

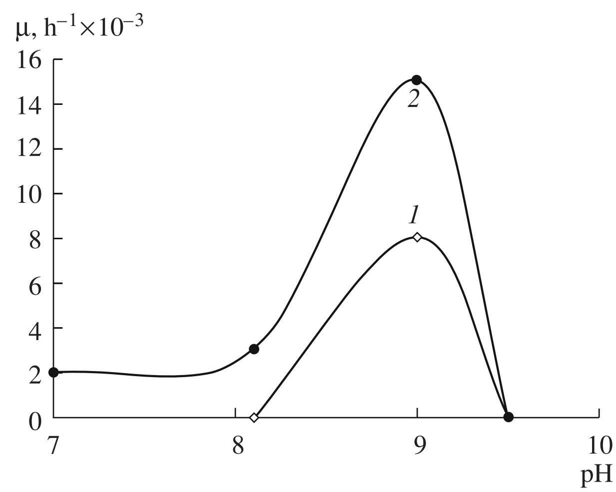

Fig. 1. Maximal specific growth rate (μ, $h^{-1} \times 10^{-3}$) of the aboriginal bacterial community under oxygen-free conditions depending on pH: on synthetic waste (*1*) and reused water (*2*).

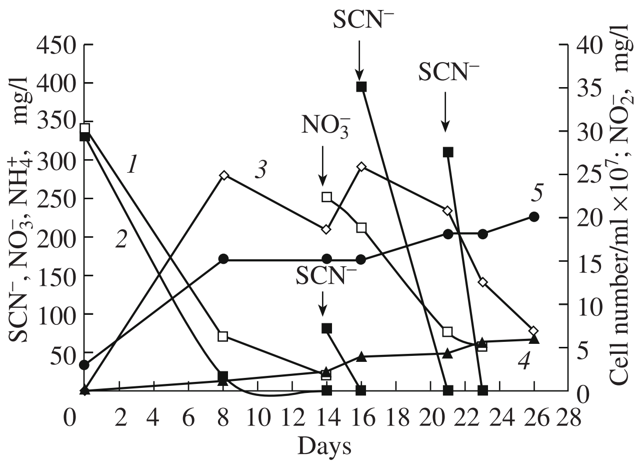

Fig. 2. Thiocyanate decomposition by the aboriginal autotrophic bacterial community via denitrification under oxygen-free conditions ($\mu = 0.008$ h^{-1}) on synthetic waste. NO_3^-, mg/l (*1*); SCN^-, mg/l (*2*); NO_2^-, mg/l (*3*); NH_4^+, mg/l (*4*); cell number/ml × 10^7 (*5*).

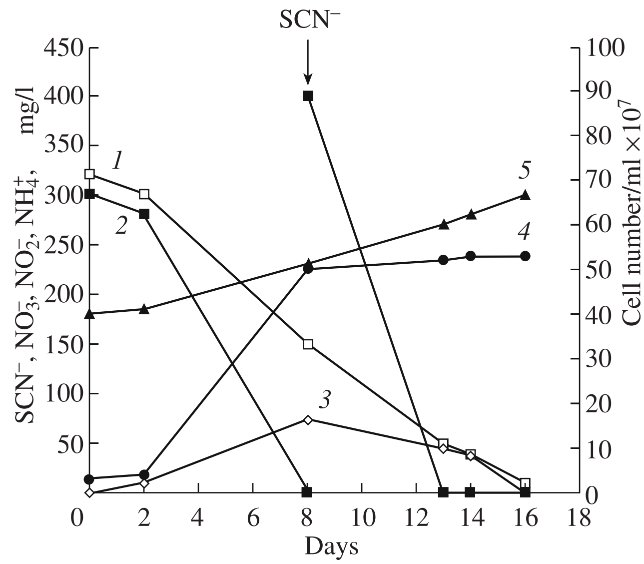

Fig. 3. Thiocyanate decomposition by the aboriginal autotrophic bacterial community under oxygen-free conditions ($\mu = 0.015$ h^{-1}) in the reused water sample. NO_3^-, mg/l (*1*); SCN^-, mg/l (*2*); NO_2^-, mg/l (*3*); NH_4^+, mg/l (*4*); cell number/ml × 10^7 (*5*).

thiocyanate oxidation. The maximum specific growth rate was 0.008 and 0.015 h^{-1} for the cultivation on synthetic waste and reused water, respectively. At the optimum pH, the rate of thiocyanate destruction was as high as 1.38 mg/l h in synthetic waste and 1.56 mg/l h in reused water. In the course of denitrification, nitrite was reduced to gaseous nitrogen; after 30 days, the concentration of the latter increased by more than an order of magnitude. Sulfate was revealed in the medium as the sulfur-containing end product of thiocyanate decomposition.

The data obtained demonstrates that the isolated bacterial community was capable of decomposing thiocyanate under oxygen-free conditions both on synthetic medium and in reused water of the metallurgical works (on addition of nitrate).

Thiocyanate decomposition by the aboriginal bacterial community under aerobic conditions. The isolated bacterial community was capable of autotrophic thiocyanate decomposition under aerobic conditions.

Synthetic waste with SCN^- and a sample of reused water with SCN^- were inoculated with the enrichment culture of autotrophic thiocyanate-decomposing bacteria. The experiments were carried out at 28°C and pH 8.9–9.0. The kinetic parameters of growth of the autotrophic aboriginal bacterial community and SCN^- decomposition under aerobic conditions on synthetic waste with thiocyanate are shown in Fig. 4. It can be seen from the figure that the bacterial community consumed thiocyanate with the formation of ammonium, similar to the case of thiocyanate decomposition under oxygen-free conditions. The maximum specific growth rate was 0.03 h^{-1}. Thus, under aerobic conditions the growth rate on synthetic waste was higher than under oxygen-free conditions (0.008 h^{-1}).

Similar patterns were observed in the experiment when thiocyanate decomposition was studied under aerobic autotrophic conditions in a sample of reused water (Fig. 5). The results of the experiment showed that ammonium accumulated in the medium in the course of thiocyanate decomposition. The maximal specific growth rate reached 0.048 h^{-1} and was compa-

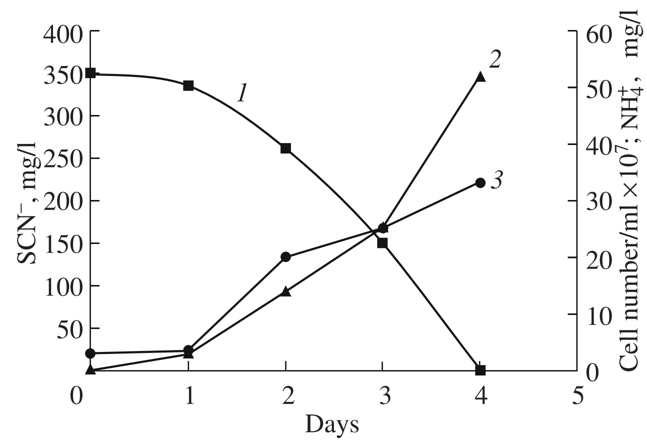

Fig. 4. Thiocyanate decomposition by the aboriginal bacterial community under aerobic autotrophic conditions at pH 9.0 ($\mu = 0.03$ h^{-1}) on synthetic waste. SCN^-, mg/l (*1*); NH_4^+, mg/l (*2*); cell number/ml × 10^7 (*3*).

rable to the growth rate on synthetic waste. The rate of thiocyanate decomposition under aerobic conditions at the optimal pH was as high as 3.65 mg/l h on synthetic waste and 4.58 mg/l h in reused water. Microscopy revealed the bacterial community to be similar to that obtained under oxygen-free conditions on synthetic waste and reused water.

In order to confirm the identity of both communities, cross transfers of the communities grown under aerobic or oxygen-free conditions were carried out. Growth on thiocyanate and its decomposition were observed in both cases.

Thus, the isolated bacterial community was capable of thiocyanate decomposition under both aerobic and oxygen-free conditions (by denitrification).

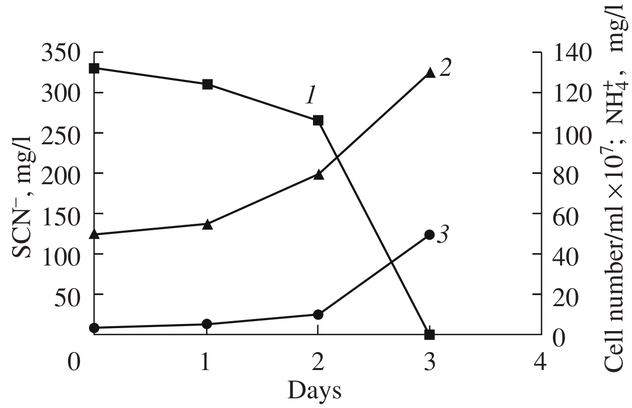

Fig. 5. Thiocyanate decomposition by the aboriginal bacterial community under aerobic autotrophic conditions at pH 9.0 (μ = 0.048 h^{-1}) in reused water. SCN^-, mg/l (*1*); NH_4^+, mg/l (*2*); cell number/ml × 10^7 (*3*).

DISCUSSION

The isolated bacterial community was formed in the waste disposal system consisting of primary settling tanks, in which bulky suspended matter (sludge) is settled from reused water, and the sludge trap, in which a partial process of waste self-purification takes place. The depth of the sludge trap (3–4 m) is sufficient for development of oxygen-free conditions. High thiocyanate concentrations create a nutrient medium for thiocyanate-utilizing bacteria both under aerobic (surface layers) and oxygen-free conditions (in the depth). A low nitrate content in reused water, the predominance of an oxygen-free zone in the sludge trap, and low average annual temperatures do not allow the self-purification process to proceed in full measure.

High mineralization of the waste (the mineral residue was 25 g/l), high pH values (8.9–9.4), and the presence of cyanide were also specific features of the conditions for the formation of the bacterial community.

The bacterial community isolated by us was capable of thiocyanate decomposition both under aerobic and oxygen-free conditions in the presence of nitrate (the molar SCN to NO_3 ratio was 2 : 1). In the course of the work, the community was cultivated on synthetic waste very close in composition to the reused water of the works. The isolated aboriginal bacterial community successfully oxidized thiocyanate (aerobically and under oxygen-free conditions) on synthetic and industrial waste. When the bacterial community was cultivated on synthetic waste with thiocyanate, the maximal specific growth rate was lower than in the samples of reused water (0.008 and 0.015 h^{-1}, respectively), which might result from the presence of organic substances at low concentrations (about 0.3 g/l of the glucose equivalent). Ammonium and sulfate were the end products of thiocyanate decomposition in both synthetic waste and reused water. Nitrite was formed in the course of denitrification, and it was subsequently utilized by the bacterial community for further thiocyanate oxidation, resulting in formation of dinitrogen gas.

The aboriginal bacterial community isolated from the waste of the metallurgical works can be described as an alkalitolerant culture (the optimum pH for growth is 9.0). This circumstance allowed the authors to suggest that the strains in this community differed from the previously isolated neutrophilic (*Th. denitrificans, Th. thioparus*) [9] and alkaliphilic (*Tv. thiocyanodenitrificans, Tv. denitrificans*) [14] cultures capable of oxygen-free thiocyanate oxidation.

Thus, it was shown in the course of the studies that reused water contained bacterial communities capable of thiocyanate detoxification under aerobic and oxygen-free conditions. However, aerobic conditions were preferable, because, for the process to proceed under oxygen-free conditions and lead to removal of all thiocyanate (17.2 mM), a higher content of nitrate is required than the concentration present in the waste.

Additional introduction of nitrate (or nitrate-containing waste) may activate the process of thiocyanate decomposition in the sludge trap under oxygen-free conditions.

The object of our further work is to identify the strains present in the isolated bacterial community.

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