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**EXPERIMENTAL ARTICLES**

# Phenotypic Characteristics of *Thiobacillus ferrooxidans* Strains

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**Abstract**—*Phenotypic* polymorphism of *Thiobacillus ferrooxidans* strains isolated from various ecological niches was studied. The strains differed both in rates of growth and oxidation of $Fe^{2+}$, $S^0$, $FeS_2$, and sulfide minerals contained in concentrate. Each strain, irrespective of its original environment, required a period of adaptation to a new substrate. Strains TFN-d, TFBk, TFO, and TFL-2, isolated from ores and concentrates rich in oxidizable substrates, showed an equal adaptation rate (five culture transfers) but differed in their adaptation efficiency. Strain TFV-1, isolated from low-grade ore and showing the lowest rates of growth and oxidation of all the substrates, required five culture transfers to adapt to $S^0$ and $FeS_2$ and seven culture transfers to adapt to the concentrate. It is concluded that the phenotypic properties of the strains correlate with their genotypic polymorphism and the environmental conditions under which their microevolution took place.

*Key words*: strains of *Thiobacillus ferrooxidans*, oxidized substrate, adaptation, growth and substrate oxidizing activity, strain polymorphism, microevolution.

The chemolithotrophic, gram-negative acidophilic bacterium *Thiobacillus ferrooxidans* oxidizes ferrous iron, elemental sulfur and its reduced compounds, and sulfide minerals. This organism plays a key role in microbial communities taking part in bacterial-chemical processes of gold recovery and metal leaching from ores and concentrates under mesophilic conditions. In natural environments, the major habitat of *T. ferrooxidans* is the deposits of sulfide ores, which are known to differ widely in their composition of minerals and concentration of metal ions and toxic elements accumulated in the liquid phase of oxidation.

The diversity of *T. ferrooxidans* strains (isolated from natural environments) and the physiological characteristics of the strains adapted to different substrates were described by several researchers [1–3]. Strains of *T. ferrooxidans* were shown to differ in their pH and temperature growth optima, to have different resistance to heavy metal ions and toxic elements, and to differ in the oxidation rate of the same substrates. Even the electrode potential of sulfides depended on the strain present. The biological mechanisms underlying this diversity of strains is not well understood. One thing clear is that it has to do with the specific conditions that the strains experienced in various ecological niches. We isolated numerous strains of *T. ferrooxidans* from ores, concentrates, and dense pulps used in the technological processes. Using the method of pulsed-field gel electrophoresis of chromosomal DNA digested with restriction endonuclease, strain polymorphism of *T. ferrooxidans* was shown [4]. This is an indication that the microevolution of a strain in each ecological niche was accompanied by changes in the nucleotide sequence of its chromosomal DNA. The structural peculiarities of the chromosomal DNA in different strains of *T. ferrooxidans* is a feature stable enough to be used in new strain identification, strain monitoring, and studies of experimental variability [5]. Thus, by analyzing restriction profiles of the chromosomal DNA, each type of ore or concentrate was found to be characterized by a particular predominant strain of *T. ferrooxidans*, adapted to the specific factors of the environment [6]. Some strains, when switched in the experiment from oxidation of ferrous iron to elemental sulfur or pyrite–arsenopyrite gold concentrate, displayed nonheritable changes in the structure of the chromosomal DNA, which vanished with the return of the strain to the original substrate [7–9]. On these grounds, the oxidized substrate was hypothesized to be among the medium factors that, in the course of the microevolution, gave rise to strain-specific polymorphism of the chromosomal DNA structure. This hypothesis was substantiated in the studies of the oxidation of $Fe^{2+}$ in a continuous culture. By raising the concentration of $Fe^{3+}$ in the medium in the course of prolonged continuous cultivation of strain TFI of *T. ferrooxidans*, a mutant strain TFI-Fe was obtained with increased resistance to high concentrations of $Fe^{2+}/Fe^{3+}$ (50 g/l) and inheritable changes in the structure of the chromosomal DNA [9]. Therefore, it is evident that the genotypic polymorphism in strains of *T. ferrooxidans* is connected with the history of their earlier existence. The objective of the present work was to study the phenotypic polymorphism of *T. ferrooxidans* strains observed in their growth and

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**Table 1.** Substrates and growth conditions for strains of *T. ferrooxidans* in technological processes

| Strain | Ore characteristics | Major sulfide minerals in concentrates and process conditions | Mineral content, % | pH of pulps | Metal concentration in solutions, g/l | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | $Cu^{2+}$ | $Zn^{2+}$ | $Fe^{2+}$ | $Fe^{3+}$ | $As^{3+}$ | $As^{5+}$ |
| TFN-d | Russia, Sakha, Nezhdaninsk deposit of gold–arsenic ores | Gravitation concentrate. Dense pulp, pilot industrial tests | | | | | | | | |
| | Minerals: | $FeS_2$ | 48.5 | 1.3–1.6 | ND | ND | 0.4–3.0 | 6.5–10.0 | 0.5–5.5 | 1.1–2.3 |
| | $FeS_2$, FeAsS, ZnS, PbS, $CuFeS_2$ | FeAsS | 35.0 | | | | | | | |
| TFBk | Kazakhstan, Bakyrchiksk deposit of carbonaceous gold–arsenic ores. Minerals: | Flotation gold–arsenic concentrate. Dense pulp, pilot industrial tests | | | | | | | | |
| | $FeS_2$, FeAsS, CuS, $CuFeS_2$ | $FeS_2$ | 14.0–33.0 | 1.5–2.6 | ND | ND | 0.5–1.9 | 3.4–15.2 | 0.6–1.2 | 0–5.6 |
| | | FeAsS | 3.6–4.5 | | | | | | | |
| TFO | Russia, Olimpiadinsk deposit of pyrrhotite gold–arsenic ores. Minerals: | Flotation gold–arsenic concentrate. Dense pulp, pilot industrial tests. | | | | | | | | |
| | $FeS_2$, FeAsS, $Sb_2S_3$, FeS, ZnS, | $FeS_2$ | 6.4–11.0 | 1.15–1.97 | ND | ND | 0–0.3 | 5.3–12.5 | 0.84–2.5 | 0–0.014 |
| | PbS, $CuFeS_2$ | FeAsS | 7.0–10.0 | | | | | | | |
| | | FeS | 25.3–36.5 | | | | | | | |
| TFL-2 | Russia, copper–zinc ore from the Uchalinsk factory. Minerals: | Tails of the manufacturing cycle of copper–pyrite intermediate product. Dense pulp, large-scale laboratory tests. | | | | | | | | |
| | ZnS, $FeS_2$, FeAsS, $CuFeS_2$, FeS, | ZnS | 19.0–20.0 | 1.6–3.1 | 2.0 | 15.6–25.0 | 0.4–8.0 | 0.4–8.0 | 0 | 0 |
| | gray copper ore | $CuFeS_2$ | 1.7–2.0 | | | | | | | |
| | | $FeS_2$ | 65.0–70.0 | | | | | | | |
| TFV-1 | Russia, Ural, heap of low-grade ore of the Volkovsk deposit. Minerals: $CuFeS_2$, $Cu_2S$, $Cu_5FeS_4$, and copper oxides | Low-grade copper ore, copper industrial heap leaching. | | | | | | | | |
| | | $CuFeS_2$ | ND | 2.15–2.8 | 0.5–1.2 | 0 | 0.34–3.3 | 0.6–1.5 | 0 | 0 |

Note: ND means that no data is available.

**Table 2.** Growth and ferrous iron oxidation parameters shown by strains of *T. ferrooxidans*

| Strain | Final pH/Eh values | Increase in the number of cells, $\times 10^8$ in 1 ml per day | Rate of $Fe^{2+}$ oxidation $V_{max}$, g/(l h) | Specific growth rate, $\mu_{max}$, $h^{-1}$ | Cell number doubling time, $t_d$, h | Substrate utilization coefficient |
|---|---|---|---|---|---|---|
| TFN-d | 2.5/805 | 4.7 | 0.200 | 0.141 | 4.9 | 0.99 |
| TFL-2 | 2.45/800 | 4.2 | 0.186 | 0.146 | 4.7 | 0.95 |
| TFBk | 2.5/800 | 3.5 | 0.175 | 0.114 | 6.1 | 0.84 |
| TFO | 2.45/795 | 3.2 | 0.171 | 0.112 | 6.2 | 0.78 |
| TFV-1 | 2.5/798 | 2.8 | 0.168 | 0.107 | 6.5 | 0.69 |

oxidation of $Fe^{2+}$, $S^0$, $FeS_2$, and sulfide-containing gold–arsenic concentrate and the adaptation of these strains to new substrates.

## MATERIALS AND METHODS

Five strains of *T. ferrooxidans* (TFV-1, TFN-d, TFBk, TFO, and TFL-2) from the collection of cultures of the Laboratory of Chemolithotrophic Microorganisms, Institute of Microbiology, Russian Academy of Sciences were used. The strains were isolated from dense pulp in reactors during the course of tests of the efficiency of new biohydrometallurgical processes in recovering gold from concentrates obtained by the enrichment of pyrite–arsenopyrite ores from several deposits and by the leaching of non-ferrous metals from a copper–pyrite–zinc intermediate product and lean copper ore (Table 1). The same strains were isolated from the corresponding natural substrates (ores and their concentrates). Their identity with the industrial strains was established on the basis of the structure of the chromosomal DNA [9].

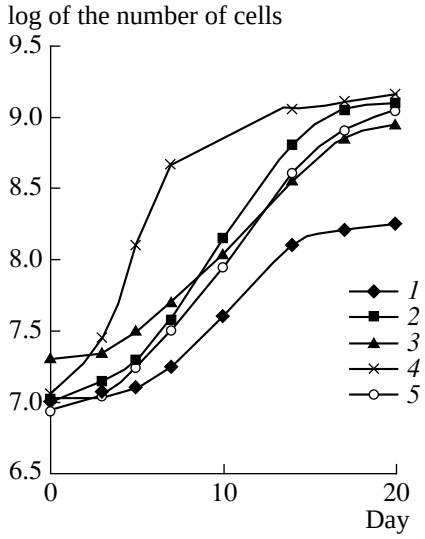


**Fig. 1.** Growth dynamics shown by strains of *T. ferrooxidans* in the first transfer to a medium with $S^0$: (*1*) TFV-1; (*2*) TFN-d; (3) TFBk; (*4*) TFO; and (*5*) TFL-2.

Strains were cultured in 500-ml Erlenmeyer flasks containing 200 ml of Silverman and Lundgren medium 9K [10] on a shaker at 150 rpm at a temperature of 28 ± 2°C. Exponential cultures of *T. ferrooxidans* were used as inoculum (10%). Strains were adapted to new substrates by sequential transfers to the Silverman and Lundgren medium containing the given substrate. No less than seven to eight culture transfers were made for the given substrate. In each passage, strains were cultured until the stationary growth phase. The adaptation sequence was terminated when rates of growth and oxidation of the new substrate reached their maximums, which remained unchanged over subsequent passages. $Fe^{2+}$, elemental sulfur, gravitation concentrate of Nezhdaninsk ore, and mineralogically pure pyrite from the Akchatau deposit (Kazakhstan) containing 50.86% sulfur and 49.14% iron were used as the source of energy. Adaptation experiments were carried out in Erlenmeyer flasks (250 ml) with 50 ml of the medium containing 0.5 g of the solid phase for the first passage and 1.0 g for the next passages. Cells of *T. ferrooxidans* were enumerated by direct counting in a Reichert microscope (Austria) equipped with a phase-contrast attachment. The $Fe^{3+}/Fe^{2+}$ ratio was determined by complexometric titration using Trilon B [11]. The sulfate ion content of the medium was determined by turbidimetric analysis [12]. The pH and Eh values were determined with an I-130.2M.1 ionometer.

The substrate-utilization coefficient was estimated as the increase in the number of cells in 1 l ($\times 10^{11}$) divided by the amount of the substrate oxidized (g/l), which was determined from the accumulated oxidation product ($SO_4^{2-}$ in the case of elemental sulfur and $Fe^{3+}$ in the case of $Fe^{2+}$ or $FeS_2$). The utilization coefficients for the gold–arsenic concentrate and for pyrite were calculated in relation to the quantity of dissolved $Fe^{3+}$, since in the course of their oxidation by *T. ferrooxidans* strains, the medium pH decreased from 2.5–2.6 to 1.2–1.5. According to Karavaiko *et al.* [3], in this range of pH values, $Fe^{3+}$ mostly occurs in the soluble form.

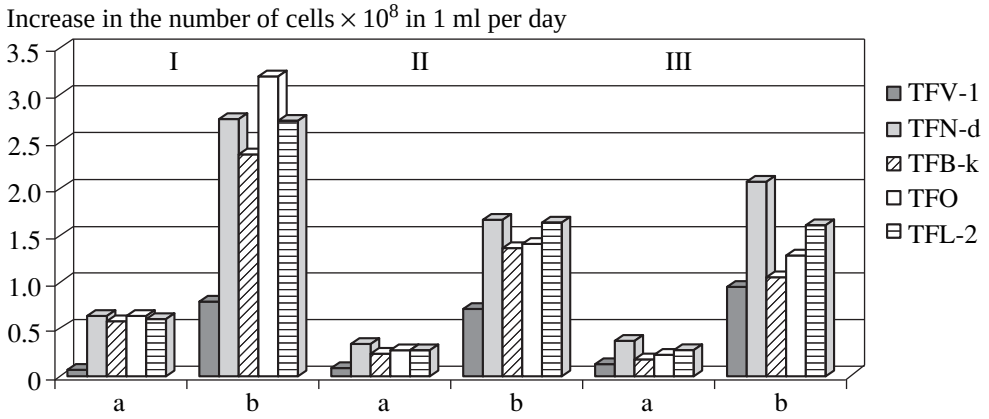


**Fig. 2.** Growth of (a) original and (b) adapted strains *T. ferrooxidans* on media with different substrates: (I) $S^0$; (II) $FeS_2$; and (III) concentrate.

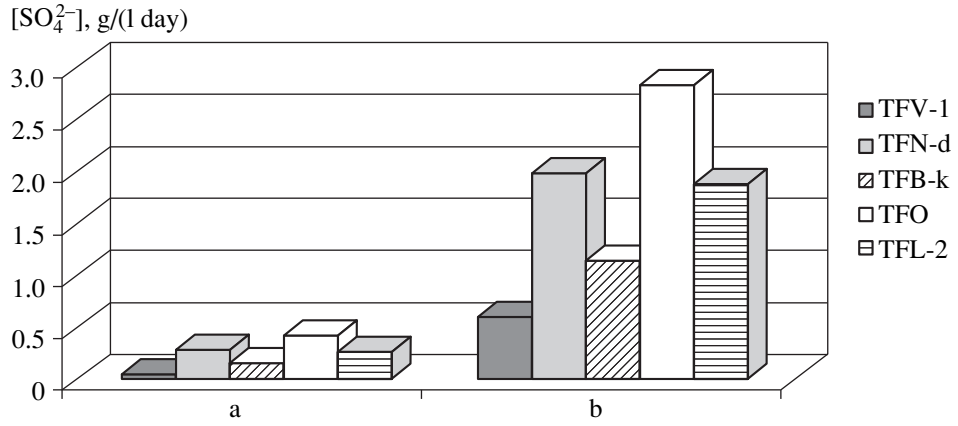


**Fig. 3.** Oxidation of sulfur by original and adapted strains of *T. ferrooxidans*. Notation as in Fig. 2.

## RESULTS AND DISCUSSION

### *Substrates and Growth Conditions for T. ferrooxidans Strains*

Table 1 lists characteristics of ores and their concentrates and the original environmental conditions for the strains under study, reflecting their history before the experiment. These substrates and environmental conditions fall into three basically different types. The first one is gold–arsenic high-sulfur ores and concentrates containing predominantly pyrite and arsenopyrite or pyrrhotite along with high concentrations of iron and arsenic ions in solutions (Olimpiadinsk, Nezhdaninsk, and Bakyrchiksk natural deposits). The second type is copper–zinc ores and intermediate products containing predominantly pyrite, sphalerite, and chalcopyrite, and, in solutions, large concentrations of copper, zinc, and iron ions (Uchalinsk deposit). The third type is constituted by low-grade copper ores from the Volkov deposit and from refuse heaps with low concentrations of metal ions in solutions.

However, microbiological analysis of pulps, solutions, and concentrates, and the analysis of chromosomal DNA of the corresponding predominant microbial cultures by molecular biology methods showed that not only the type of the substrate but also its qualitative and quantitative characteristics are important for the microevolutionary processes taking place at the strain level. From all of the five substrates tested, strains of *T. ferrooxidans* were isolated; however, they exhibited different restriction profiles of the chromosomal DNA [9]. On this basis, it was hypothesized that the phenotypic properties of strains (which reflect their genotypic polymorphism) and the conditions under which the strains had existed (their prehistory) might be correlated. Below, we present our findings on the strain growth and oxidation of the major substrates and on the patterns of strain adaptation to these substrates.

**Oxidation of $Fe^{2+}$.** Ferrous iron is known to be the most readily oxidizable substrate for *T. ferrooxidans* As seen from Table 2, all strains of *T. ferrooxidans* used in our experiments showed the following kinetic parameters during $Fe^{2+}$ oxidation: $\mu_{max}$ = 0.107–1.146 $h^{-1}$ and

**Table 3.** Growth and elemental sulfur oxidation parameters shown by strains of *T. ferrooxidans*

| Strain | $\mu_{max}$, $h^{-1}$ | | Cell number doubling time, $t_d$, h | | Substrate utilization coefficient | |
|---|---|---|---|---|---|---|
| | original strain | adapted strain | original strain | adapted strain | original strain | adapted strain |
| TFO | 0.025 | 0.059 | 27.7 | 11.7 | 1.39 | 1.13 |
| TFN-d | 0.016 | 0.054 | 43.3 | 12.8 | 2.01 | 1.38 |
| TFL-2 | 0.015 | 0.052 | 46.2 | 13.3 | 2.17 | 1.43 |
| TFBk | 0.012 | 0.046 | 57.8 | 15.1 | 3.77 | 2.05 |
| TFV-1 | 0.010 | 0.030 | 69.3 | 23.1 | 1.73 | 1.37 |

**Table 4.** Growth and pyrite oxidation parameters shown by strains of *T. ferrooxidans*

| Strain | $\mu_{max}$, $h^{-1}$ | | Cell number doubling time, $t_d$, h | | Substrate utilization coefficient | |
|---|---|---|---|---|---|---|
| | original strain | adapted strain | original strain | adapted strain | original strain | adapted strain |
| TFN-d | 0.012 | 0.034 | 57.8 | 20.4 | 2.81 | 1.86 |
| TFL-2 | 0.011 | 0.030 | 63.0 | 23.1 | 3.65 | 2.16 |
| TFO | 0.010 | 0.028 | 69.3 | 24.8 | 3.22 | 2.01 |
| TFBk | 0.009 | 0.027 | 77.0 | 25.7 | 3.66 | 1.91 |
| TFV-1 | 0.007 | 0.026 | 99.0 | 26.7 | 1.51 | 1.14 |

the cell doubling time of 4.7–6.5 h. The obtained values are reasonably close to those found in the literature for similar batch conditions [13]. Even so, these strains can be roughly divided into the following three groups: most actively growing, TFN-d and TFL-2; those with medium growth activity, TFBk and TFO; and the strain with the lowest growth rate, TFV-1. This division is confirmed by the data on the cell yields per day and $Fe^{2+}$ oxidation rates (Table 2). The ferrous iron utilization coefficients for these strains decreased in the same order. These data reflect the individual phenotypic strain characteristics, which apparently arise as a result of strain microevolution occurring in the corresponding environments (Table 1).

### *Adaptation of T. ferrooxidans Strains to New Oxidation Substrates*

Strains cultured for a long time on medium 9K with $Fe^{2+}$ were used as inoculum in studies of the dynamics of their adaptation to new substrates such as elemental sulfur, pure pyrite, and complex gold–arsenic concentrate obtained from ores of the Nezhdaninsk deposit containing a mixture of sulfide minerals (Table 1). All these products are typically present in sulfide ores and in pulps used in the technological processes. Meanwhile, the major phenotypic features of different strains of *T. ferrooxidans* have not so far been studied in the context of the speed and efficiency of strain adaptation to these substrates, and the limits of such an adaptation remain unclear. The speed of adaptation means the least number of transfers to be made for a strain to attain the maximum rates of growth and oxidation, while the efficiency of adaptation relates to these maximum rates of growth and oxidation.

**Oxidation of $S^0$.** The growth and sulfur oxidation activities of different strains of *T. ferrooxidans* in the first culture transfer are shown in Figs. 1, 2 (Ia), and 3a. Like in the case of $Fe^{2+}$, several strain groups can be identified. The most active strain was TFO, isolated from sulfur-rich (pyrrhotite) concentrate (Table 1). It is followed by strains TFN-d, TFL-2, and TFBk, isolated from pyrite- and arsenopyrite-rich concentrates and intermediate products. The lowest activity level is shown by strain TFV-1, isolated from lean ores of the Volkov deposit. The values of $\mu_{max}$ and the cell number doubling times are in agreement with this division (Table 3).

Except for TFV-1, the final figures of the biomass yield were fairly close for all strains. There was a correlation between $S^0$ oxidation rates and growth rates (Fig. 2 (Ia) and 3a). The highest coefficient of $S^0$ utilization in the first culture transfer was shown by strain TFBk, and the lowest one by TFO (Table 3). In the latter strain, a relatively smaller fraction of energy derived from $S^0$ oxidation was used to build up the number of cells. In subsequent strain transfers to a medium with sulfur, rates of cell growth and substrate oxidation increased regularly to attain the maximum for the given strain in the fifth passage (Figs. 2 (Ib) and 3b). This was the limit of the adaptation capacity for all strains. The time periods over which the maximum biomass was accumulated in each transfer (i.e., the time periods needed to reach the stationary phase) differed among the strains. In adapted strains, the specific growth rate increased and the generation time decreased markedly

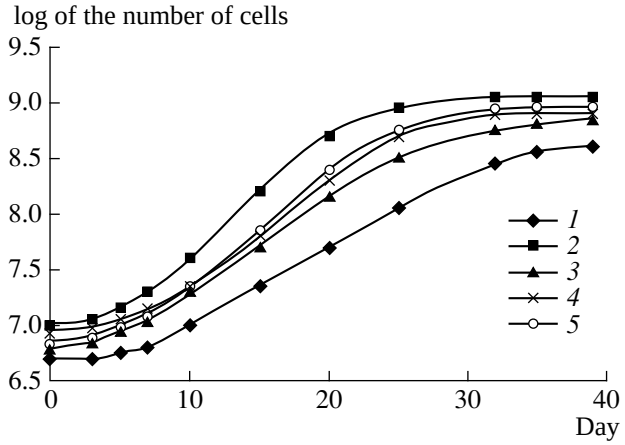


**Fig. 4.** Growth dynamics shown by strains of *T. ferrooxidans* in the first transfer to a medium with $FeS_2$. Notation as in Fig. 1.

(Table 3). The rate of sulfur oxidation in adapted strains was also higher and varied with each strain. The highest efficiency of adaptation to sulfur was exhibited by strain TFO, and the lowest one by TFV-1. Interestingly, in adapted strains, the coefficient of substrate utilization was lower and, therefore, the rate of waste oxidation of $S^0$ was greater than in the original strains. The highest rate of waste oxidation was noted in strain TFO, isolated from a substrate with a high concentration of sulfur (Table 1).

### *Oxidation of $FeS_2$ and Sulfide Minerals in Concentrates and Semiproducts*

Bacterial-chemical oxidation of sulfide minerals is a complex process, determined by their crystal and chemical peculiarities and electrochemical interactions. Also formed in the course of oxidation of sulfide minerals are elemental sulfur and its reduced compounds. In other words, a whole range of different substrates is simultaneously available to different strains of *T. ferrooxidans* active in different deposits and technological processes. It was reported that $Fe^{2+}$ and $S^0$ can be concurrently oxidized by *T. ferrooxidans* via independent pathways [14–16].

**Oxidation of $FeS_2$.** Pyrite is one of the most common sulfide minerals occurring both in ore deposits and in concentrates and semiproducts obtained from ores (Table 1). Since no crystal chemical analysis was made of the pyrite from the Akchatau deposit used in our tests and of pyrites in ores and concentrates wherefrom the studied strains were isolated, the degree of strain adaptation to the Akchatau pyrite under natural and industrial conditions remains unclear. As seen from the data in Figs. 4, 2 (IIa) and 5 (Ia) and Table 4, growth rates and pyrite oxidation rates shown by all the strains in the first passage were relatively low. Therefore, it can be concluded that pyrites from the ores and concentrates wherefrom the strains used in this study were isolated were different in their properties from the pyrites of the Akchatau deposit. The rates of growth and pyrite oxidation differed among the strains. The most and the least active strains were TFN-d and TFV-1, respectively, with the activity levels of other strains falling within this range. The order of strain activity never changed up to and including the fifth passage (Figs. 2 (IIb) and 5 (Ib)).

Like in the case of sulfur, the maximum rates of growth and $FeS_2$ oxidation and the limits of adaptation capacity were attained in the fifth passage, where the difference among the strains was not so pronounced. In strains adapted to $FeS_2$, the rates of biomass accumulation and substrate oxidation increased sharply, but the efficiency of substrate utilization decreased. This

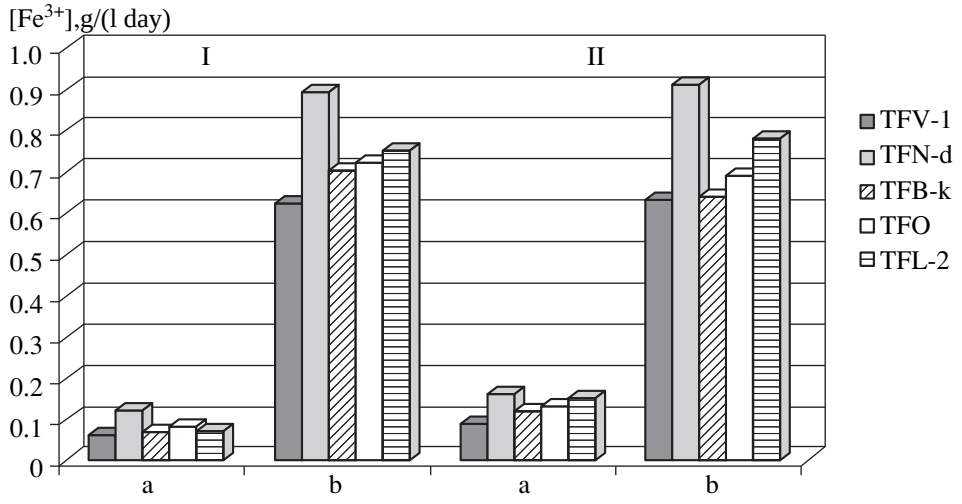


**Fig. 5.** Oxidation of (I) $FeS_2$ and (II) concentrate by (a) original and (b) adapted strains of *T. ferrooxidans*. Notation as in Figs. 1 and 2.

**Table 5.** Parameters of growth and oxidation of gold–arsenic concentrate shown by strains of *T. ferrooxidans*

| Strain | $\mu_{max}$, $h^{-1}$ | | Cell number doubling time, $t_d$, h | | Substrate utilization coefficient | |
|---|---|---|---|---|---|---|
| | original strain | adapted strain | original strain | adapted strain | original strain | adapted strain |
| TFN-d | 0.014 | 0.035 | 49.5 | 19.8 | 2.48 | 2.33 |
| TFL-2 | 0.012 | 0.028 | 57.8 | 24.8 | 1.83 | 2.08 |
| TFO | 0.009 | 0.026 | 77.0 | 26.7 | 1.85 | 1.85 |
| TFBk | 0.008 | 0.025 | 86.6 | 27.7 | 1.61 | 1.78 |
| TFV-1 | 0.007 | 0.027 | 99.0 | 25.7 | 1.33 | 1.51 |

means that in strains adapted to $FeS_2$, a larger fraction of the substrate was used in waste oxidation than in the unadapted strains. It can be therefore assumed that, in the course of adaptation to a new substrate, the substrate oxidation activity of a culture outstrips its growth activity. It should be remembered that, because the rate of oxidation of pyrite was estimated in terms of $Fe^{2+}$ oxidation alone, and the oxidation of $S^{2-}$ was not taken into account, this is only a relative conclusion, applicable only to adapted and unadapted cultures. A pH decrease to 1.0–1.5 in the course of strain growth both on $S^0$ and on $FeS_2$ and concentrate indicated that, in the two latter cases, elemental sulfur was also oxidized.

### *Oxidation of Sulfide Minerals in Concentrates*

Gravitational gold–arsenic concentrate produced from ores of the Nezhdaninsk natural deposit contained mostly pyrite and arsenopyrite (Table 1). In the electrochemical couple formed by these two compounds, the first to be oxidized by *T. ferrooxidans* is FeAsS, because it has a lower electrode potential and acts as the anode in this system. In this case, the adaptation of *T. ferrooxidans* strains to arsenopyrite played a major role. Such a system is characterized by large concentrations of metal ions, specifically, $As^{3+}$, $As^{5+}$, and $Fe^{3+}$. Thus, in the course of adaptation to concentrates, bacteria have to face a larger diversity of factors than in their adaptation to sulfur or pyrite alone. The maximum cell increase over one day exhibited by the strains in the first transfer is illustrated by Figs. 2 (IIIa) and 6. The ranking of strains in terms of their growth activity on concentrate was the same as that on $FeS_2$. The values of the kinetic growth parameters ($\mu_{max}$ and $t_d$) were also virtually the same as on $FeS_2$ (Tables 4 and 5). At the same time, the utilization coefficients of concentrate were lower for all strains than those of pyrite. This difference was the largest in strains TFBk, TFL-2, and TFO. The efficiency of pyrite oxidation by strain TFV-1 in the first transfer was a little higher than the efficiency of concentrate utilization and equaled that of the adapted strain. In subsequent transfers, the rates of growth and sulfide mineral oxidation increased to the individual maximums attained in five transfers for strains TFN-d, TFL-2, TFO, and TFBk, and in seven transfers for strain TFV-1 (Table 5, Figs. 2 (IIIb) and 5 (IIb)). In strain TFN-d cultured on concentrate, the biomass increase per unit time was somewhat larger than that on $FeS_2$. This might be explained by the higher amenability of arsenopyrite to oxidation by bacteria or by oxidation of elemental sulfur, released when FeAsS is chemically or electrochemically oxidized in the given system. In strains TFBk and TFO adapted to concentrate, the biomass increase per unit time was a little smaller than that on $FeS_2$, possibly because the main oxidized mineral in the Bakyrchik ore concentrate was pyrite and that in Olimpiadinsk concentrate is pyrrhotite (Table 1). Unlike the adaptation to $S^0$ and $FeS_2$, in adaptation to concentrate, the coefficient of substrate utilization increased somewhat in strains TFV-1, TFBk, and TFL-2; remained the same as in the unadapted culture in strain TFO; and decreased a little in strain TFN-d. A possible explanation is that the substrate utilization coefficient was calculated on the basis of $Fe^{3+}$ formation, which may not be the only oxidation product. Interestingly, the highest efficiency of adaptation to concentrate was exhibited by strain TFN-d, isolated from the same concentrate, i.e., the indigenous strain. Its advantage was evident at all steps of the adaptation.

## CONCLUSIONS

Investigation of strain diversity of chemolithotrophic bacteria is a key problem in the development of biohydrometallurgical technologies. The adaptation capabilities of strains have to be known to exploit the most active ones with the highest control potential. In this study, not only the variation of phenotypic properties of *T. ferrooxidans* strains (depending on their prehistory) was shown but also the limits of their adaptation capacity. These data testify to a certain genome response in each strain to a change in the substrate utilized and, possibly, to other extreme environmental factors. A new leader strain having the highest adaptation efficiency emerges with the change in the oxidation substrate. In our case, the original and adapted strains, put in the order of decreasing cell yields and substrate oxidation rates, would form the following sequences (Figs. 2, 3, 5):

$Fe^{2+}$: TFN-d, TFL-2 > TFBk, TFO, > TFV-1;

$S^0$: TFO > TFN-d, TFL-2 > TFBk > TFV-1;

$FeS_2$: TFN-d > TFL-2, TFO > TFBk, TFV-1;

Concentrate: TFN-d > TFL-2, TFO > TFBk ≈ TFV-1.

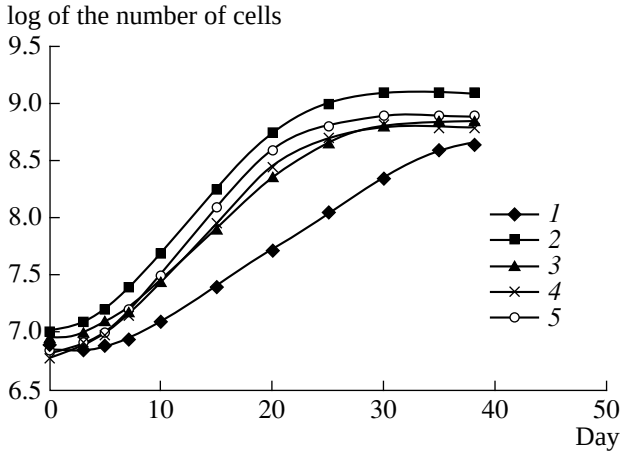


**Fig. 6.** Growth dynamics shown by strains of *T. ferrooxidans* in the first transfer to a medium with concentrate. Notation as in Fig. 1.

On all substrates, except sulfur, the leader strain was TFN-d, isolated from gold–arsenic concentrate and dense pulp used in its processing. On elemental sulfur, the leading position was held by strain TFO, isolated from dense pulp used in the treatment of high-sulfur pyrrhotite concentrates at the Olimpiadinsk gold recovery plant. This strain was less active in oxidizing $Fe^{2+}$, $FeS_2$, and the gold–arsenic concentrate from the Nezhdaninsk natural deposit. Finally, a fact to be noted is the leadership of TFN-d over TFO and TFBk on its native concentrate. The two latter strains, which were also isolated from gold–arsenic concentrates but produced from other ores, were outstripped by TFN-d in terms of kinetic growth parameters and oxidation of sulfide minerals (even after a period of adaptation). These processes must be, apparently, controlled by subtler mechanisms having to do with the crystal chemical properties of different sulfide minerals. The strain least active on all substrates was TFV-1, isolated from low-grade copper ore used in heap copper leaching. The ultimate limits of its capacity were evidently found. This strain is the least "talented" and this has to do with the history of its existence.

Despite the fact that all strains of *T. ferrooxidans* are able to oxidize $Fe^{2+}$, elemental sulfur, its reduced compounds, and different sulfide minerals, which is an evolutionary feature of this species, a period of adaptation is needed when the substrate to be oxidized is changed. Even strain TFN-d, grown for a long time on $Fe^{2+}$, requires a protracted adaptation to the concentrate from which it was isolated to attain maximum rates of growth and substrate oxidation. This process is, therefore, inducible. As shown above, different strains may have close adaptation speeds but different adaptation efficiencies. This might be due to different activities of the control systems of the genes responsible for the oxidation of new substrates and the efficiency of their utilization or even to different mechanisms of gene control. We see that the genotypic strain polymorphism of *T. ferrooxidans*, which evolved through microevolution induced by various environmental factors, becomes manifest in the form of phenotypic strain diversity. Most saliently, this diversity is manifested in the response of the strains to a change in the oxidation substrate.

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