

THERMODYNAMIC PROGNOSIS OF MICROBIAL INTERACTION WITH IRON COMPOUNDS

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Abstract. Microbial redox-transformation of iron compounds is closely related to the environmental conditions defined by a set of physical and chemical factors, including the pH and Eh value. Thermodynamic calculations of all theoretically feasible ways of microbial interaction with iron compounds (mobilization/immobilization, oxidation/reduction) are presented. It was theoretically grounded that microorganisms were capable to perform a high activity in the biogeochemical cycles of carbon and iron. Obtained results can be used to prognose the transformation of iron compounds by microorganisms in natural and man-made ecosystems and create biotechnologies of microbial purification of water sources and wastewater from iron compounds.

Key words: microorganisms interaction with iron, thermodynamic prognosis, iron reduction, biogeochemical role of microorganisms, microbial biotechnologies.

INTRODUCTION

Life is a continuous chain of organic and inorganic compounds transformation. It involves chemical elements of the periodic system in biogeochemical cycles. Microorganisms provide cyclicality and balance

of macro- and microelements in natural and man-made ecosystems [18].

Energy metabolism of microorganisms is based on redox-reactions by which energy is released and stored in phosphorylated compounds (ATP, etc.). (Fig. 1) [14].

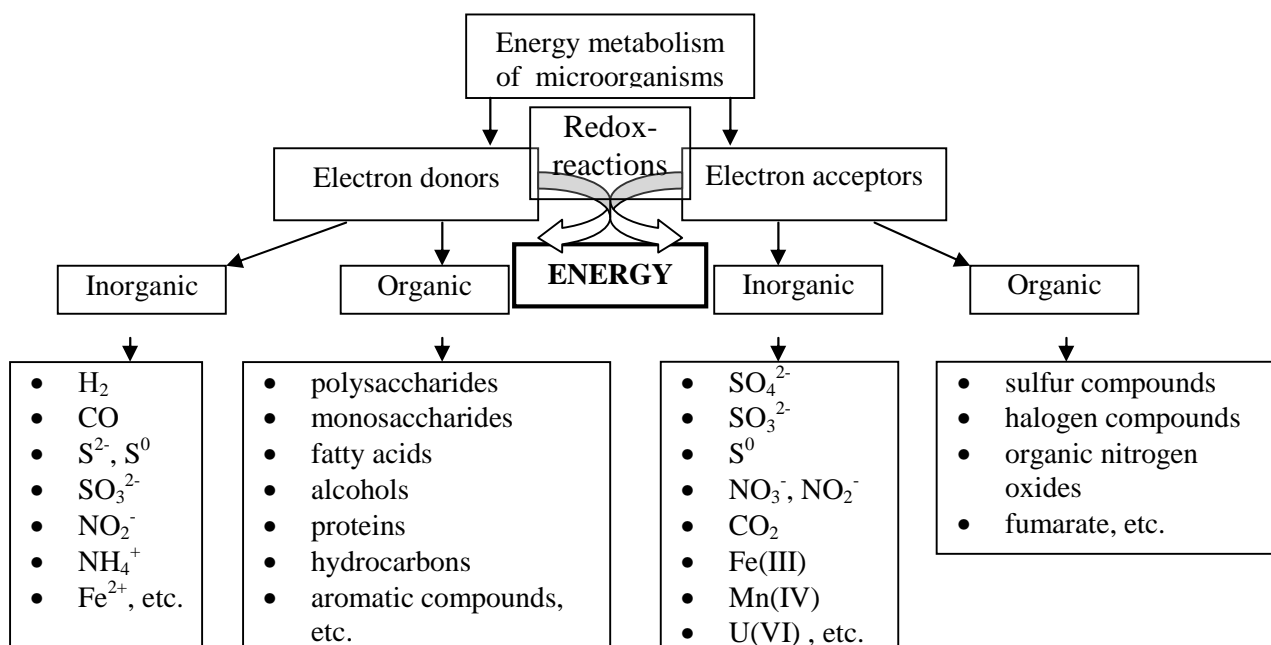


Fig. 1. Compounds serving as electron donors and acceptors in the reactions of energy metabolism of microorganisms

Oxidation of organic matter coupled with $Fe(III)$ reduction has a significant role in biogeochemical cycles [9, 10]. Iron compounds are widespread in natural ecosystems (soils, sediments of seas and freshwater, etc.) at a concentration of several tens of milligrams to 3,5 grams per 1 kg of dry deposits (soil, etc.) [1]. This implies that $Fe(III)$ can be reduced by microorganisms coupled with organic matter oxidation in stoichiometric ratios. Therefore conjugated cycle of organic compounds oxidation and $Fe(III)$ reduction by microorganisms may

play a significant role in the distribution of vector carbon and energy flows in ecosystems (Fig. 2).

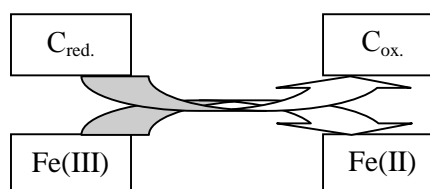


Fig. 2. Conjugated redox-reaction of organic matter oxidation and $Fe(III)$ reduction

Involvement of microorganisms in the transformation of iron compounds is known from the beginning of the twentieth century. [9, 15]. However, the complete significance of microorganisms in the biogeochemical cycles of iron compounds transformation has not been determined completely [10].

Microorganisms' ability to generate energy reducing Fe(III) as a terminal electron acceptor is proved by studies held during XX century [5, 6, 7, 10]. On the other hand, Fe(III) can be reduced by microorganisms nonspecifically, i.e. by exometabolites – reducing agents [2, 5]. However, currently there is no generalized theoretical concept of iron compounds transformation by microorganisms in biogeochemical cycles. That is why the aim of the work was to develop the method of thermodynamic prognosing of microbial interaction with iron compounds in order to determine all possible pathways of iron transformation in biogeochemical cycles in natural and man-made ecosystems.

MATERIALS AND METHODS

Thermodynamic and mathematical calculating methods were used to estimate microbial interaction with iron compounds [12, 14, 17].

RESULTS AND DISCUSSION

There are both soluble and insoluble compounds of Fe(II) and Fe(III) in the environment due to the physical and chemical conditions. Iron is a part of insoluble minerals such as hematite (Fe_2O_3), magnetite (Fe_3O_4), goethite ($\alpha\text{-FeO(OH)}$), lepidokryt ($\gamma\text{-FeO(OH)}$), pyrite (FeS_2), siderite (FeCO_3) and others. Soluble forms of iron are ions or soluble complexes with organic acids, amino acids, etc. [6, 8, 9, 13].

Generalized pathways of microbial interaction with iron are as follows: "mobilization-immobilization" and "oxidation-reduction". The scheme of iron compounds transformation is presented on Fig. 3.

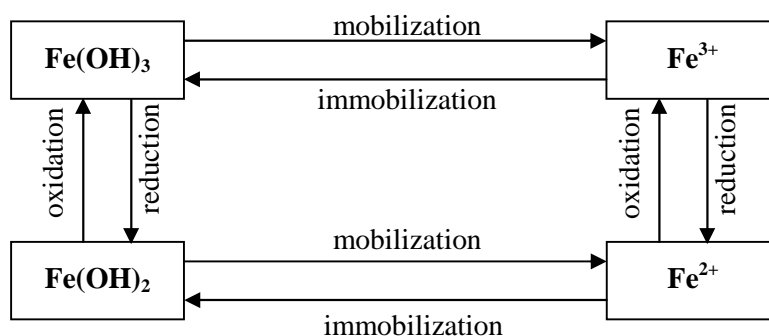


Fig 3. The scheme of iron compounds transformation

The course of theoretically feasible metabolic pathways of microbial iron compounds transformation essentially depends on the pH and Eh value. Depending on the pH value and redox-potential Fe(II) and Fe(III) may be in the form of soluble (ions and chelated complexes) and insoluble (oxides, hydroxides, carbonates, sulfides, etc.)

compounds. Reaction equations and Pourbaix diagrams of elements [12] allow to calculate the pH and Eh of inverse reactions of soluble and insoluble iron compounds formation (Table 1).

Let's consider possible ways of soluble and insoluble iron compounds formation depending on the pH.

Table 1. Formation of soluble and insoluble iron compounds depending on their concentration and the pH value of environment

№	Iron compounds		The pH value of iron compounds transformation at a concentration	
	Soluble	Insoluble	0,01 mole/l	1 mole/l
1	Fe^{3+}	Fe_2O_3	0,43	0,0
2	Fe^{3+}	Fe(OH)_3	2,28	1,61
3	Fe^{2+}	Fe(OH)_2	7,65	6,65

Insoluble Fe(III) oxides (Fe_2O_3) are formed at the pH values above 0,0. Ions of Fe^{3+} at the concentration of 1 mole/l are stable in the medium at the $\text{pH} \leq 1,61$. At higher values of the pH Fe(III) forms an insoluble

hydroxide – Fe(OH)_3 . That is why iron is insoluble in oxygenic zone of environment. Iron(II) in the form of Fe^{2+} cations at the concentration of 1 mole/l is stable at the $\text{pH} \leq 6,65$. At the $\text{pH} > 6,65$ insoluble

hydroxides of iron(II) – $\text{Fe}(\text{OH})_2$ – are formed. As the concentration of iron ions in the environment decreases, the pH value at which the transformation of soluble compounds into insoluble takes place

increases (Fig. 4). For example, at the concentration of 1 mole/l of Fe^{3+} its hydroxide is formed at the $\text{pH} > 1,61$, and at the concentration of 0,01 mole/l – at the $\text{pH} > 2,28$ (Table 1).

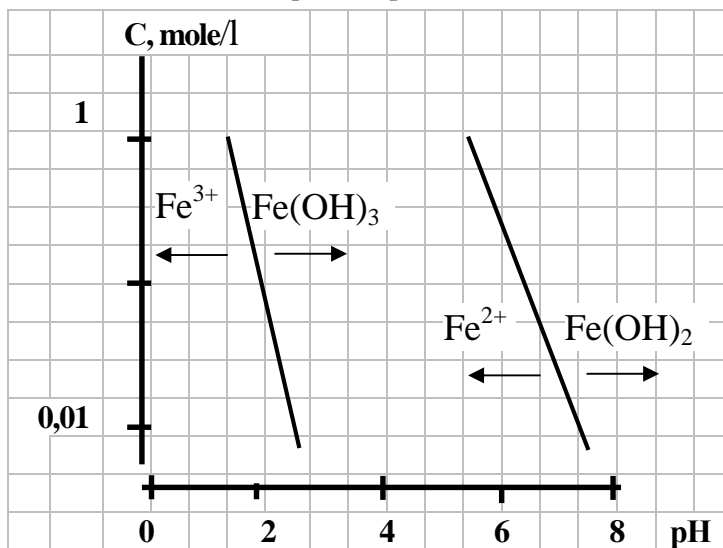


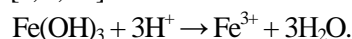
Fig. 4. The pH dependence on iron compounds concentration during their transformation from soluble into insoluble

Depending on the direction of the microbial metabolism there is a possibility of iron compounds mobilization or immobilization in accordance with the Table 1.

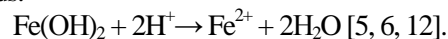
Mobilization of iron compounds occurs due to the acidification of medium or the formation of metal-organic complexes with microbial exometabolites-chelators.

Let's consider iron mobilization at the acidification of medium. Insoluble Fe(III) compounds in the form of oxides and hydroxides are stable in alkaline, neutral and even acidic conditions. Cations of Fe^{3+} are stable in the narrow pH range 0,0...1,6. Acidification of the culture medium occurs when protons that can be regarded as typical exometabolites are accumulated. Acidification due to H^+ takes place during fermentation of organic compounds in the absence or deficiency of O_2 . The mobilization of Fe(III) compounds in anaerobic conditions occurs during the accumulation of protons in the culture medium by autotrophic microorganisms such as *Thiobacillus ferrooxidans*, *Sulfolobus acidocaldarius*. Thus, mobilization of insoluble Fe(III) compounds can be

carried out by microorganisms that strongly acidify the environment [5, 6, 12]:



Insoluble Fe(II) compounds in the form of hydroxides are stable in neutral and alkaline conditions. Cations of Fe^{2+} are stable at the wide range of $\text{pH} = 0,0-6,7$. So, typical chemoorganotrophic microorganisms (for example, *Shewanella putrefaciens*, *Pseudomonas* sp.) that reduce the $\text{pH} < 7$ during the growth can mobilize insoluble Fe(II) compounds:



Microbial exometabolites form soluble complex compounds with iron ions and hydroxides. There is the possibility of chelated complexes formation ($\text{R}_1\text{-Fe}^{2+}\text{-R}_2$, $\text{R}_1\text{-Fe}^{3+}\text{-R}_2$) during the interaction of iron with amino acids or organic acids (acetate, citrate, etc.). They are stable in soluble form in a wide range of the $\text{pH} = 0,0...12,0$.

Fig. 5 generally shows possibility of insoluble iron compounds mobilization due to the interaction with exometabolites that are the products of fermentation, and also acidification of the medium.

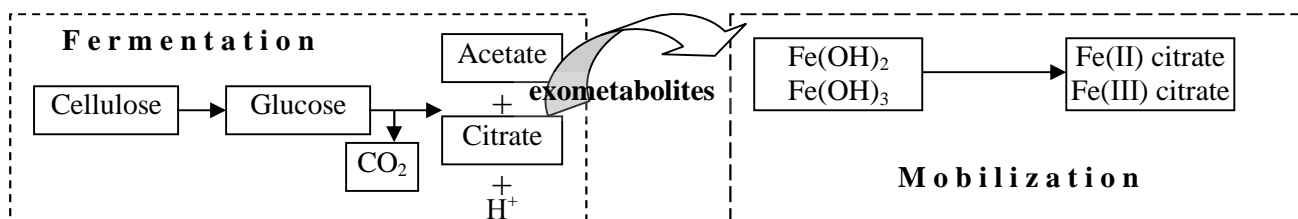


Fig. 5. Mobilization of insoluble iron compounds by microbial exometabolites – fermentation products

Immobilization of iron compounds may occur due to the destruction of metal-organic complexes or medium alkalization by microorganisms.

In the first case, immobilization of soluble iron occurs due to the organic radicals of complex Fe(II) and Fe(III) compounds destruction by microorganisms. It leads to the formation of insoluble iron oxides and hydroxides in neutral and alkaline conditions. In the second case, we assume that some microorganisms such

as denitrifying bacteria may indirectly form insoluble iron compounds according to the following mechanism. The alkalization of culture medium containing KNO_3 as electron acceptor takes place due to the accumulation of alkali K^+ cation during the reduction of NO_3^- -group of KNO_3 to molecular nitrogen (N_2). Thus, the pH shift to the values more than 7,5 leads to the precipitation of iron. Soluble iron cations transform into insoluble hydroxides (Fig. 6, Table 1):

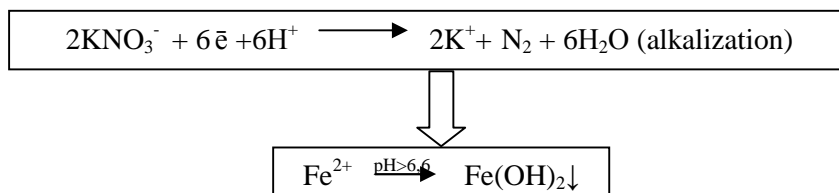
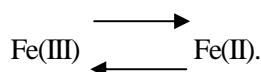
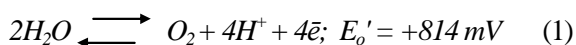


Fig. 6. Possible immobilization of soluble iron compounds with the participation of denitrifying bacteria

The end result of microorganisms interaction with iron compounds significantly depends on the redox-potential [14]. The prognosis of microbial interaction with iron compounds is presented in Fig. 7 in the pH and Eh coordinates. Let's consider the possibility of microbial Fe(III) reduction depending on the pH and Eh values. Microbial redox-transformation of iron compounds is described by one reaction:



Microbial reduction of metals is determined by the thermodynamic properties of water as a habitat for microorganisms, energy characteristics of microorganisms and redox-characteristics of metals. Microbial catabolism is possible only inside the zone of thermodynamic stability of water in the Eh-range of - 414 to +814 mV (pH = 7,0), where the upper and lower limit are determined by the reactions:



In the first case, if the Eh value goes beyond the resulted limits water acts as a reducing agent and is oxidized to O_2 (equation 1). In the second case, when $\text{Eh} \leq -414 \text{ mV}$, the proton of water serves as an oxidant and is reduced to H_2 (equation 2) [17].

The reactions № 1-3 are in the area of water thermodynamic stability (from -414 mV to +814 mV at the pH = 7,0). So microorganisms are able to reduce Fe(III). Reactions of Fe(II) reduction to Fe^0 are outside

of this zone (reactions № 4, 5). Thus, microbial reduction of Fe(II) compounds is strongly prohibited.

Reduction of soluble Fe^{3+} is possible at the $\text{pH} \leq 1,6$, because Fe(III) is in the ionic form only in highly acidic conditions (reaction № 1). Reduction of insoluble iron hydroxide (Fe(OH)_3) takes place at the $\text{pH} > 1,6$. Redox-potential of Fe(OH)_3 reduction (reactions № 2, 3) is pH-dependent, as H^+ is involved in the reaction. For example, for the reaction № 2 at the $\text{pH} = 2,0$ $\text{Eh} = +702 \text{ mV}$, but at the $\text{pH} = 6,0$ $\text{Eh} = -7 \text{ mV}$.

The difference of potentials between the electron donor system and Fe(III) that is an electron acceptor determines the possibility of microbial Fe(III) reduction. According to our conception, metabolically active microorganisms and their redox-enzymes serve as "the electron donor system". Reduction of Fe(III) is possible either with energy obtaining or without its emission that is a side process.

On our opinion, the difference of potentials between donor and acceptor systems at least 100 mV is the necessary and sufficient condition for coupled electron donor oxidation and Fe(III) reduction. In summary, metabolically active bacteria that decrease the Eh to negative values due to the substrate oxidation (the source of carbon and energy) is the donor system. Oxidized iron compounds (Fe^{3+} , Fe(OH)_3 , $\text{R}_1\text{-Fe}^{3+}$ - R_2 , etc.) are the acceptor system. So, decreasing of the Eh by microorganisms leads to inevitable reduction of Fe(III) to Fe(II).

Thermodynamic prognosis allows to generally estimate theoretically feasible contribution of microorganisms in the biogeochemical cycles of iron compounds transformation in the environment.

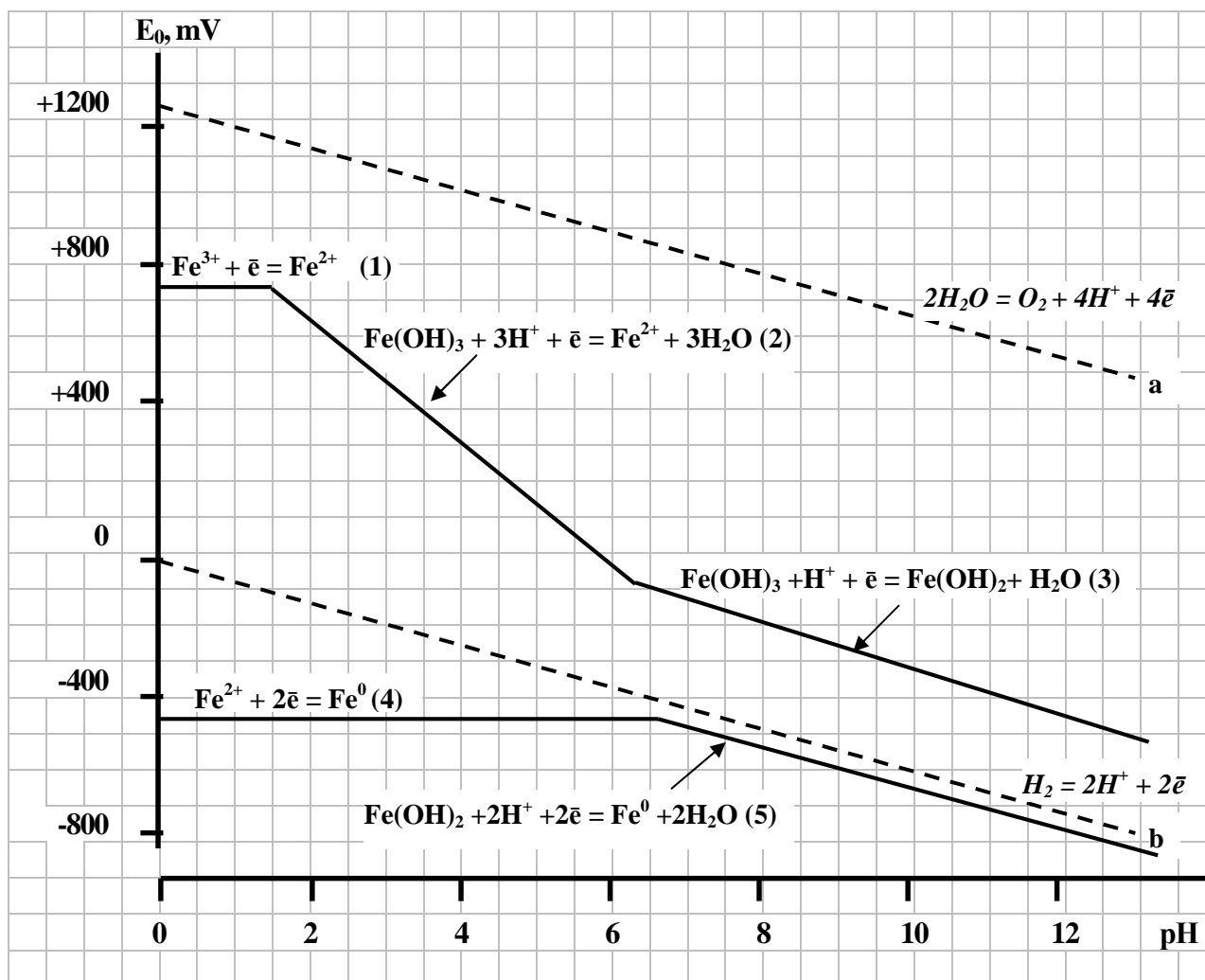


Fig. 7. Zones of redox-stability of Fe(II) and Fe(III) compounds

Note: a (top) and b (lower) - limit of thermodynamic stability of water:

a – is described by the equation: $O_2 + 4H^+ = 2H_2O$ and $Eh = 1,228 - 0,0591 \cdot pH - 0,0295 \lg PH_2$;

b – is described by the equation: $2H^+ + 2e = H_2$ and $Eh = 0,000 - 0,0591 \cdot pH - 0,0591 \lg PH_2$.

Determination of iron stability fields allows to prognose any possible way of microbial interaction with iron compounds (Fig. 8).

Microbial Fe(III) reduction is possible only in the area of water thermodynamic stability [14]. The upper limit of this zone is described by the equation $O_2 + 4H^+ = 2H_2O$. For this reaction, the standard potential¹ (E_0') is equal to +814 mV. Standard potential of the reaction that describes the lower limit ($H_2 = 2H^+ + 2e$) is -414 mV (Fig. 8).

Such compounds as Fe^{3+} , Fe^{2+} , Fe_3O_4 , $Fe_2O_3 \cdot nH_2O$, $Fe(OH)_3$, $Fe(OH)_2$, $FeCO_3$, FeS_2 and other are stable in

the area of water thermodynamic stability. It is possible to calculate any number of options for the ratio of iron compounds depending on their concentration and the pH and Eh value. We must consider not only the concentration of iron, but also carbonates and sulfides in the environment to calculate the conditions required for the formation of insoluble $FeCO_3$ and FeS_2 . This need is related to the fact that carbon dioxide and its derivatives (HCO_3^- , CO_3^{2-}), and sulfide (H_2S , HS^- , S^{2-}) are common exometabolites of microorganisms. In this regard, it is theoretically possible to calculate the conditions of soluble iron sedimentation with such compounds as HCO_3^- , CO_3^{2-} , $R-SH$, H_2S and S^{2-} . We used the generalized scheme shown on Fig. 8 for calculations.

¹ Standard potential (E_0') means that the concentration of oxidized and reduced forms is equal to 1 mole and the $pH = 7,0$.

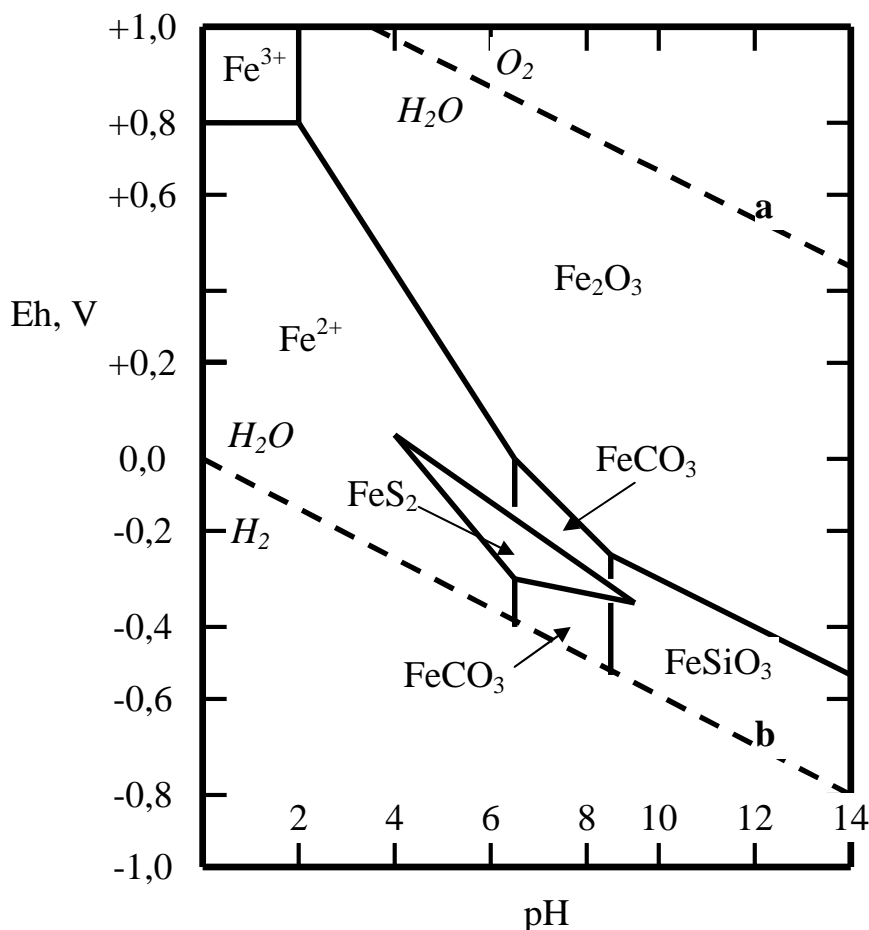


Fig. 8. Stability fields of iron compounds [3]

Note: a (top) and b (lower) - limit of thermodynamic stability of water:

a – is described by the equation: $O_2 + 4H^+ = 2H_2O$ and $Eh = 1,228 - 0,0591 pH - 0,0295 \lg PH_2$;

b – is described by the equation: $2H^+ + 2e = H_2$ and $Eh = 0,000 - 0,0591 pH - 0,0591 \lg PH_2$.

Stability field of Fe^{3+} is in the range of $Eh = +770...+1100$ mV and $pH = 0,0-2,0$. Cation of Fe^{2+} , in contradiction to Fe^{3+} , is stable in a wider area of the pH-Eh. The range of Fe^{2+} stability in terms of Eh is $-400...+770$ mV and $pH = 0,0-6,0$. At the $pH > 2,0$ Fe^{3+} transforms into insoluble $Fe(OH)_3$ or $Fe_2O_3 \cdot nH_2O$. Hydroxide, $Fe(OH)_3$, is stable in the range of $Eh = -550...+770$ mV and $pH = 2,0-14,0$. Soluble form of Fe^{2+} is converted into insoluble FeS_2 in the range of $Eh = -400...+100$ mV and $pH = 6,0-9,0$. Iron carbonate $FeCO_3$ is stable in the range of $Eh = -450...0$ mV and $pH = 6,0-8,0$. Insoluble $FeSiO_3$ can be formed within $Eh = -800...0$ mV and $pH = 8,0-14,0$.

Physical and chemical parameters of ecosystems allow to determine the state of iron without comprehensive direct chemical analyzes of natural samples of soil, etc. Measurement of the pH and Eh in the ecosystem can prognose the dominant redox-

forms of iron – Fe(II) or Fe(III) and soluble (insoluble) compounds. For example, if the $Eh = 0$ mV at the $pH = 6,0$ iron can exist in insoluble ($Fe(OH)_3$, Fe_2O_3) and in soluble (Fe^{2+}) form. And at the $Eh = 0$ mV and the $pH = 8,0$ only insoluble form ($Fe(OH)_3$ or Fe_2O_3) can exist.

Basing on thermodynamic properties of iron we can create conditions (selective media) for microbial reduction of soluble and insoluble forms of iron.

To mobilize insoluble Fe(III) compounds it is required to create such selective conditions where microorganisms decrease $pH < 2,0$. It is possible during the fermentation of carbohydrates that leads to the strong acidification of medium (Table 1). Also mobilization of insoluble Fe(II) and Fe(III) is possible during production of exometabolites-chelators (Table 1)

On the other hand it is required to move the pH value in the alkaline zone for the immobilization of

soluble iron compounds (Table 1). It is possible during the growth of denitrifying bacteria and any other metabolic pathways that lead to the alkalization of the environment. The formation of insoluble carbonates and sulphides of iron can take place in slightly alkaline conditions due to the accumulation of exometabolites (CO_2 and H_2S). Solubility product¹ of iron carbonate (FeCO_3) is $3,5 \cdot 10^{-11}$, and pyrite (FeS_2) – $6,3 \cdot 10^{-31}$ [11].

On our opinion, formation of iron carbonates is one of the dominant process in ecosystems in neutral, slightly alkaline and alkaline conditions. It can be used for water purification from iron compounds by microorganisms. Carbon dioxide is produced during the organic compounds oxidation by microorganisms. Interaction of carbon dioxide in the form of HCO_3^- or CO_3^{2-} with iron (Fe^{2+}) leads to the formation of insoluble FeCO_3 in slightly alkaline conditions and purification of water from soluble iron compounds.

In acidic conditions ($\text{pH} \leq 2$) where Fe(III) is found mainly in cationic form (the reduction potential is equal to +770 mV) (Fig. 7), thiobacilli or acid resistant chemoorganotrophic bacteria can reduce Fe^{3+} to Fe^{2+} .

Compounds of Fe(III) form insoluble oxides and hydroxides at the pH range from 2 to 8. The redox-potential of these compounds is lower than of the cationic form. It ranges from -100 to +770 mV (Fig. 7). Reduction of Fe(III) oxides and hydroxides can be provided by a wide range of microorganisms belonging to different taxonomic groups (*Clostridium*, *Bacillus*, *Pseudomonas*, *Geobacter*, *Shewanella*, *Rhodospirillum rubrum*, etc.). These microorganisms can significantly affect the reallocation of carbon and iron in ecosystems [5, 6, 9].

We suppose that alkaliphilic microorganisms can also reduce insoluble Fe(III) compounds, such as $\text{Fe}(\text{OH})_3$, at the $\text{pH} > 8$. The redox-potential of these compounds at the $\text{pH} > 8$ ranges from -400 to -100 mV (Fig. 7). The energy output of the reduction is low and has few advantages for microorganisms. Therefore, it is likely that under these conditions nonspecific reduction of the Fe(III) compounds in the stationary growth phase by microbial exometabolites is possible.

¹ Solubility product is the mathematical product of ions activity of slightly soluble electrolyte in its saturated solution. Its value indicates the degree to which a compound dissociates in water. The higher is the solubility product, the more soluble is the compound.

Basing on the Pourbaix diagrams [12], we calculated the standard potentials of iron compounds reduction reactions and substantiated theoretically acceptable microbial reduction of certain iron compounds (Table 2).

Obviously, that microorganisms can reduce Fe(III) to Fe(II) (Table 2, reactions 1-4). It is possible because the standard potentials of Fe(III) reduction reactions are within the range of water thermodynamic stability ($-414 \leq E_0' \leq +814$ mV). However, further reduction of Fe(II) to Fe^0 is impossible, because the value of the standard potential is outside the water thermodynamic stability. So there is a strong thermodynamic ban for this reactions (Table 2, reactions 5 and 6).

Table 3 presents the redox-potentials of iron reduction reactions. Table 4 shows redox-potentials of electron donors oxidation reactions. Basing on these data, we calculated the change of free energy of coupled redox-reactions [14] (Table 5):

$$\Delta G_0' = -nF\Delta E_0' = -n \cdot 23,067 \cdot \Delta E_0' \text{ (kcal/mole)}, \quad (3)$$

$$\Delta E_0' = E_{0A}' - E_{0D}' \text{ (V)}, \quad (4)$$

where n – number of electrons participating in the redox-reaction, F – Faraday constant, and $\Delta E_0'$ – the difference of potentials between the electron acceptor and donor. The value of $\Delta E_0'$ is brought to the $\text{pH} = 7,0$ and a molar concentration of oxidized and reduced forms of electron donors and acceptors.

For example, for the reaction $\text{H}_2 + 2\text{Fe}^{3+} = 2\text{H}^+ + 2\text{Fe}^{2+}$ the difference of potentials between the electron acceptor (Fe^{3+}) and donor (H_2) is ($\text{pH} = 1,6$):

$$\Delta E_0 = +0,771 + 0,095 = 0,866 \text{ V} \quad (5)$$

The change of free energy for given redox-reaction is:

$$\Delta G_0 = -nF\Delta E_0 = -2 \cdot 23,067 \cdot 0,866 = -39,95 \text{ kcal/mole}. \quad (6)$$

The value of reaction is negative, i.e. $\Delta G_0 < 0$, so the reaction occurs with energy release. This implies the possibility of microbial reduction of Fe(III) to Fe(II) by the shown reaction with release of energy.

The necessary condition for any metal reduction is that the redox-potential of metabolically active microorganisms and respective complex of redox-enzymes has to be more electro negative than metal reduction potential. In this case the $\Delta G_0'$ takes negative values. It means that the redox-reaction of metal reduction occurs with the release of free energy ($\Delta G_0' < 0$) [14].

Possibility of microbial Fe(III) reduction is confirmed by our calculations, shown in Table 5 for such electron donors in energy metabolism as H_2 , NADH_2 , succinate. For H_2 as an electron donor in reactions 1-4 the $\Delta G_0'$ (kcal/mole) has negative values:

-39,95; -12,5; -10,61; -12,18 respectively. It suggests the possibility of using such reactions for obtaining energy by microorganisms. In contrast reactions 5 and 6 are characterized by positive values of $\Delta G_0'$ (respectively +1,2; +2,12 kcal/mole). In this case, the potential of electron acceptor is lower than the potential of donor.

Therefore bacteria can not carry out these reactions since these reactions lead to the energetic depletion of microorganisms. Transferring electrons from succinate and NADH₂ the course of reactions 7-10 and 13 is theoretically possible, while reactions 11, 12 and 14-18 are energetically prohibited.

Table 2. Thermodynamic prognosis of microbial reduction of iron compounds

№	Fe _{ox.}	Fe _{red.}	Reaction equation	pH	Eh, V	Possibility of reduction
1	Fe(III)	Fe(II)	$\text{Fe}^{3+} + \bar{e} = \text{Fe}^{2+}$	1,6*	+0,771	+
2	Fe(III)	Fe(II)	$\text{Fe}(\text{OH})_3 + \text{H}^+ + \bar{e} = \text{Fe}(\text{OH})_2 + \text{H}_2\text{O}$	7,0	-0,143	+
3	Fe(III)	Fe(II)	$\text{Fe}(\text{OH})_3 + 3\text{H}^+ + \bar{e} = \text{Fe}^{2+} + 3\text{H}_2\text{O}$	7,0	-0,184	+
4	Fe(III)	Fe(II)	$[\text{Fe}^{3+} \cdot \text{citrate}] + \bar{e} = [\text{Fe}^{2+} \cdot \text{citrate}]$	7,0	-0,150	+
5	Fe(II)	Fe(0)	$\text{Fe}^{2+} + 2\bar{e} = \text{Fe}^0$	7,0	-0,440	-
6	Fe(II)	Fe(0)	$\text{Fe}(\text{OH})_2 + 2\text{H}^+ + 2\bar{e} = \text{Fe}^0 + 2\text{H}_2\text{O}$	7,0	-0,460	-

Note: *- Fe³⁺ cation is stable only in strongly acidic conditions. Reaction $\text{Fe}^{3+} + 3\text{H}_2\text{O} = \text{Fe}(\text{OH})_3 + 3\text{H}^+$ corresponds to the equation $\lg\{\text{Fe}^{3+}\} = 4,84 - 3\text{pH}$. If $\{\text{Fe}^{3+}\} = 1$, $\lg\{\text{Fe}^{3+}\} = 0$. Herefrom $3\text{pH} = 4,84$. Namely, $\text{pH} = 1,6$ means, that in excess of this value Fe³⁺ is transformed into insoluble form – Fe(OH)₃.

Table 3. Standard redox-potentials of iron reduction reactions

№	Reaction equation	pH	E ₀ , V
1	$\text{Fe}^{3+} + \bar{e} = \text{Fe}^{2+}$	1,6	+0,771*
2	$\text{Fe}(\text{OH})_3 + \text{H}^+ + \bar{e} = \text{Fe}(\text{OH})_2 + \text{H}_2\text{O}$	7,0	-0,143
3	$\text{Fe}(\text{OH})_3 + 3\text{H}^+ + \bar{e} = \text{Fe}^{2+} + 3\text{H}_2\text{O}$	7,0	-0,184
4	$[\text{Fe}^{3+} \cdot \text{citrate}] + \bar{e} = [\text{Fe}^{2+} \cdot \text{citrate}]$	7,0	-0,150
5	$\text{Fe}^{2+} + 2\bar{e} = \text{Fe}^0$	7,0	-0,440
6	$\text{Fe}(\text{OH})_2 + 2\text{H}^+ + 2\bar{e} = \text{Fe}^0 + 2\text{H}_2\text{O}$	7,0	-0,460

Note: *- for reaction № 1 ΔE_0 value is given to the value $\text{pH} = 1,6$.

Table 4. Redox-potentials of electron donors oxidation reactions

№	Reaction equation	pH	E ₀ , V
1	$\text{H}_2 = 2\text{H}^+ + 2\bar{e}$	1,6	-0,095
		7,0	-0,414
2	$\text{NADH}_2 = \text{NAD}^+ + 2\text{H}^+ + 2\bar{e}$	1,6	-0,158
		7,0	-0,320
3	succinate = fumarate + 2H ⁺ + 2e ⁻	1,6	+0,354
		7,0	+0,03

According to our calculations, the efficiency of Fe(III) reduction is as higher, as the greater energy output for the redox-reactions is. In the considered redox-reactions, microorganisms carry out reduction of Fe³⁺ ions to Fe²⁺ (reaction 1, 7, 13) the most effectively using H₂, NADH₂ and succinate as electron donors. Hydroxide

and citrate of Fe(III) are to be reduced with less efficiency, if H₂ and NADH₂ serve as electron donors. When succinate acts as an electron donor microorganisms can not reduce Fe(III) hydroxide and citrate.

Similarly, the energy output can be calculated for any pair of electron donors and acceptors.

The phenomenon of Fe(III) reduction in the environment has been the subject of controversy between supporters of chemical and biological nature of the process for a long time. The possibility of both types of the processes existence is proved now. Thus, chemical Fe(III) reduction is possible by microbial reducing metabolites: organic acids, sulfide containing amino acids or inorganic compounds such as hydrogen sulfide. In addition, Fe(III) may be involved in dissimilatory metabolism of bacteria as a terminal electron acceptor [16].

There are three metabolic pathways of Fe(III) reduction:

1. Using of Fe(III) as a terminal electron acceptor by microorganisms in catabolic processes obtaining energy;

2. Non-specific reduction of iron by metabolic products that are low-potential reducers [5];

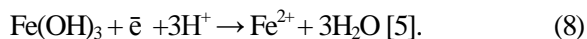
3. Non-specific reduction of iron due to the redox-analogy of metal and the terminal electron acceptor (for example, NO₃⁻), that is used by microorganisms in the respiratory chain. Because of the proximity of their redox-potential values microbial redox enzyme systems may “mistakenly” reduce Fe(III) compounds [17]. In this case reduction of Fe(III) doesn't lead to energy obtaining.

Table 5. Energetical prognosis of Fe(III)-reduction by microorganisms

№	Reaction equation (acceptor and donor)	pH	E_0', V	$\Delta E_0', V$	$\Delta G_0',$ kcal/mole
1*	$Fe^{3+} + \bar{e} = Fe^{2+}$ $H_2 = 2H^+ + 2\bar{e}$	1,6	+0,771*	+0,866	-39,95
			-0,095		
2	$Fe(OH)_3 + H^+ + \bar{e} = Fe(OH)_2 + H_2O$ $H_2 = 2H^+ + 2\bar{e}$	7,0	-0,143	+0,271	-12,5
			-0,414		
3	$Fe(OH)_3 + 3H^+ + \bar{e} = Fe^{2+} + 3H_2O$ $H_2 = 2H^+ + 2\bar{e}$	7,0	-0,184	+0,23	-10,61
			-0,414		
4	$[Fe^{3+} \cdot citrate] + \bar{e} = [Fe^{2+} \cdot citrate]$ $H_2 = 2H^+ + 2\bar{e}$	7,0	-0,150	+0,264	-12,18
			-0,414		
5	$Fe^{2+} + 2\bar{e} = Fe^0$ $H_2 = 2H^+ + 2\bar{e}$	7,0	-0,440	-0,026	+1,2
			-0,414		
6	$Fe(OH)_2 + 2H^+ + 2\bar{e} = Fe^0 + 2H_2O$ $H_2 = 2H^+ + 2\bar{e}$	7,0	-0,460	-0,046	+2,12
			-0,414		
7*	$Fe^{3+} + \bar{e} = Fe^{2+}$ $NADH_2 = NAD^+ + 2H^+ + 2\bar{e}$	1,6	+0,771*	+0,929	-42,86
			-0,158		
8	$Fe(OH)_3 + H^+ + \bar{e} = Fe(OH)_2 + H_2O$ $NADH_2 = NAD^+ + 2H^+ + 2\bar{e}$	7,0	-0,143	+0,177	-8,17
			-0,320		
9	$Fe(OH)_3 + 3H^+ + \bar{e} = Fe^{2+} + 3H_2O$ $NADH_2 = NAD^+ + 2H^+ + 2\bar{e}$	7,0	-0,184	+0,136	-6,27
			-0,320		
10	$[Fe^{3+} \cdot citrate] + \bar{e} = [Fe^{2+} \cdot citrate]$ $NADH_2 = NAD^+ + 2H^+ + 2\bar{e}$	7,0	-0,150	+0,17	-7,84
			-0,320		
11	$Fe^{2+} + 2\bar{e} = Fe^0$ $NADH_2 = NAD^+ + 2H^+ + 2\bar{e}$	7,0	-0,440	-0,12	+5,54
			-0,320		
12	$Fe(OH)_2 + 2H^+ + 2\bar{e} = Fe^0 + 2H_2O$ $NADH_2 = NAD^+ + 2H^+ + 2\bar{e}$	7,0	-0,460	-0,14	+6,46
			-0,320		
13*	$Fe^{3+} + \bar{e} = Fe^{2+}$ succinate = fumarate + $2H^+ + 2\bar{e}$	1,6	+0,771*	+0,417	-19,24
			+0,354		
14	$Fe(OH)_3 + H^+ + \bar{e} = Fe(OH)_2 + H_2O$ succinate = fumarate + $2H^+ + 2\bar{e}$	7,0	-0,143	-0,173	+7,98
			+0,03		
15	$Fe(OH)_3 + 3H^+ + \bar{e} = Fe^{2+} + 3H_2O$ succinate = fumarate + $2H^+ + 2\bar{e}$	7,0	-0,184	-0,214	+9,87
			+0,03		
16	$[Fe^{3+} \cdot citrate] + \bar{e} = [Fe^{2+} \cdot citrate]$ succinate = fumarate + $2H^+ + 2\bar{e}$	7,0	-0,150	-0,18	+8,3
			+0,03		
17	$Fe^{2+} + 2\bar{e} = Fe^0$ succinate = fumarate + $2H^+ + 2\bar{e}$	7,0	-0,440	-0,47	+21,68
			+0,03		
18	$Fe(OH)_2 + 2H^+ + 2\bar{e} = Fe^0 + 2H_2O$ succinate = fumarate + $2H^+ + 2\bar{e}$	7,0	-0,460	-0,49	+22,6
			+0,03		

Note: * – for reaction № 1, 7, 13 values are given to the value pH = 1,6, because at the pH > 1,6 Fe(III) is precipitated: $Fe^{3+} + 3OH^- \rightarrow Fe(OH)_3$

Generally reduction of $Fe(OH)_3$ occurs in accordance with these coupled redox-reaction:



Microorganisms can use a wide range of inorganic and organic compounds as electron donors for Fe(III) reduction. These compounds are listed in Table 6.

Table 6. Electron donors for Fe(III)-reducing microorganisms

№	ē donor	Reaction equation	Microorganisms			Ref.
			Aerobes	Facultative anaerobes	Anaerobes	
1	H ₂	$H_2 + 2Fe(III) \rightarrow 2H^+ + 2Fe(II)$	<i>Pseudomonas</i> sp.	<i>Shewanella putrefaciens</i>	<i>Desulfovibrio</i> sp.	5, 6, 7, 9
2	S ⁰	$S^0 + 6Fe(III) + 4H_2O \rightarrow HSO_4^- + 6Fe(II) + 7H^+$	<i>Thiobacillus ferrooxidans</i> , <i>T. thiooxidans</i> , <i>Sulfolobus acidocaldarius</i>	–	–	5, 6, 9
3	C ₆ H ₁₂ O ₆	$C_6H_{12}O_6 + 24Fe(III) + 12H_2O \rightarrow 6HCO_3^- + 24Fe(II) + 30H^+$	<i>Bacillus</i> sp.	<i>Vibrio</i> sp., <i>Bacillus</i> sp.	<i>Clostridium butyricum</i>	5, 9
4	lactate	$lactate^- + 4Fe(III) + 2H_2O \rightarrow acetate^- + HCO_3^- + 4Fe(II) + 5H^+$	<i>Pseudomonas</i> sp.	<i>Shewanella putrefaciens</i>	<i>Desulfovibrio</i> sp., <i>Desulfotomaculum nigrificans</i>	5, 6, 9
5	acetate	$acetate^- + 8Fe(III) + 4H_2O \rightarrow 2HCO_3^- + 8Fe(II) + 9H^+$	–	–	<i>Geobacter metallireducens</i> , <i>Desulfuromonas acetoxidans</i>	6
6	formate	$formate^- + 2Fe(III) + H_2O \rightarrow 2HCO_3^- + 2Fe(II) + 2H^+$	–	<i>Shewanella putrefaciens</i>	–	5, 6
7	pyruvate	$pyruvate^- + 2Fe(III) + 2H_2O \rightarrow acetate^- + HCO_3^- + 2Fe(II) + 3H^+$	–	<i>Vibrio</i> sp., <i>Shewanella putrefaciens</i>	–	5, 6, 9
8	phenol	$phenol + 28Fe(III) + 17H_2O \rightarrow 6HCO_3^- + 28Fe(II) + 34H^+$	–	–	<i>Geobacter metallireducens</i>	5, 6

Note: “–” – no data.

CONCLUSIONS

Thus, the thermodynamic prognosis allows to calculate main directions of biogeochemical ways of microbial interaction with iron compounds in man-made and natural ecosystems (Fig. 9). The transformation of iron compounds is based on the pH and Eh values of the environment. It includes redox-

reactions and reactions of soluble iron compounds transformation into insoluble and vice versa. It is possible to calculate all theoretically feasible kinds of microbial interaction with iron compounds and to determine the necessary and sufficient conditions for the regulation of this interaction according to Pourbaix diagrams and respective redox-equations.

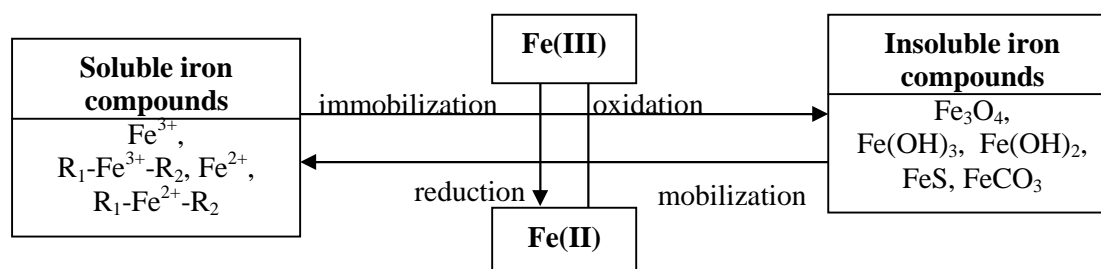
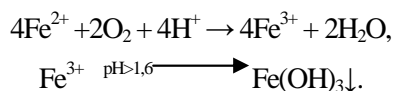


Fig. 9. The model of inverse microbial transformation of soluble iron compounds into insoluble and oxidized into reduced

Microorganisms can carry out conjugated cycle of carbon compounds oxidation and Fe(III) reduction. Using Fe(III) in dissimilatory metabolism simultaneously with such common terminal electron acceptors as O_2 , NO_3^- , SO_4^{2-} , etc. Fe(III)-reducing microorganisms are involved in the distribution of vector carbon and energy flows in ecosystems.

Microorganisms can mobilize iron of minerals, destroy soil minerals, facilitate migration of its soluble compounds in the earth crust. They also can immobilize iron, concentrate it, deposit of iron and form minerals (Fig. 9).

Controlled regulation of microbial transformation of iron compounds enables the development of effective water purification biotechnologies from iron. In this case, there must be created conditions for conversion of soluble iron compounds into insoluble form. Since Fe^{2+} cations are stable in a wide pH range (Table 1), it must be oxidized to Fe(III). It can be done using microbial associations with aeration of media. For example, *Thiobacillus ferrooxidans* oxidizes Fe(II) to Fe(III) in low-acidified conditions with next formation of insoluble $Fe(OH)_3$ as follows [4]:



As organic compounds are present in water they may form metal-organic complexes with Fe(III). Chelated iron is stable in a wide pH-range. Organic compounds must be destroyed by microorganisms to remove Fe(III) from the solution. Cation of Fe^{3+} is unstable at the $pH > 1,6$. Because at the $pH > 1,6$ Fe(III) compounds are stable only in insoluble form, such as $Fe(OH)_3$. It leads to the formation of insoluble iron oxides and hydroxides and, consequently, to iron removal from water. Interaction of Fe(II) and Fe(III) compounds with such microbial exometabolites, as H_2S and CO_2 , leads to the formation of insoluble iron carbonates and sulphides. It also helps to remove iron from water.

It is possible to calculate theoretically and choose the most effective microbial pathway of iron compounds transformation basing on the thermodynamic prognosis of microbial interaction with iron. Thermodynamic prognosis allows to quantify the contribution of microorganisms in the biogeochemical cycles of iron compounds transformation in the environment, and create cost-effective and environmentally friendly biotechnologies of water purification from iron or use microorganisms for Fe(II) and Fe(III) mobilization from metal ore.

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ТЕРМОДИНАМИЧНИ ПРОГНОЗИ ЗА ВЗАИМОДЕЙСТВИЯТА НА МИКРООРГАНИЗМИТЕ С ЖЕЛЯЗОСЪДЪРЖАЩИ СЪЕДИНЕНИЯ

Вира Говоруха, Олга Радченко, Александър Таширев

Резюме. Микробната редокс-трансформация на железните съединения е свързана с условията на околната среда, дефинирани като група от физически и химически фактори, включително рН и Eh. В работата са представени всички теоритично изпълними пътища на взаимодействието на микроорганизмите с железни съединения (подвижни/ имобилизирани, окисление/редукция). Теоритично обосновано е, че микроорганизмите са способни да проявяват висока активност по отношение на биогеохимичните цикли на въглеродо и желязото. Получените резултати могат да бъдат използвани за прогнозиране на микробните трансформации на железни съединения в природата и екосистемите създадени от човека и за създаване на биотехнологии за почистване на водни източници и отпадни води от желязосъдържащи съединения.

Ключови думи: микроорганизми, железни съединения, термодинамични прогнози, редукция на желязото, биогеохимична роля на микроорганизмите, микробни биотехнологии.

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