

Model of Biomineralization of Ferrum Compounds by *Gallionella* Cells Immobilized on Contact Loading of Bioreactor

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Received: November 21, 2017. Revised: December 03, 2017. Accepted: December 06, 2017

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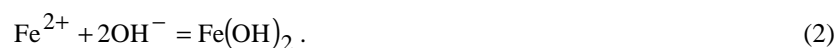
Abstract

In the underground waters not polluted by organic compounds the bivalent iron occurs in the form of hydrocarbonates. An inseparable part of ferrum ions are ferrum bacteria. As a result of the literature review carried out it is determined that up till present no mechanism of the impact of additional source of non-organic carbon on the processes of in-cellular metabolism of *Gallionella* type bacteria had been established. The aim of the paper is the determination of the effect of the additional source of non-organic carbon in the form of Na_2CO_3 on processes of metabolism and the rate of biochemical oxidation of iron compounds by bacteria *Gallionella sp.* and the development of the possible mechanism of the assimilation of non-organic carbon in the recovered pentose phosphate cycle. The comprehensive scheme of activating bacteria metabolism developed by us testifies to the possibility of increasing the energy capacity of recovered pentose phosphate cycle, the acceleration of metabolism cycles and the rate of pumping electrons via the fermentative cell system. The totality of presented processes results in speeding up the fermentative oxidation of Fe^{2+} on the surface of a cell with the final formation of matrix structures of biominerals and the increased efficiency of bioreactors operation.

Keywords: biomineralization of iron compounds; iron bacteria; electrons; respiratory chain.

1. Introduction

In the underground waters non-polluted by organic compounds the bivalent iron occurs, as a rule, in the form of hydrocarbonates. In non-oxidized underground waters iron ions (II) are subjected to hydrolysis with the formation of complexes:



An inseparable component of ferrum ions in underground waters are various kinds of iron and of manganese oxidizing bacteria. The presence of these or other kinds of iron bacteria depends on a number of factors. The main ones include the level of pH – Eh of the medium, the availability of dissolved organics or of mineral carbon, and also the presence of Fe^{2+} ions. In acid waters with pH=3.5–5.0 the development of *Th.ferrofidans* bacteria is observed. In waters with the pH level =5.5–7.5 and Eh=100 – 300mB there are *Gallionella* gram negative chemo-

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This paper should be cited as: O. Kvartenko, I. Gryuk, L. Sabliy. Model of biomineralization of ferrum compounds by *Gallionella* cells immobilized on contact loading of bioreactor. Energy Eng. Control Syst., 2017, Vol. 3, No. 2, pp. 51–56. <https://doi.org/10.23939/jeeecs2017.02.051>

litho-autotrophs the energy source for which is a non-organic carbon contained in the form of free carbon dioxide or hydrocarbonates. Bacteria of this type develop in microaerophile conditions with the concentration of dissolved oxygen from 0.1 to 3.0 mg/dm³ with $t = 8-16\text{ }^{\circ}\text{C}$ and concentration of Fe^{2+} from 1.0 to 30 mg/dm³.

In waters with the pH level = 6.0–7.5, under anoxide conditions with the concentration of oxygen 1.0–3.0 mg/dm³, $t = 10-40\text{ }^{\circ}\text{C}$, there is observed the active development of gram negative heterotrophic bacteria of *Lepthothrix* type. The concentration of dissolved oxygen for this type of bacteria is not a limiting factor. The oxidation of ferrum by these bacteria is not connected with receiving energy but is a consequence of the oxidization by products of their in-cellular metabolism – by hydrogen peroxide. The source of carbon is organic acids and sugar. Bacteria of *Lepthothrix* type are able to develop under the conditions of the pollution of underground waters by dissolved humin acids. The works by Czekalla, 1985; Seppänen H. 1991; Mouchet, 1992; Mencha M. N., 2000; Zhurba M.G., 2006; [1–5] are dedicated to studying possibilities of using iron bacteria in the branch of treating underground waters.

However, most of them study only the natural mechanism of the iron compounds oxidation with the help of various types of iron bacteria [1, 2, 4], and also optimal values of dissolved oxygen, pH – Eh values of medium [2, 3, 5], of the rate of filtering in bioreactors with permanent water consumption [3, 5].

Up till present, there had been no studies of the optimal value of bicarbonate alkalinity of outlet water, and also of using the additional source of non-organic carbon of *Gallionella* chemo-litho-autotrophs. Thus, an urgent task is the disclosure of the effect of the additional source of non-organic carbon on processes of mass transfer, rate of biochemical oxidation of ferrum compounds and the development of the mechanism for assimilating non-organic carbon into recovered pentose phosphate cycle exemplified by *Gallionella* bacteria.

2. Analysis of recent publications and research

At present, several mechanisms are known which describe the movement of electrons along the breathing chain of chemo-litho-autotrophs of bacteria. Fig. 1 presents the mechanism of the electrons transport in the breathing chain of chemo-litho-autotrophic ferrum bacteria [6].

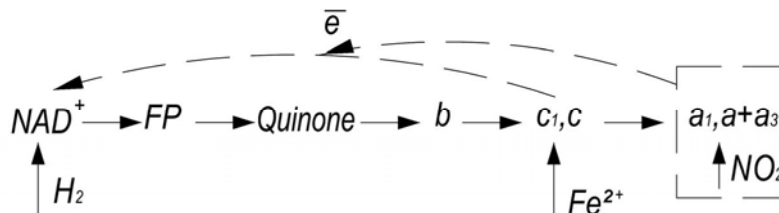


Fig. 1. Scheme of electrons movement for aerobic chemo-litho-autotrophic iron bacteria [6]

As a result of the wide spectrum of research concerning physiology, genomics and systematics of fresh water ferrum bacteria conducted by a group of scientists under the guidance of David Emerson, Eric Field and Caren Davenport in Bigelow Laboratory for Ocean Sciences, in East Boothley Harbor, in Los Alamos National Laboratory, in DOE Joint Denome Institute, in Walnut Creek, USA the improved scheme was suggested of the transport of electrons from Fe^{2+} ions, in cyto-plasmatic membrane of ferrum bacteria *Gallionella* (Fig. 2).

The scheme is based only on genomic analysis Mt_0A and shows the possibility of protein to initiate to initiate the oxidation of Fe^{2+} to Fe^{3+} . In work [7] it is shown that the mechanism of electrons transport from the exterior cell of membrane to cytoplasm is still unknown. As a result of studies [7] is determined that bacteria of *Gallionella* ES-2 type have no bc_1 chromosomes and it was assumed that ACIII pool may substitute their functions. The organism has bd type of cytochrome oxidase, which may interact with quinones. According to Emerson researches the chain of electrons movement consists of five separate complexes [8]. *Gallionella* organism is fully completed with genomes for NADH dehydrogenase, and also with complex of dehydrogenase succinate (Fig. 2).

The microorganism has quinon with the help of which the splitting off and the transfer of the electron from NADH dehydrogenase to cytochrome complex ACIII takes place. As was noted down in papers [7,8] the microorganism has a cluster which includes molybdopterin oxidoreductase. This [ACIII] complex is able to interact with pools of quinions and movements of electrons to the terminal of oxidaze [9] as a result of which a microorganism is able to use it instead of

cytochrome *bc₁* complex. The microorganism has also cytochrome of *cbb₃* oxidase which have larger inclination for oxygen [10]. Besides, the microorganism codes cytochrome of *bd*-oxidase which is also the inclination for normal development in the conditions for the presence of oxygen and is connected directly with quinion pool [11].

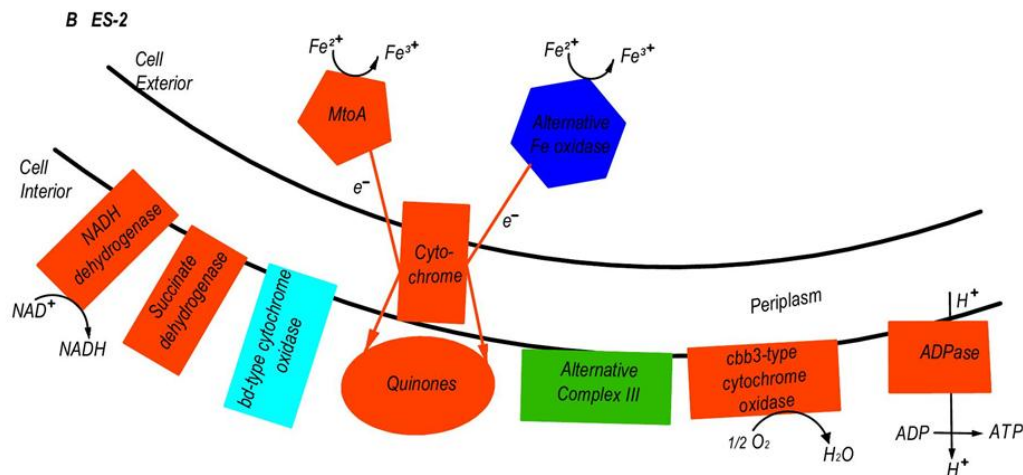


Fig. 2. Scheme of electron motion from Fe^{2+} ions to the respiratory chain at the *Gallionella* CPM [7]

Ferrum bacteria of *Gallionella* type are well adapted to chemolithoautotrophy, including the assimilation of CO_2 and have the system of electrons transport adapted for their development in the medium with low concentrations of oxygen. Correspondingly to [7] *Gallionella* contain additional genes for producing polysaccharide and for fixing CO_2 , and also are adapted to the action of heavy metals.

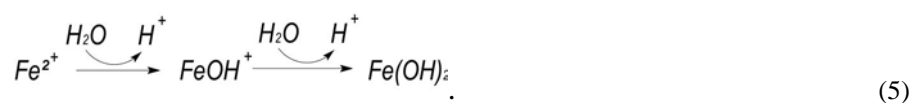
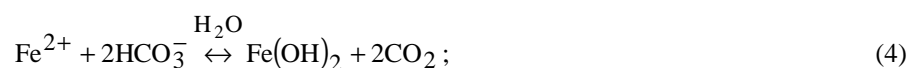
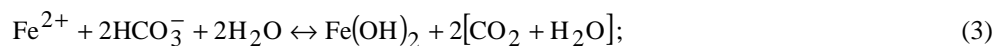
3. Forming paper aim

The aim of theoretical researches was to reveal the impact of the additional source of non-organic carbon in the form of Na_2CO_3 on processes of mass transfer and rate of biochemical oxidation of ferrum compounds into *Gallionella sp.* The development of the possible mechanism of the assimilation of nonorganic carbon with Na_2CO_3 in recovered pentose phosphate cycle.

4. Presentation and discussion of research results

On the basis of the theory of electrons movement mechanism from Fe^{2+} ions to breathing mechanism on cytoplasmic membrane ferrum bacteria of *Gallionella* type [7] we for the first time suggested the mechanism for activating processes of electron transport, the assimilation of carbon dioxide, the operation of proton cellular pump in ferrum bacteria of *Gallionella* type with removing products of hydrolysis outside the boundaries of reaction in bioreactors while using the additional source of carbon in the form of calcinated soda (Fig. 3).

In underground waters iron is contained in the presence of HCO_3^- ions both in the form of Fe^{2+} , $\text{Fe}(\text{OH})^+$ and in the molecular form of $\text{Fe}(\text{OH})_2$. Under the insufficient quantity of dissolved oxygen in water and the presence of other reducers the oxidation of ferrum compounds (II) is preceded by hydrolysis.



Therefore, in the block “source waters” (Fig. 3) two processes connected with the hydrolysis of ferrum compounds are simultaneously considered. I – hydrolysis of ferrum hydrocarbonate $\text{Fe}(\text{HCO}_3)_2$, with formation of

Fe(OH)₂ and two molecules of CO₂. II – hydrolysis of the small quantity of Fe²⁺ ions with the formation of FeOH → Fe(OH)₂ and the removal of two protons which acidize water. This process is simplified (without the influence of dissolved organic substances, sulfides and hydrosulfides) and presented in Fig.3 in block “source water”.

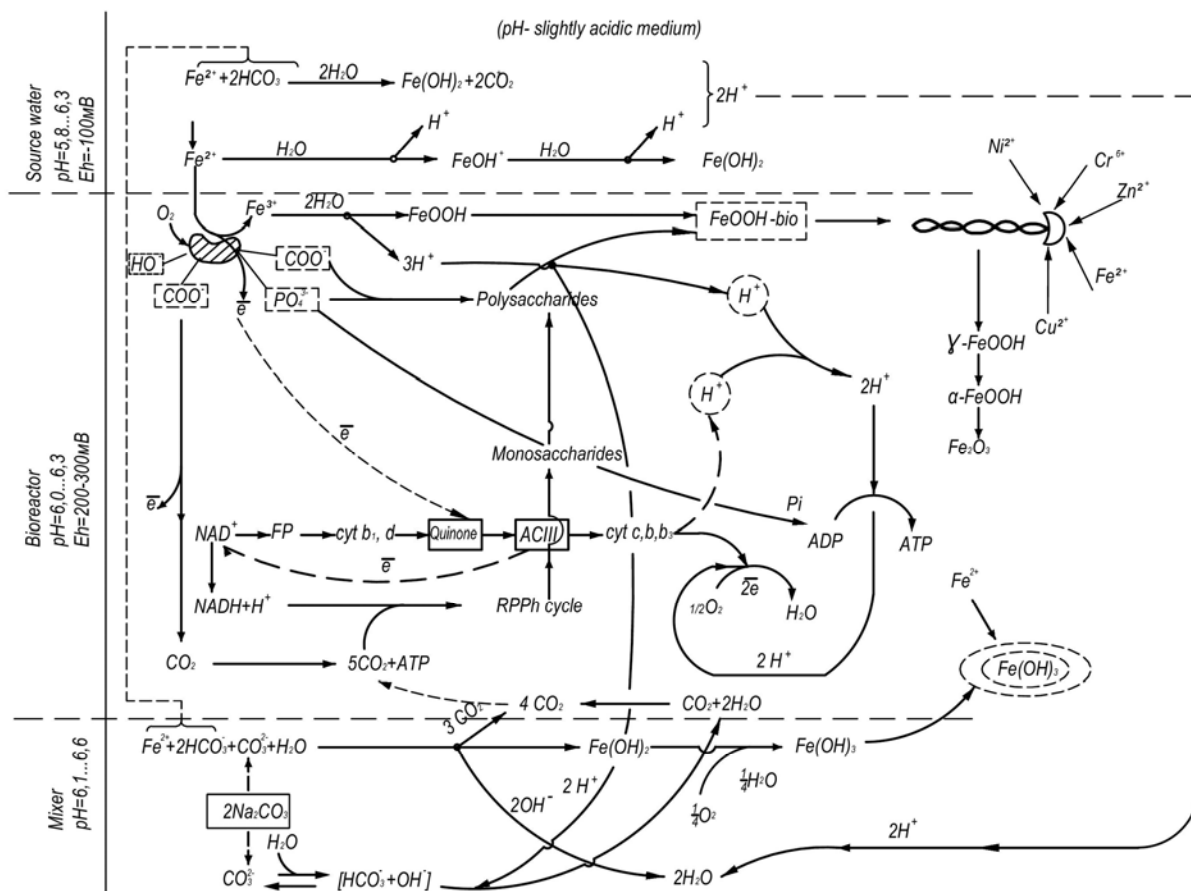
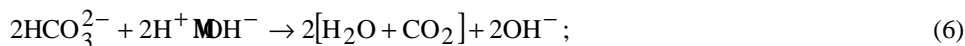


Fig. 3. The complex scheme of activation of processes of intracellular metabolism of bacteria *Gallionella*, fixed on contact loading of a bioreactor using the additional source of carbon in the form of calcinated soda

In the mixer, there takes place the stage by stage introduction into the source water of the solution of calcinated soda and air oxygen. In soda solution introduction, there takes place the hydrolysis of Na₂CO₃ by anion with the formation of two molecules CO₂ and two molecules of ions hydroxide which neutralize two protons created in outlet water as a result of the hydrolysis of Fe²⁺ and which are removed as water from the zone of reaction:



When introducing the calculated concentration of dissolved oxygen 1/4 O₂ there takes place the oxidation of the part of ferrum compounds (II) to ferrum hydroxides (III) with their removal outside the boundaries of the zone of the biological process of treatment. When introducing calcinated soda, there takes place the increase in pH and the bicarbonate alkalinity of the medium, the speeding up of the processes of hydrolysis. Simultaneously with this, the removal of products of hydrolysis from the zone of reaction contributes to more complete formation of Fe(OH)₃ in the mixer. Carbon which is introduced at this stage to mixer, is an additional source for building cellular material, along with hydrocarbonates and carbon dioxide of source water. Carbon is built-in to the recovered pentose phosphate cycle as a result of which monosaccharides are formed which later on are able to being polymerized into polysaccharides complexes with fermentative oxidation on the surface of a cell by Fe²⁺ ion transforming into biominerals FeOOH-bio [12], which have a characteristic form of spiral-like stems of *Gallionella* iron bacteria.

The presence of which, in matrix structures of interporous space of contact loading of bioreactors, was confirmed by results of researches carried out at stations of biological deferrization in a settlement of Rokytne and t. Berezne of Rivne region [13]. The spectral analysis of bacteria covers, carried out with the help of a raster-type microscope FEI Quanta (USA) equipped with Roentgen spectroscopy for conducting the microanalysis (EDAX), showed the presence of not only of Fe, but also of such elements as C, N, P, which testifies about their biological origin [13].

Characteristic is the availability of negatively charged groups of anions COO^- , PO_4^{3-} , OH^- on the surface of a cell on which the adsorption takes place of ions Fe^{2+} [14]. Assimilated on the surface of a cell anions groups COO^- , PO_4^{3-} are used by a cell in subsequent cycles: phosphate non-organic $\text{P}_i(\text{PO}_4^{3-})$ is utilized in cycle $\text{ADP} - \text{ATP}$; carbinatation COO^- is in the role of one of carbonate sources in cycles of building polysaccharides, and also in recovered pentose phosphate cycle during electron loss and restoration to free form of carbon dioxide CO_2 .

Larger part of ions Fe^{2+} is tied via exterior cellular ferment – restuzian, losing one electron which is transported from the surface of the ferment to chinon and further transferred to complex AC III from where by the known scheme of back transport [7] a part of electrons against gradient is transferred to complex $\text{NAD}^+ \rightarrow \text{NADH} + \text{H}^+$ and introduces together with carbon to the first stage of Calvin cycle. The higher is the value $\Delta\mu_{\text{H}^+}$, the quicker will be the process of pumping electrons from complex ACIII to complex NAD^+ with formation $\text{NADH} + \text{H}^+$ as a result of which on the surface of a cell under the impact of polysaccharides takes place the speeding up of hydrolysis processes of ions Fe^{3+} with the formation of matrices of biominerals which in time undergo the chain of transformations. Thus, the process of fermentative oxidation of ions Fe^{2+} takes place on the account of the transfer and building-in of the electron into the breathing chain of a bacterium. As a result the reaction of biochemical transformation $\text{Fe}^{3+} \rightarrow \gamma\text{FeOOH}$ takes place with the removal into the environment of three protons, two of each are tied with complex $[\text{HCO}_3^- + \text{OH}^-]$ created as a result of the hydrolysis of one of molecules of calcinated soda in the mixer as a result of which an additional molecule of carbon dioxide comes into the bioreactor. Besides, another three molecules of carbon dioxide formed as a result of the hydrolysis of ferrum hydrocarbonate and calcinated soda come into the bioreactor from the mixer. Another molecule of carbon dioxide from carboxylate-ion COO^- (Fig.3) joins these molecules in the bioreactor. Thus, a pool is created from five molecules of carbon dioxide which are assimilated by a cell at the first stage of recovered pentose phosphate cycle with stage-by-stage formation of mono- and polysaccharides.

As was pointed down earlier, as a result of the hydrolysis of ions Fe^{3+} as a consequence of fermentative reactions three protons are created two of which are neutralized by a complex $[\text{HCO}_3^- + \text{OH}^-]$ with the formation of CO_2 and two molecules of water. A proton which remained together with the proton singled out by a cell in the process of breathing uses a cellular proton pump for transferring into the cycle the creation ATP of the number of protons in the environment of a cell decreases, and a proton freed as a result of the reaction hydrolysis of Fe^{3+} is transferred to the free place. Thus, the speeding up of the process of hydrolysis of ions Fe^{3+} takes place in the presence of polysaccharides with the formation of matrix structure of biominerals.

5. Conclusion

Based on the consideration of the presented mechanism, we can see that after introducing the additional source of non-organic carbon, the energy capacity of recovered pentose phosphate cycle increases, cycles of in-cellular metabolism and the rate of electrons pumping via exterior fermentative system of a cell are accelerated. The totality of presented processes results in speeding up the fermentative oxidation of ions Fe^{2+} on the surface of a cell with the final formation of matrix structures of biominerals. The presented scheme shows the balancing of the process of the biochemical oxidation of Fe^{2+} by ferrum bacteria of *Gallionella* type at once in three directions: first – the arrival of H^+ to proton pump with the formation of ATP; second – the creation of polysaccharides, via recovered pentose phosphate cycle as a result of the supply of the additional source of carbon; third – the acceleration of "pumping" electrons with the help of the restuzian ferment.

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Модель біомінералізації сполук заліза клітинами *Gallionella*, іммобілізованими на контактному завантаженні біореактора

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Анотація

У підземних водах, незабруднених органічними сполуками, двовалентне залізо трапляється у формі гідрокарбонатів. Невід’ємним супутником іонів заліза є залізобактерії. У результаті проведеного літературного огляду встановлено, що дотепер не вивчено механізмів впливу додаткового джерела неорганічного вуглецю на процеси внутрішньоклітинного метаболізму бактерій роду *Gallionella*. Метою роботи є встановлення впливу додаткового джерела неорганічного вуглецю у вигляді Na₂CO₃ на процеси масообміну та швидкість біохімічного окиснення сполук заліза бактеріями *Gallionella sp.* та розроблення можливого механізму асиміляції неорганічного вуглецю до відновленого пентозофосфатного циклу. Комплексна схема активації метаболізму бактерій, яку ми розробили, свідчить про можливість підвищення енергетичної потужності відновленого пентозофосфатного циклу, прискорення циклів метаболізму та швидкості перекачування електронів через ферментативну систему клітини. Сукупність наведених процесів призводить до прискорення ферментативного окиснення іонів Fe²⁺ на поверхні клітини з кінцевим утворенням матриксних структур біомінералів та підвищення ефективності роботи біореакторів.

Ключові слова: біомінералізація сполук заліза; залізобактерії; електрони; дихальний ланцюг.