Study of biogenic iron oxides by neutron activation analysis and x-ray diffraction

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Abstract: Bacteria from the Sphaerotilus-Leptothrix group of the iron oxidizing bacteria contribute to the biogeochemical cycle of the iron that occurs in the lithosphere. It is a fundamental geological process of iron oxidation performed by microorganisms where iron first is mobilized, then it is used and assimilated by the bacteria and finally it is immobilized and deposited. The deposition of iron ions is extracellular in the form of biogenic products contained in tubular structures (sheaths). We report on the determination of the elemental constitution and the structure of biogenic iron oxides/(oxy) hydroxides resulting from the bacterial metabolism. The Fe(II)-oxidizing organism was isolated from freshwater wetland surface sediments in Vitosha Mountain.

Key words: sheath-forming bacteria, Leptothrix bacterium, biogenic iron oxides, PGAA, NAA

Introduction

The Fe(II)-oxidizing bacteria (FeOB) and Fe(III)-reducing bacteria (FeRB) were among the first groups of microorganisms to be recognized for carrying out a fundamental geological process - the bacterial iron redox cycling (Weber et al., 2006). "Iron bacteria" are found naturally in soils and water. When groundwater flows through iron-bearing soil or rock it picks up Fe along the way. In general, wherever there is oxygen, water, and iron, there is potential for iron bacteria to develop. When Fe(II) comes into contact with O_2 or other suitable oxidants (e.g. NO_3^-, Mn(IV)), Fe(II) can be reoxidized to Fe(III).

Iron is one of the most common elements found in nature accounting for at least 5% of the earth's crust. A large part of Earth's sedimentary iron deposits can be attributed directly or indirectly to microbial activity (Schieber, 2004). Banded Iron Formations (BIF), for instance, is thought to have formed from the precipitation of iron from the Earth's ancient oceans. Photosynthetic bacteria produced, for possibly the first time in the young Earth's oceans, free oxygen which oxidized the dissolved Fe(II) that at those times existed amply. Oxidized iron Fe(III) is not soluble in water and thus it would precipitate out of the waters and onto the mud-covered sea floor. Bacteria are believed as the earliest life forms on the Earth and eventually the oxygen producing varieties formed the BIF and helped transform the Earth.

The notable feature of some FeOB are the unique morphological structures they produce, such as powders, sheaths or stalks, that act as organic matrices upon which the deposition of hydrous ferric oxides can occur (Cornell et al., 2003). They are capable of accumulating metals by binding them as cations to the cell surface in a passive process as well gaining energy for growth from the oxidation of ferrous iron with O_2 as terminal electron acceptor. Emerson et al. (2010) provided a historical overview of research on circumneutral bacterial Fe(II) oxidation, as well as the physiology and systematics of known lithotrophic FeOB.

Sheath-forming iron- and manganese-depositing bacteria belonging to the Sphaerotilus-Leptothrix group (SLG) are widespread in natural and artificial water systems. Known requirements for their growth include the presence of organic substrates and molecular oxygen. They are capable of oxidizing Fe^{2+} and Mn^{2+} and as a result of their metabolism, they form biogenic iron oxides/(oxy)hydroxides accumulated in their "sheaths". The sheaths may appear yellow to dark brown because of the deposition of iron and manganese oxides.

In this report we complement the information on the biogenic oxides' structures and the capabilities for accumulation of iron in the bio-products of Leptothrix bacteria grown in the elective nutrient media known as the medium of Adler and the Isolation medium (Angelova et al., 2015), also for the case of the silicon–iron–glucose–peptone (SIGP) nutrient medium.

Experimental Part

The sampling region is a stream located in Vitosha Mountain where deposits with characteristic texture and brown-red colour due to the ferroxides were formed (Figure 1, a).

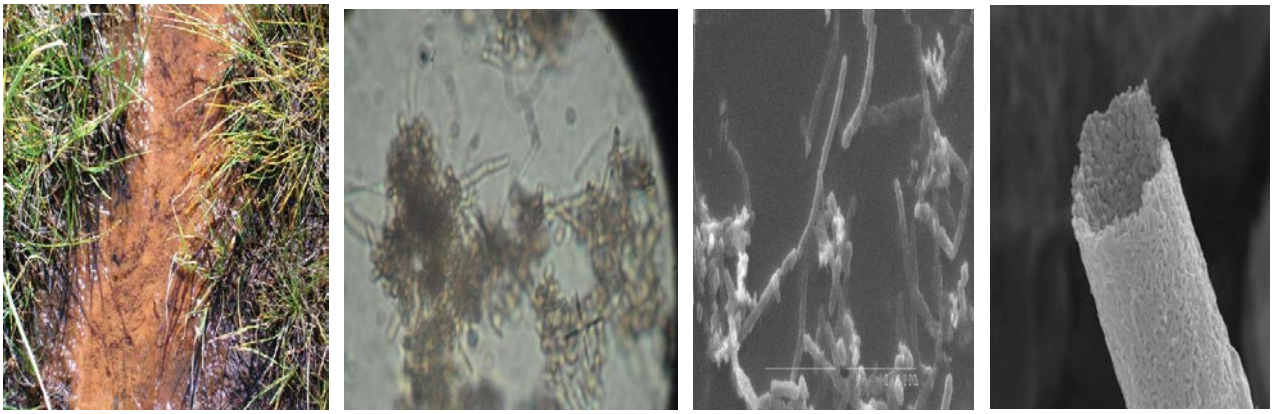

Figure 1. **(a)** Typical bacterial deposits in the water flow in Vitosha Mountain (Aleko locality, 1783 m altitude); Microscopic images of the microtubules produced by Leptothrix ochracea in SIGP medium: **(b)** light microscopy image, (**c)** scanning electron microscopy image (JEOL, 5000x); **(d)** microstructure of the wall of a tubule.

Two types of cultivation – static and dynamic were carried out. The dynamic cultivation was achieved both in Erlenmeyer flasks by shake at 70 rpm and in specially constructed fermenter with additional aeration. The period of cultivation was from 7 to 120 days. The cultivation was carried out at 3 different temperatures - 10°C, 20°C и 37°C. Periodically samples were taken and microscopic analyses of the cultures in the process of cultivation were performed. Samples of cultivated in different feeding media biomass were obtained and are noted further in the text according to the name of the used medium as follows:

- One sample denoted Reference sample is a biomass picked from a natural source in Vitosha Mountain;
- Two samples denoted '2 Adler-DE' and '2 Adler-DP' were products after cultivation of the bacteria in AM with ammonium iron (II) at different periods of cultivation: sample 2 Adler-DE – 56days cultivation in Erlenmeyer flasks of Leptothrix sp, Adler-DP – 88days cultivation in Roux flasks, enriched culture;
- Three samples denoted 1 Isolation-MP; 1 Isolation-MY and 1 Isolation-MF were products from bacterial action of the bacteria in IM with (NH4)2Fe(SO4)2·6H2O, as follow: sample 1MP – 103 days cultivation on Isolation medium in Roux flasks; sample 1MY – 103 days cultivation in bioreactor; sample 1MF – 103 days cultivation on Isolation medium in Fernbach flask ;
- One sample denoted SIGP E,1 is from monobacterial Leptothrix with nutrient silicon–iron–glucose–peptone (SIGP) medium (Sawayama et al., 2011): 1 g glucose, 1 g Bacto peptone, 0.2 g Na_2SiO_3_$9H_2O$, 0.044 g $CaCl_2$_$2H_2O$, 0.041 g $MgSO_4$_$7H_2O$, 0.076 g Na_2HPO_4_$12H_2O$, 0.02 g $KH_2PO_4.2H_2O$, 2.838 g HEPES (N-2hydroxyethelpiperazine-N'-2-ethanesulfonic acid) and 0.05 mM $FeSO_4$ in 1000 ml distilled H_2O (pH 7.0).

The analysis of the population of the iron bacteria in the biomass was performed on the basis of the specific morphology of the bacteria according to the literature by microscopy of fresh and fixed preparations. After isolation of pure cultures they were inspected with respect to morphological and physiological characteristics according the classical taxonomic scheme (Angelova et al., 2015). The key morphological characteristics analysed so far were: a) cell shape; b) Gram*stain; c) motility; d) presence of capsule. The list of growth characteristics includes: 1) ability to grow on different selected media; 2) ability to oxidize Fe^{2+}; 3) Preferable source of Fe^{2+}.

The initial characterization of the biogenic iron oxides/(oxy)hydroxides was carried out by laboratory x-ray diffraction (XRD) using a Bruker D8 diffractometer in the Bragg–Brentano reflection geometry with Cu Kα radiation (λ = 1:5418 Å). SEM and TEM images were taken in studying the products morphology.

Neutron activation analysis (NAA) and prompt gamma activation analysis (PGAA), both using the k0-standardization method, were implemented for determination of the concentrations of major and trace elements in the initial biomass and the bio-products of cultured bacterial origin. NAA and PGAA are nuclear analytical methods for identifying and quantifying element concentrations in samples simultaneously. Essentially, they are multi-elemental, multi-isotopic techniques providing evidence for the average composition of the irradiated volume and consequently, they can be very accurate for homogeneous samples.

The samples were irradiated in the reactors BRR (Budapest) and FRM-II (Munich) and the concentrations of about 30 elements and their radionuclides were determined. The major practical difference between the two methods originates from the gamma detection. PGAA is based on the detection of the prompt gamma rays (leaving the compound nuclei in 10^{-12}-10^{-9} s) emitted by the target during neutron irradiation whereas NAA utilizes the delayed gamma rays from the radioactive daughter nucleus (with short or long half-lives), observed after the irradiation, Figure 2. Consequently, NAA and PGAA require different experimental setups and procedures for sample preparation, data acquisition and spectrum evaluation; furthermore, the sensitivity for the elements varies between the two methods. Details can be found in (Gmeling et al., 2014).

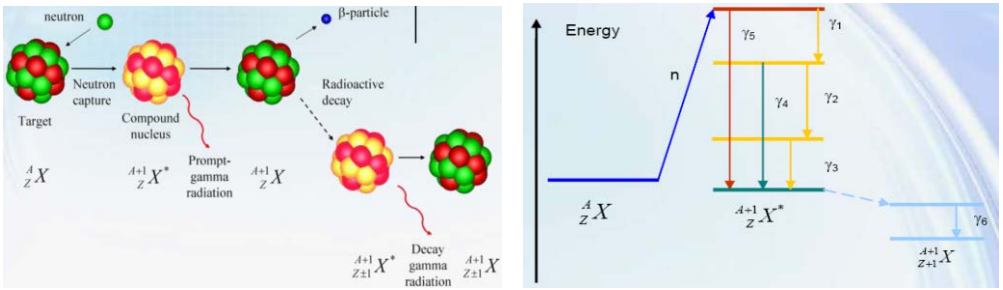

Figure 2. Diagram illustrating the radiative neutron capture - $^{A}X(n, \gamma)^{A+1}X$ reaction and the later decay reaction. The gamma energy is characteristic for the element or isotope whereas the gamma-ray intensity is characteristic for the quantity of the element or isotope.

Results and Discussion

Filtered and dried at room temperature biomass from nature (Reference sample) and the cultivation vessels was used. The strain Leptothrix spp. isolated from the site in Vitosha Mountain and grown in the artificial culture medium SIGP formed the typical sheaths (Fig.1,b). The optimal growth of the cultures is observed at 20 ^{o}C under dynamic conditions. The formation of the sheaths started after a seven-day cultivation period. The structures disintegrated completely approximately after 90 days since cultivation. The sheaths' formation under laboratory conditions depended mainly on the nutrient medium and cultivation types. A change of the dimensions of the tubular structures (sheaths) formed could be observed; the average diameter was in the range of 0.4 - 1 μm with the length reaching approximately 7 μm.

Generally, PGAA gives precise results for major elements (Si, Ti, Al, Fe, Mn, Mg, Ca, Na, K and—as a unique method—for H), for some of the light trace elements as B and Cl, as well as for Sc, S, Cr, Co, Ni, Cd, Nd, Sm and Gd. NAA is sensitive for the rare earth elements, and for many major (Ti, Al, Fe, Mn, Mg, Ca, Na, K) and trace elements (e.g.: Sc, V, Cr, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Zr, As, Sb, Ce, Ba, Hf, Ta, W). For most major elements the results obtained by the two methods show very good agreement.

Short-term irradiations were performed for determination of elements such as Al, Ca, Cu, Mg, Mn, S and V. Within a maximum decay period of 120 s the samples were packed into polyethylene capsules and measured for 10 min and again later for 20-30 min. Long-term irradiation was used for the determination of elements producing medium- or long-lived isotopes ($T_{1/2}$≥6 h). Each sample was measured three times. After a typical decay time of 2-3 days, the radionuclides ^{76}As, ^{82}Br, ^{47}Ca, ^{42}K, ^{140}La, ^{24}Na, ^{122}Sb, ^{239}Np(U) and ^{233}Pa(Th) were counted. To improve the detection limit for several radionuclides, a second measurement was made after 7-12 days (when the ^{24}Na isotope had decayed). After a decay period of about 20-30 days (when the ^{82}Br isotope decayed), the samples were counted for 5-15 hours and the radionuclides ^{110m}Ag, ^{131}Ba, ^{141}Ce, ^{60}Co, ^{51}Cr, ^{134}Cs, ^{152}Eu, ^{59}Fe, ^{86}Rb, ^{124}Sb, ^{46}Sc, ^{65}Zn and ^{187}W were measured.

Table 1 clearly indicates the strong increase in the iron content, which depends on the culture medium and is superior in the so-called Isolation medium. The enrichment rate varied between 3.8 and 7.4 as compared with the reference samples (product of nature). It deserves noting that the concentrations of S, As, Ca, Fe, K and Na were found significant in the samples studied. Additional interest comes from the registered highly selective increase of several essential elements in support to the ability of the NAA technique to reveal and quantify the presence of specific trace elements in the biosphere. However, the details are out of the scope of the present report and these data will be reported and discussed elsewhere.

The XRD analyses yielded as well the amounts and particle sizes (below 30 nm) of the resulting oxide products. Fig. 3 shows typical XRD patterns. The XRD pattern of a powder sample of SIGP-cultivated bacteria is revealing a poorly-crystalline single-phase of lepidocrocite (γ-FeOOH) with an average diameter of the particles of 8 nm.

Table 1 INAA results for the samples 2 Adler-D, 1 Isolation-M and SIGP**

Elem./ Unit	Reference sample (Vitosha)	2DE** 56 days	2DP* 88 days	1MP* 56 days	1MF* 88 Days	1MY* 103 days	SIGP** 90 days
Fe, g/kg	93±4	375±17	354±16	457±26	486±22	689±40	453±40

* Enriched; ** Pure

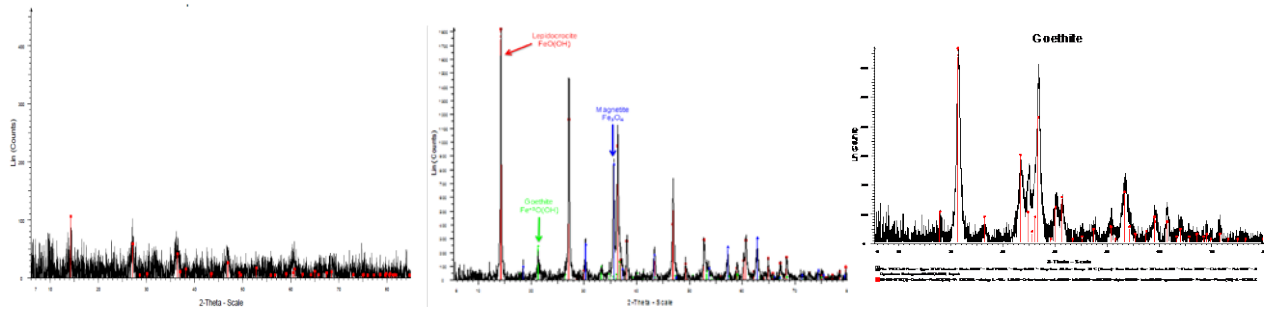

Figure 3. XRD phase analysis of biogenic powder from cultivated bacteria in an elective medium: (a) SIGP: lepidocrocite (γ-FeOOH); (b) Adler: lepidocrocite – 59.67 % - 29.931 nm, magnetite – 21.56 % - 23.860 nm, goethite – 18.77 % - 12.025 nm; (c) Isolation-MY: goethite – 100 % - 5.306 nm; Isolation-MF: goethite – 100 % - 6.198 nm

Summary

A biogenic nanostructured material is obtained after growing Leptothrix spp. in SIGP, Adler's and Isolation nutrient media. High enrichment level of iron was found by the PGAA and INAA techniques in cultivated isolates as compared to the reference sample (product of nature). The enrichment rate varied between 3.8 times for the Adler's medium and 7.4 times for the isolation medium. Three types of iron oxide compounds were found after cultivation in Adler's medium: lepidocrocite (γ-FeOOH), non-stoichiometric magnetite ($Fe_{3-x}O_4$) and goethite (α-FeOOH). The cultivation in the isolation medium and SIGP medium yielded a single phase bacterial product – goethite and lepidocricite, respectively.

Acknowledgements: The partial financial support through the HAS (RISSPO) – BAS (INRNE) bilateral agreement for collaboration 2013/2015 and the EU-FP7 NMI3 project is gratefully acknowledged. This work was supported by the Bulgarian National Science Fund under project DID02/38/2009.

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Резюме

Бактериите от групата Sphaerotilus-Leptothrix на желязо-окисляващите бактерии допринасят за биохимичния кръговрат на желязото в литосферата. Това е основен геоложки процес на окисление на желязо, който се извършва от бактериални микроорганизми, при което железните йони първоначално стават подвижни, след което се използват и усвояват от бактериите и накрая желязото се обездвижва и отлага. Отлагането на железни йони е извънклетъчно под формата на биогенни продукти, съдържащи се в тръбни структури (обвивки). Ние докладваме за определянето на елементния състав и структурата на биогенните железни оксиди / (окси) хидроокиси, произведени в резултат от метаболизма на бактерията. Fe (II) -окисляващият организъм се изолира от повърхностни седименти на сладководен източник в местността Алеко на Витоша.