

MECHANISM OF FORMATION WATER MOLECULES AND CHEMICAL BONDS IN LEPTOTHRIX MATERIALS

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Abstract. The provided study involves investigating chemical interactions in Leptothrix biomaterial through infrared spectroscopy. The analysis explores variations in chemical relationships based on the nature of chemical bonds and biomaterial coordinates. The primary chemical bonds identified in the biomaterial are associated with Fe-O vibrations and resonances of [OH] functional groups. Additionally, the study presents model representations detailing the formation mechanism of water molecules within the Leptothrix matrix. Model calculations for a two-vacancy cluster defect structure in Fe₂O₃ and Fe₃O₄ reveal lifetimes of 180 ps and 174 ps, respectively.

Keywords: Chemical bonds, Leptothrix biofilms, iron oxide, Fourier-transform infrared spectroscopy, lattice vibration.

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1. Introduction

In recent years, issues related to drinking water have continued to pose serious concerns in various parts of the world (Kumari *et al.*, 2021; Akanksha *et al.*, 2022). The swift industrial expansion occurring in an era when water sources, rivers, and lakes face contamination from hazardous heavy elements poses a critical threat to the well-being of

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humans, animals, and plants. As industries release pollutants into water bodies, the presence of toxic elements intensifies, jeopardizing ecosystems and public health (Abdullayeva *et al.*, 2020). It is well-established that iron can undergo diverse processes, particularly in aquatic environments, including deposition and precipitation within water systems. Under specific conditions, iron may form solid particles that deposit or precipitate out of the water, influencing its quality and ecological dynamics (Emerson *et al.*, 2010). Additionally, the increasing impact of anthropogenic factors on a daily basis contributes to the spread of heavy elements in the environment (Mitra *et al.*, 2022). The concentration of heavy metals exceeding normative limits in drinking water sources has severe implications for human health, leading to various allergic reactions, liver enlargement, changes in blood morphology, memory loss, and gastrointestinal diseases (Briffa *et al.*, 2020). Despite the availability of numerous water purification devices and filters, fully resolving the challenges associated with purifying heavy elements is not feasible. Nevertheless, alternative methods can be explored to address the issue at hand. In this context, one of the modern methods for purifying drinking water involves the use of biotechnological means, specifically, the use of microorganisms. In this category of microorganisms, Leptotrix bacteria are considered as a unique method for the removal of heavy elements from drinking water. Leptotrix bio-organisms induce the formation of tubular structures based on the absorption of Fe^{3+} and Fe^{2+} ions, and extensive scientific research has been conducted in this direction (Mirzayeva *et al.*, 2023). The varied nature of natural aquatic habitats, and thus the diverse conditions for bacterial colony development, including the iron-transforming Leptothrix sp., forms a basis for continuous research endeavors. These efforts are directed at obtaining a more comprehensive understanding of the structure of iron-containing materials generated through bacterial activities, as highlighted in the work by (Thomas Arrigo *et al.*, 2022). Such material is easily distinguishable by its specific orange-brown color of the fluffy sediments that form on the surface of small slow-flowing streams, in various wetlands, lakes, swamps and in wastewater treatment systems (Matýsek *et al.*, 2021; Singh *et al.*, 2018; Atakishiyeva *et al.*, 2023). The precipitate's color is due to a mixture of Fe (III) oxides and oxyhydroxides resulting from the reaction of the aforementioned iron oxidizing bacteria (FeOB) *Leptothrix* (Thomas Arrigo *et al.*, 2022; Sowers *et al.*, 2019). In nature, the processes of abiotic and biotic oxidation of iron Fe (II) to Fe (III) are highly competitive, with the advantage of one over the other highly dependent on the different conditions, but the outcome as a composition of the sediments is approximately the same, i.e. Fe (III) oxides and oxyhydroxides (Sowers *et al.*, 2019). The unique fingerprints of the biogenic origin in such sediments are the specific bacterial tubular structures formed (Singh *et al.*, 2018; Hajiyeva *et al.*, 2022), which add some structural particularities to the iron-containing mixture. Physical studies of such materials from different locations, where the presence of specific tubular formations with micron dimensions prove their biotic origin, significantly contribute to enriching the knowledge so far regarding their specific structure.

One of the species of aquatic Fe/Mn-oxidizing bacteria, genus *Leptothrix*, is characterized by the formation of an extracellular microtubular shell covered with Fe-nacop (Suzuki *et al.*, 2013). When isolated strains are cultured on artificial media containing Fe source, most of the sheaths envelop the bacterial cells in the early stages of incubation. However, most of the envelopes, especially those that appear thick and yellow-brown, subsequently become at least partially empty, resembling most of the old *L. ochracea* envelopes collected from natural aquatic environments (Suzuki *et al.*, 2013).

Emerson et al. noted that only about 10% of the shells contained a chain of bacterial cells at their active growth stage. According to Wu, *L. ochracea* forms and releases Fe-coated shells very rapidly in an enriched culture with low nutrient and Fe content, leaving a large mass of empty shells (Wu *et al.*, 2020). However, why and how *Leptothrix* cells die within the shells has not yet been studied. Internal transcribed spacer (ITS) analysis of ribosomal DNA revealed that OUMS1 is close to the known species *L. cholodnii* with a difference of only two base pairs (Sawayama *et al.*, 2011). The self-destructive final stage of the bacterial life cycle, called autolysis, has been studied for more than 100 years. Bacterial autolysis is influenced by a number of factors including growth phase, pH of the medium, osmotic pressure, temperature, and availability of oxygen, nitrogen, and carbon source (Rice *et al.*, 2008). On the other hand, the mechanism of formation of various elements and molecules in the structure of biomaterials was investigated using the PLT method. Positrons penetrating a solid can be used as a kind of microprobes that indicate places in the crystal lattice where the atomic density is less than the average density in the volume, i.e. vacancies and vacancy clusters. The annihilation aspects of a positron trapped in free space or void nano-cavities are different from those of a positron trapped in hydrogen-containing nano-cavities. Thus, based on this aspect, structural analyzes for binding of H₂O molecules inside structural defects were performed on Fe₂O₃ and Fe₃O₄. The primary objective of the research is to study the nature of chemical bonds in *Leptotrix* biomaterial that has absorbed iron ions. The method used for this study is Fourier-transform infrared spectroscopy (FTIR).

2. Materials and methods

Typical ochre-colored flocs located in a small unnamed stream in a wooded area within the city of Dubna (RF), close to Volga River (less than 200 m away, having no contact with it), were chosen for sampling procedure (Fig. 1) (Mirzayeva *et al.*, 2023).



Fig. 1. The area distribution of the biomaterial (Russian Federation, Dubna city, close to Volga River) and the sample preparation process

The functional groups and chemical bonds of the irradiated samples were investigated by FTIR spectroscopy (FTIR Varian-640), in order to examine the structural changes in samples (Demir *et al.*, 2019). The FTIR-spectroscopy method used is based on the measured spectra of translucent compressed tablets (Mirzayev, 2020). These tablets were measured by mixing (1:300) boron carbide using KBr, under $6.0 \times 10^4 \text{ N} \times \text{m}^{-2}$ pressure conditions at room temperature in thin layers of 3-7 μm thickness and 8·30 mm^2 sizes in the range of up to 5500 cm^{-1} . Atomic force microscopy (AFM)-based techniques are used widely to study the mechanical behavior of the cellular structure and its interaction with the extracellular matrix or other cell-to-cell interactions. The basic contact mode is well-adapted for solid samples and in order to determine complex spatial characteristics of a given surface, fractal analysis can be applied to the images recorded using AFM instrument. Atomic force microscopy (AFM) is used for quantitative and qualitative data based on different properties like morphology, size, surface roughness, and texture (Neov *et al.*, 2022). The calculations was obtained by the MIKA package with his local density approximation (LDA) method, without relaxation in a conventional scheme using TCDFT (Makkonen *et al.*, 2006; Boronski *et al.*, 1986). We provide a brief introduction to the functional that accounting for the exchange-correlation interaction (Kohn *et al.*, 1965).

$$E_{xc}[n(r)] = \int n(r) \varepsilon_{xc}[n(r)] dr, \quad (1)$$

$\varepsilon_{xc}[n(r)]$ is the one-particle functional of the exchange-correlation energy. The exchange correlation energy is included in the effective Kohn and Sham potential by means of a one-particle equation:

$$\left\{ -\frac{1}{2} \nabla^2 + V_{eff}[n(r), r] \right\} \varphi_i(r) = \varepsilon_i \varphi_i(r). \quad (2)$$

The DFT formalism proposed by Kohn, and Sham is suitable for the interaction between electrons and positrons (Kohn *et al.*, 1965). For the positron wave function was used parametrization (Popov *et al.*, 2023). The annihilation rate λ is the reciprocal value of the positron lifetime τ , in an inhomogeneous electron gas.

$$\lambda = \pi r_0^2 c \int dr n_+(r) n_-(r) \gamma = \pi r_0^2 c \sum_i \int dr, \quad (3)$$

where r_0 is the classical electron radius “c” is the speed of light, and γ is the enhancement factor of the electron density at the positron. The program's iterative procedure converges to twelve decimal places. By using, a mathematical model a study of supposed effects was performed on ongoing processes in Fe_2O_3 and Fe_3O_4 in the conditions of synthesizing balance, as observed in the environment where the bacterium lives.

3. Results and discussion

Leptothrix bacteria primarily inhabit and form in aquatic environments (Kunoh *et al.*, 2015). The chemical composition of water, pH, the quantity of oxides and natural radionuclides, the degree of turbidity, and other factors influence the physical parameters of bio-tubes (Safarik *et al.*, 2017; Angelova *et al.*, 2017). Research results have shown that as the chemical composition of water approaches the determined standards for the chemical composition of drinking water, the diameter and length of Leptothrix bio-tubes increase (Kunoh *et al.*, 2016). Infrared spectroscopic studies of water indicate the mobility of OH and H-O-H chemical bonds at 3357 cm^{-1} , 1670 cm^{-1} , and 700 cm^{-1} vibration frequencies (Palencia *et al.*, 2018). The asymmetric stretch resonances of water molecules

are observed at 3357 cm^{-1} , scissor bend resonances at 1670 cm^{-1} , and fingerprint zone vibration resonances at 700 cm^{-1} (Marcechal *et al.*, 2011; Qiang *et al.*, 2013). In the infrared (IR) spectroscopy analyses, the $2800\text{--}3600\text{ cm}^{-1}$ interval characterizes the vibrations of OH functional groups and the formation mechanism of the hydrate system (Mirzayev, 2020). In the crystal structure of Leptothrix bio material, the chemical bonds were obtained with FTIR transmittance spectroscopy analysis and use of potassium bromide (KBr) mixed completely with KBr in the ratio of (1:300). FTIR graph of sample of background are shown in the Fig. 2. In the Fig. 2, shows the FTIR spectrum of up to 4000 cm^{-1} in background mode for experimental accuracy. At 2300 cm^{-1} , the vibrational mode for water and OH functional groups in the air where observed. This is in agreement with the data reported by (Palencia *et al.*, 2018).

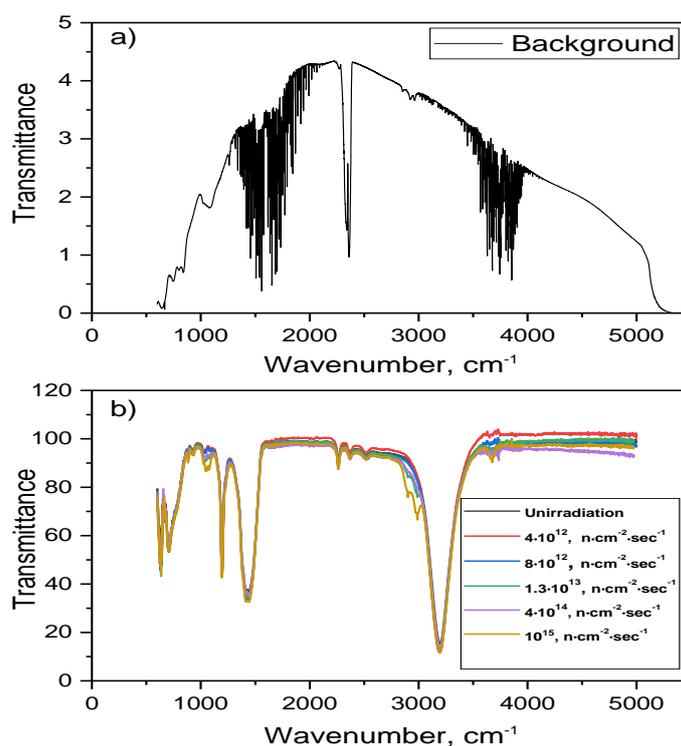


Fig. 2. FTIR spectrum of background.

Figure 3 presents the FTIR spectra Leptothrix bio samples from various coordinates ($56^{\circ}44'51.7''\text{N } 37^{\circ}06'58.4''\text{E}$, $56^{\circ}44'42.2''\text{N } 37^{\circ}07'05.6''\text{E}$, and $56^{\circ}44'42.3''\text{N } 37^{\circ}07'10.2''\text{E}$), with a pH of 8.0 and permanganate oxidizability in water of $6.32\text{ mg(O}_2\text{)/L}$. Fig.3 shows minor and major vibration modes of groups Fe_2O_3 , Fe_3O_4 , Fe(OH) and OH hydroxyl groups which are up to 2000 cm^{-1} . It also shows that the FTIR spectra which is divided into the more parts.

The chemical composition analysis of the water in which Leptothrix bacteria live reveals that iron constitutes 39.88% of the total composition. The abundant amounts of Ca, Mg, Mn, and Si elements make up 3.95%, 0.9%, 0.56%, and 0.045%, respectively. As observed in Fig. 3, infrared spectroscopy modes up to 2000 cm^{-1} represent the presence of airborne water and OH functional groups. Modes up to 2000 cm^{-1} display small and large vibration modes of Fe_2O_3 , Fe_3O_4 , and Fe(OH) groups in various portions.

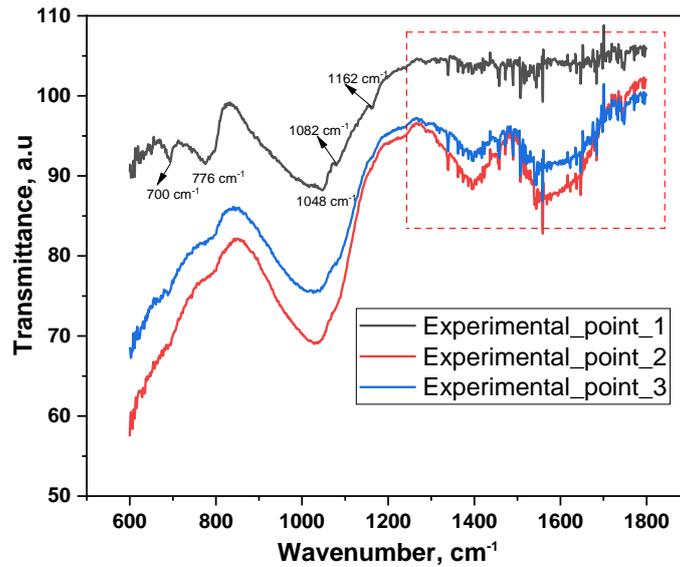


Fig. 3. FTIR spectrum of the Leptothrix biogenic material. FTIR spectrum in the range from 400 to 2000 cm^{-1} is presented.

The 700 cm^{-1} and 776 cm^{-1} Fe-O-Fe modes belonging to the first functional groups in the large vibration group correspond to Fe_2O_3 under different angles of inclination. The frequencies of 1048 cm^{-1} , 1082 cm^{-1} , and 1116 cm^{-1} belong to Fe_3O_4 , representing Fe-O stretched resonances. Simultaneously, 1023 cm^{-1} , 1042 cm^{-1} , 1059 cm^{-1} , 1077 cm^{-1} , and 1086 cm^{-1} characterize the stretched resonances of di-ferrite and tetra-ferrite groups (Nalbandian et al., 2016). The symmetric stretched resonances of Fe-O-Si at 1248 cm^{-1} and 1370 cm^{-1} were not been observed. The functional groups observed at higher, 2263 cm^{-1} , 2369 cm^{-1} , and 2519 cm^{-1} , coincide with the Fe-(OH) stretching modes. All the mentioned functional groups align with literature reports (Aghazadeh *et al.*, 2019). In Fig.4, 2D surface morphology images obtained through atomic force microscopy of Leptothrix bio bacteria are presented. The microstructure acting on the surface of the samples is depicted within a $20\times 20\text{ }\mu\text{m}$ interval. As observed in the 2D morphology of the structure, the dynamics of oxide distribution are weak, and the areas and sizes of particle distribution vary in the microstructure.

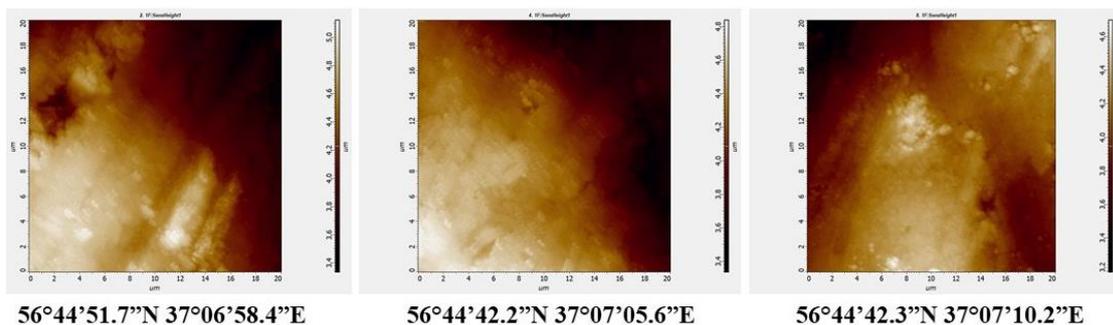


Fig. 4. 2D surface morphology of Leptothrix biobacteria studied by atomic force microscope (AFM).

The particle size ($60\text{-}70\text{ }\mu\text{m}$) increases and/or remains constant ($65\text{ }\mu\text{m}$) in the morphology of samples taken from different coordinates (Demir *et al.*, 2020). Different-sized amorphous regions have been identified on the surface of the samples at different

locations. The growth rate of amorphous regions on the sample surface, the dislocation of the microstructure, and the occurrence of dislocations are determined based on the coordinates. The examination of dislocation in the microstructure formation mechanism suggests that there is a greater occurrence of plastic deformation on the surface. In Fig.5 shows the vacancy cluster defects at Fe_2O_3 and Fe_3O_4 implanted within the water molecules, as expected to be available in an aggressive aqueous environment.

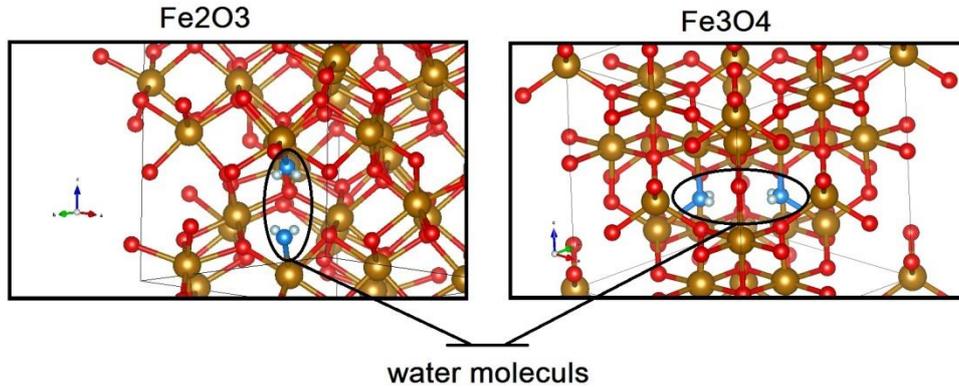


Fig. 5. Schematic position of defects and implanted water molecules in lattices of Fe_2O_3 and Fe_3O_4

The case that was not considered in our previous work (Mirzayeva *et al.*, 2023b) is the accumulation of water molecules in the vacancy clusters of the two materials that we consider (Fe_2O_3 and Fe_3O_4). The unit cell and spatial parameters we used are fixed as follows: for Fe_2O_3 : $a, b, c = 9.56 \text{ \AA}$, $\alpha, \beta, \gamma = 90^\circ$ / $Ia/3$ (206), for Fe_3O_4 : $a, b, c = 8.44 \text{ \AA}$, $\alpha, \beta, \gamma = 90^\circ$ / $Fd/3m$ (229) (Agayev *et al.*, 2020; Mirzayeva *et al.*, 2019; 2022a). Two water molecules were added in both cases in a vacancy cluster of two iron vacancies. Directly in an aggressive water environment, these molecules are expected to interact with the valence bonds of the iron atoms, near the vacancy. In Fig.5 shows both cases. The corresponding numerical results for τ (positron lifetime) for a vacant cluster with an increasing number of hydrogen atoms in it are presented in Fig. 6.

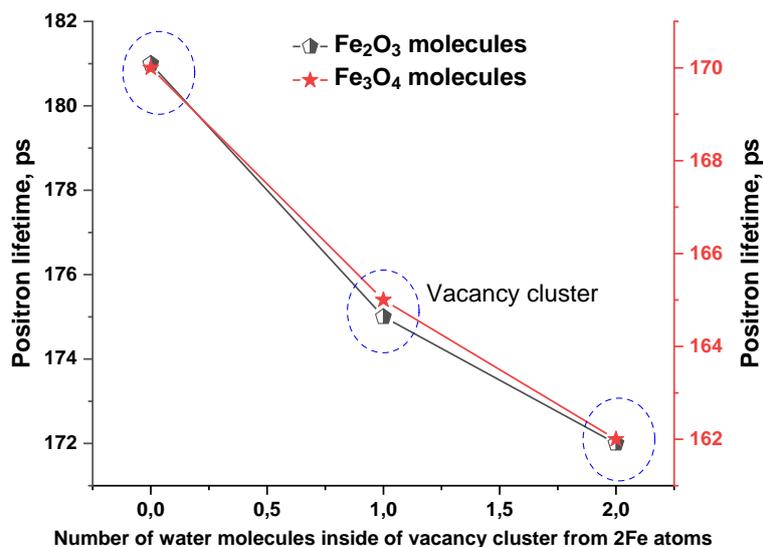


Fig. 6. Calculation results from MIKA for Fe_2O_3 and Fe_3O_4 , with implanting of H_2O molecules in defects.

The results of the graph confirm the statement in our transitional work, namely that Fe_3O_4 is a better agent in aggressive aqueous media than Fe_2O_3 . The Fe_2O_3 configuration implies larger volumes with low electron density, and therefore for that we observe no change in the τ results for 1 and 2 water molecules in the vacancy cluster.

4. Conclusions

In *Leptothrix* bio samples taken from various coordinates, infrared spectroscopy modes up to 2000 cm^{-1} depict the presence of oxides, water, and OH functional groups. Different frequencies of small and large vibration modes of Fe_2O_3 , Fe_3O_4 , and Fe(OH) groups have been observed. The Fe-O-Fe modes at 700 cm^{-1} and 776 cm^{-1} belonging to the first functional groups in the large vibration group correspond to Fe_2O_3 under various angles of inclination. Frequencies of 1048 cm^{-1} , 1082 cm^{-1} , and 1116 cm^{-1} , representing Fe_3O_4 , reveal Fe-O stretched resonances. Functional groups observed at low frequencies, 2263 cm^{-1} , 2369 cm^{-1} , and 2519 cm^{-1} , characterize the Fe-(OH) stretching modes. The representations of the surface morphology investigated with atomic force microscopy indicate the areas and sizes of particle distribution remain constant in the microstructure. The lack of change in electron density upon saturation of the free volume with water molecules for Fe_2O_3 is suggested by the larger volumes of low electron density than those in Fe_3O_4 .

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