NEW IRON OXIDES/HYDROXIDES BIOMATERIALS FOR APPLICATION IN ELECTRONICS AND MEDICINE

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НОВИ БИОМАТЕРИАЛИ ОТ ЖЕЛЕЗНИ ОКСИДИ/ХИДРОКСИДИ ЗА ПРИЛОЖЕНИЕ В ЕЛЕКТРОНИКАТА И МЕДИЦИНАТА

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Abstract: *Biotechnologies could supply inexpensive, environment friendly and effective materials for new nonstandard and concurrent solutions. The present work's focus was on acquiring new physical data for application in electronics and medicine of biogenic materials obtained due to the metabolism in laboratory conditions of iron-oxidizing bacteria from the genus Leptothrix. Powders and coatings on glass samples were under investigation. XRD and Raman Spectroscopy analysis of the data collected show that the Fe^{2+}-depending from the growth media could transformed into Fe^{3+} or $Fe^{2.5+}$ in the form of two types of oxides/hydroxides such as magnetite (Fe_3O_4) and lepidocrocite (γ-FeOOH), all with nanostructured morphology. Biotechnology for obtaining one biogenic iron phosphate hydroxide (ferrian giniite) - ($Fe_5(PO_4)_4(OH)_3.2H_2O$) was also developed. The ferrian magnetic material is dispersed in the walls of biogenic tubular structures. The average particle's size and crystalline structures of bio-products were investigated. Results based on PPMS measurements on the magnetic properties were reported. The new biogenic materials showed superparamagnetic behaviour and high sensitivity to electromagnetic radiation and have real potential for application in electronic and information technologies.*

Keywords: IRON OXIDES/HYDROXIDES BIOMATERIALS, IRON-OXIDIZING BACTERA, LEPTOTHRIX, BIO-MAGNETIT, BIO-LEPIDOCROCITE, BIO-FERRIAN GINIITE, SUPERPARAMAGNETIC BIO-PARTICALS

1. Introduction

Molecular electronics, biosensors and biocomputers are scientific areas which demonstrate extremely fast progress in the last decade. Nanosized biogenic iron oxides/(oxy)hydroxides are promising alternative to polymers applied in molecular electronics and reveal new perspectives. Biotechnologies could supply inexpensive, environment friendly and effective materials for new nonstandard and concurrent solutions. Superparamagnetic iron oxide hybrid nanoparticles having high bio-compatibility have been used "in vivo" experiments in NMR, for better contrast, repair of tissue. The first patent for artificially preparation of nanosized biogenic tubes [1] appeared in 2012. A large part of Earth's sedimentary iron deposits can be attributed directly or indirectly to microbial activity [2]. Microorganisms, so called Fe-oxidizing bacteria (FeOB) are able to oxidize Fe^{2+} and form Fe^{3+} bioproducts. Ones of the bacteria competing with the kinetically more favorable abiotic Fe^{2+} oxidation are those from the group *Sphaerotilus – Leptothrix*. As a result of the bacteria-mediated iron oxidation, red/orange microbial mats are formed, which comprise iron (oxy)hydroxides in a polysaccharide matrix in the specific form of tubular sheaths, with the exact composition of the sheaths yet to be fully determined. The question about the function of the iron oxidation by the bacteria is still open for debate due to the lack of sufficient information and difficulties with their cultivation under laboratory conditions.

The present work's focus was on acquiring new physical data for application in electronics and medicine of the biogenic material obtained due to the metabolism in laboratory conditions of FeOB from the genus *Leptothrix*. Depending on the media for cultivation used, we obtained different iron-containing nanostructured materials with crystalline identification as lepidocrocite, magnetite and ferrian giniite. Powders, coatings and bio-micro tubes from these materials were under investigation.

2. Materials and Methods

The procedure for sample preparation under laboratory conditions is outlined in Fig 1.

In this research we used a pure culture of the bacteria from the genus *Leptothrix* isolated from a natural stream in Vitosha Mountain, located at altitude of 1783 m [3, 4]. Two types of growth media for obtaining desired biogenic oxides and tubular structures (sheaths) were used: Adler's medium (AM) [5] and Silicon Iron Glucose Peptone medium (SIGP) [6] and Fernbach, Roux and Erlenmeyer flasks for the cultivation under static conditions. The cultivation is described in detail by Angelova R. et al. [3, 4]. The specific shape of the selected vessels provides a high volume and surface for aeration of the culture fluid in order to allow the bacteria to grow. The flasks were inoculated (10% v/v) and cultivated at a temperature of 20 °C. The additional iron source (iron cuttings) was added in each growth medium. The iron source for AM and SIGP media was ammonium iron (II) sulfate ($(NH_4)_2Fe(SO_4)_2·6H_2O$) and ferrous sulfate ($FeSO_4·7H_2O$), respectively.

Biotechnology for obtaining of a biogenic iron phosphate hydroxide (ferrian giniite) - ($Fe_5(PO_4)_4(OH)_3.2H_2O$) was developed based on the laboratory cultivated *Leptothrix* bacteria in SIGP with $Pb(NO_3)$. The technology is growing up to the pattent documents and will be called provisory SIGPP.

To characterize the biogenic materials obtained we used X-ray diffraction, Raman spectroscopy and TEM imaging. The magnetic measurements were performed on a Physical Properties Measurement System (PPMS) up to 1 T. All samples were obtained after 40 days of cultivation, filtering and drying of the biomass at room temperature, excluding the method for obtaining ferrian giniite, which the cultivation period was 14 days.

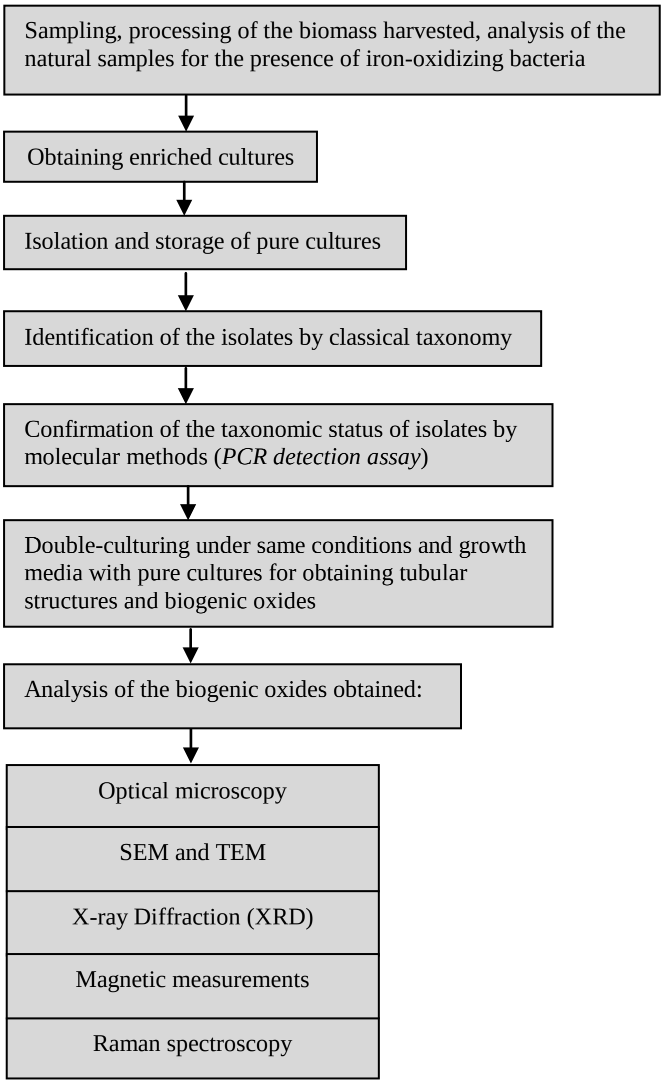
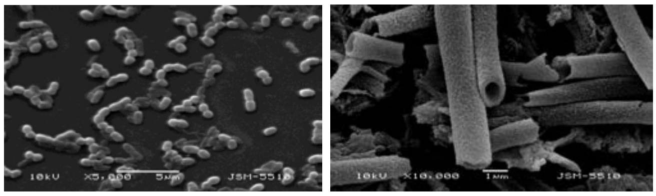


Fig. 1 *Schematic illustration of the preparation and characterization procedure of the investigated materials [14]*

3. Results and Discussion

Choosing modified Adler's medium was a decision based on assessing the results from the quantity of biomass obtained in grams, in connection with the growth time in days, with these media showing the best results. In order to achieve any type of application, the quantities of iron-bearing material obtained biotically have to compete with those obtained inorganically. Figure 2a) shows SEM images of FeOB cultivated in AM. The typical morphology of cells can be seen with shape characteristic of the genus *Leptothrix*. Hollow sheaths were revealed by SEM and light microscopy only in SIGP medium. However, none of the samples investigated contained the typical *Leptothrix* sp. hollow sheaths, as revealed by numerous SEM and light microscopy observations, even under longer periods of cultivation. This confirmed some previous investigations, which concluded that these bacteria, in most cases, lose their ability to form iron-containing sheaths when they have been cultivated in vitro [6, 5]. The cultivation in AM did not lead to the formation of such structures, but we were able to prepare sufficient quantities of biomass with ochre coloring obviously containing various types of iron oxides/(oxy) hydroxides.

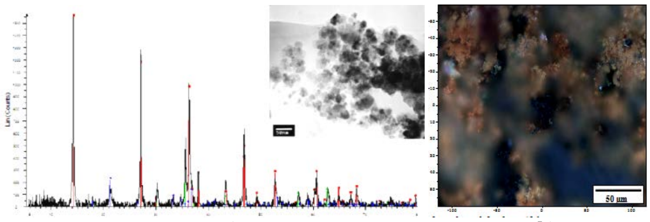
The cultivation in SIGP medium gives the possibilities due to the metabolism of *Leptothrix* sp. to obtain submicron and nanosized sheaths. The cultivation in SIGPP medium allows to form tubular structures with nanosized spheres of a biogenic iron phosphate hydroxide (ferrian giniite) - ($Fe_5(PO_4)_4(OH)_3.2H_2O$) discreetly dispersed in the sheath walls. The typical sheaths structures are illustrated in Fig. 2b).



a) **b)**

Fig. 2 *SEM of bacteria from the genus Leptothrix cultivated in* ***a)*** *Adler's medium: panoramic view and* ***b)*** *tubular structures obtained after cultivation in SIGP medium.*

The biogenically derived materials from the cultivation were characterized by XRD. Figure 3a) presents XRD spectra of samples obtained from AM. The analysis of the spectra reveals that the phase composition is mixed. For the material collected from the AM-cultivated *Leptothrix* strain, it was as follows: lepidocrocite (γ-FeOOH) – 60 %; magnetite (Fe_3O_4) – 22 % and goethite (α-FeOOH) – 18 %. The average size of all particles was calculated independently, all of them being on the nanometer scale. It was as follows: lepidocrocite particles – 30 nm; magnetite particles – 24 nm and goethite particles – 12 nm.



a) **b)**

Fig. 3a) *XRD spectra of biogenic material obtained in AM after cultivation of Leptothrix bacteria and inserted a TEM image of the powder obtained and* ***b)*** *Optical microscope photographs of the bacteria-derived material deposited on glass slides from AM.*

Glass slides from AM (see Fig. 3b) with different biofilms depending on the growth media used were prepared from FeOB cultivated for 40 days by three drop-wise depositions of collected and filtered biomass. Each layer was dried at room temperature. Thus prepared, the samples were examined by Raman micro-spectroscopy using a LabRAM HR Visible single spectrometer equipped with a microscope and a Peltier-cooled CCD detector. The 633-nm He-Ne laser line was used for excitation. The Raman measurements were performed at room temperature.

Raman spectra of the material are systematized in Table 1 depending in AM growth medium used, the corresponding iron by-products detected and their positions in the Raman signal. We have to note that a strong luminescence signal was also present during the measurements arising from all samples containing iron oxides/(oxy) hydroxides, which greatly impeded our investigation. It stretched over the whole spectral range (100 to 3400 cm^{-1}) of the spectra obtained.

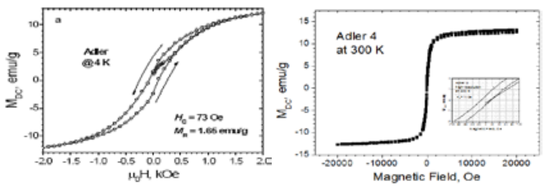
Table 1: *Raman scattering data of the biogenic material from AM.*

Growth medium (sample)	Raman peak positions detected in sample* (cm^{-1})	Assigned iron oxides/hydroxides**
Adler's medium (AM)	221, **254**, 312, 350, **381**, 465, **530**, 650	lepidocrocite (γ-FeO(OH))
	310, 540, 670	magnetite (Fe_3O_4)

* *The prominent peaks positions in the Raman signals detected are shown in bold.*

** *The assignment of spectra is on the basis of literature data [9,10,11]. Since, to the best of our knowledge, Raman studies on biogenic material have not been performed before, the comparison was made with data for natural and synthetic minerals, corrosion products etc.*

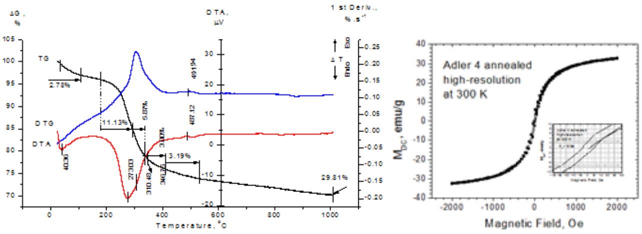
The single broad band at 670 cm^{-1} is well-known feature of magnetite (Fe_3O_4). Magnetite's other two very weak bands at 310 and 530 cm^{-1}. Magnetite is a ferrimagnetic material with iron in a mixed valence state. Its structural formula can be written as $(Fe^{3+})_A[Fe^{2+}Fe^{3+}]_BO_4$, where "A" and "B" are magnetite's tetrahedral and octahedral sublattices. Its appearance in the AM sample to the amount of around 22 % (as calculated from the XRD spectra) of the entire iron-bearing mass cannot straightforward be explained yet. One possible scheme for its formation can be deduced from a small very weak spectral feature, which is not, by any means, characteristic for lepidocrocite. The band at around 460 cm^{-1} is a fingerprint for only one iron-bearing compound, namely, ferrous hydroxide $Fe(OH)_2$. It cannot be ruled out that these small traces of $Fe(OH)_2$ are of biogenic origin, because the mechanism of the reactions involving the hydrogen peroxide (H_2O_2) derived from the *Leptothrix* sp. metabolism and the nutrition present are not fully understood, especially on the interface bacterial wall/iron source. Only some tips elevated higher of the biomass level are colored differently from reddish-brown and also in the fact that the 460 cm^{-1} peak is present in the lepidocrocite spectra only. Such small areas with concentrated $Fe(OH)_2$ can act as centers for nucleation of magnetite. It is more thermodynamically stable than the hydroxide and it evolves most probably via the scheme described by Herane M. et al.[11]. In the scheme summarized there, the formation of magnetite passes through an intermediate phase – green rust, which is unstable in air. No Raman peak positions of goethite were detected, although the XRD spectra of the sample show that it was present, with nearly 18 % of the volume. In our opinion, this is due to the specifics of the Raman spectroscopy probing having to do with the laser beam being focused on spots with diameter below 50 µm in a search for a flat surface. On the other hand the XRD data showing the good crystallinity of the lepidocrocite detected and that the goethite is in poor crystallinity state and really could speak for the trace of him in mix with the lepidocrocite. Material obtained from the AM exhibited a strong response to a weak DC magnetic field, indicative of the magnetite-phase leading role regardless of its lower content. The magnetic properties were investigated by PPMS. For the AM sample, we measured magnetic hysteresis up to 2 T at 4 and 300 K; they are presented in Fig. 4 a) and b). The coercive fields H_c are 73 and 14 Oe at 4 and 300 K, respectively. Magnetization values more typical for magnetite, rather than any antiferromagnetic or paramagnetic material, can be seen. The result shows superparamagnetic behavior of the AM sample at room temperature (300 K - see the inserted Fig.4b). The main contributor to the magnetic behavior of the biogenic product is the nanosized magnetite. The existence of a "none-zero" low coercive - Fig. 4b) field of 14 Oe could be due to the presence of a small amount of magnetite particles with a diameter larger than the superparamagnetic limit (30 nm) for this kind of particles or small contribution from the antiferromagnetic particles. The remanent magnetization (MR) is higher for the lower temperature.



a) ***b)***

Fig.4 *Hysteresis loops M(H) of AM sample at* ***a)*** *4 K and* ***b)*** *300 K.*

The sediment from AM sample was dried at 200°C up to 2 hours. Figure 5a) shows the thermal analysis of the AM sample heated up to 1000°C at air with velocity 10°C/min. Strong endothermic effect was observed in the interval 200–370°C which has summary character including: (i) dehydration, (ii) chemical transformation of γ-FeOOH → γ-Fe_2O_3 in the interval 180–300°C and (iii) burn out of the organic mass - the high level of the weight losses in the sample. Obviously after annealing could awaiting two ferrimagnetic phase in the powder, respectively magnetite Fe_3O_4 (bulk Ms=93 emu/g) and maghemite γ-Fe_2O_3 (bulk Ms=73–74 emu/g). Magnetic measurements show that the ferrimagnetic phase increases with 71% (inserted M_s in Fig. 5b). The magnetic investigations show that the powder keeps their superparamagnetic behavior. The superparamagnetic nature of the AM biogenic product makes it a perfect candidate for a magnetic component in biocompatible ferrofluids [12], as the currently used Gd-based contrast agents are also paramagnetic in nature.



a) ***b)***

Fig. 5a) *DTA, TG and DTG investigation of AM samples and* ***b)*** *Hysteresis loop of annealed AM powder.*

In the SIGP medium as a result of the bacterial metabolism submicron and nanosized sheaths have been formed (Fig. 6). The XRD pattern (Fig. 6) of the tubes obtained from the modified SIGPP medium shows a single-phase crystalline structure of the inorganic inclusions of a biogenic iron phosphate hydroxide (ferrian-giniite) - $(Fe_5(PO_4)_4(OH)_3.2H_2O)$. The ferrian magnetic material is discreetly dispersed in the walls of these biogenic tubular structures. In the scientific literature the magnetic data of this bio-material are very limited.

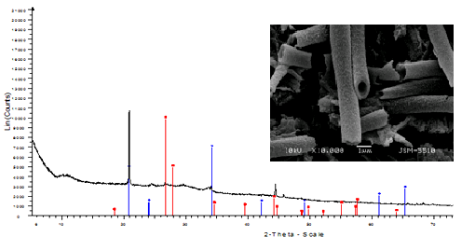
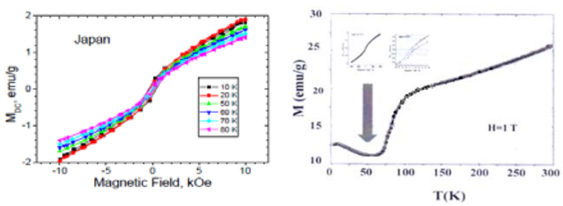


Fig. 6 *XRD spectra of biogenic material from SIGPP sample after cultivation of Leptothrix bacteria and an inserted image of the typical tubular structures obtained after cultivation in SIGP medium.*

Magnetic measurements (Fig. 7a) show a typical ferrimagnetic behavior of the sample with tubular structure. In contrast to the crystals of ferrian-giniite obtained by convenient chemical technologies [13] this bio-product has a weak hysteresis behavior which is probably defined by the nanosized dimension of the particles.

Figure 7b) presents the fitted data of the magnetic saturation with an applied magnetic field H=1T and inserted the hysteresis behavior at 1T where the curve goes down up to inverse point. Investigation by PPMS from 4 K up to 80 K show the superparamagnetic hysteresis loops typical for a single domain magnetic particles. After 80 K the magnetic moment abruptly rise but without saturation till the maximum of applied magnetic field.



a) ***b)***

Fig. 7 *Magnetic properties of SIGP sample:* ***a)*** *Superparamagnetic M(H) up to 80 K and* ***b)*** *Non-saturated magnetic moment up to 10 T.*

4. Conclusions

Bionanotechnology was employed to produce biogenic nanosized iron oxide powders and nanotubes containing magnetic nanoparticles due to the cultivation of the bacteria from the genus *Leptothrix* under laboratory conditions. The transformation of Fe^{2+} to Fe^{3+} due to bacterial metabolism in the powders was around 78 %, with the rest being found to be in a mixed 2,5 valance state in magnetite. The average size of all particles was calculated independently and found to be on the nanometric scale. The magnetic measurements results showed a superparamagnetic behavior of the iron oxide/hydroxide powders at room temperature (300 K). The main contributor to the magnetic behavior of the biogenic product is the nanosized magnetite and after annealing to 200^oC the ferrimagnetic mix from Fe_3O_4 and γ-Fe_2O_3. In the SIGPP sample as a result of the bacteria metabolism submicron and nanosized tubes has been formed with the inorganic inclusions in the walls of a biogenic iron phosphate hydroxide (ferrian giniite) - $(Fe_5(PO_4)_4(OH)_3.2H_2O)$. The understanding of the structural and magnetic properties of these materials is important for fundamental research, but also could suggest some development of potential applications in magnetoelectronics and information technologies.

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5. Literature

1. Takada J. et al., *US Patent* - US2012/0315437A1.
2. Schieber J. 2004. Groundwater-Fed Iron-Rich Microbial Mats in a Freshwater Creek: Growth Cycles and Fossilization Potential of Microbial Featur, *Lunar Planet Sci* 35:1369-1370.
3. Angelova R., M. Iliev, M. Mitova, V. Voynova, L. Tasheva, L. Slavov, V. Groudeva, S. Antonova-Nikolova, “Optimization the cultivation of neutrophilic iron bacteria in laboratory conditions”. *J. BioSci. Biotechnol.* 2014, SE/ONLINE: p. 67-70, ISSN: 1314-6246.
4. Angelova R., M. Iliev, L. Slavov, T. Koutzarova, R. Closset, I. Nedkov, V. Groudeva, “Formation of artificial sheaths of *Leptothrix* sp. under laboratory conditions”, *J. BioSci. Biotechnol.* 2015, SE/ONLINE: 165-170, ISSN: 1314-6246.
5. Ellis D.,2003, “Microbiology of the Iron-Depositing Bacteria”. *Wexford College Press*.
6. Sawayama M., T. Suzuki, H. Hashimoto, T. Kasai, M. Furutani, N. Miyata, H. Kunoh, J. Takada, “Isolation of a *Leptothrix* Strain, OUMS1, from Ocherous Deposits in Groundwater”, *Curr. Microbiol.* 2011, 63:173-180.
7. Spring S., P. Kampfer, W. Ludwig et al., “Polyphasic characterization of the genus *Leptothrix mobilis* sp. nov. and *Leptothrix discophora* sp. nov. nom. rev. and emended description of *Leptothrix cholodnii* emend”. *Syst. Appl. Microbiol.* 1996,19:634-643.
8. Das S., M.J. Hendry, “Application of Raman spectroscopy to identify iron minerals commonly found in mine wastes”, *Chem. Geol.* 2011, 290:101-108.
9. Bellot-Gurlet L., D. Neff, S. Réguer, J. Monnier, M. Saheb, Ph. Dillmann, “Raman studies of corrosion layers formed on archaeological irons in various media”, *J. Nanopart. Res.* 2009, 8:147-156.
10. Colomban Ph., “Potential and drawbacks of Raman (micro) spectrometry for the understanding of iron and steel corrosion”. INTECH *Open Access Publisher*, 2011, 568-584.
11. Herane M, Garcia-Garcia F, Sawtell D, Montes-Atenas G. 2007. Study on lepidocrocite (γ − FeOOH) performance as adsorbent for heavy metals from aqueous aerated polluted solutions. Ciencia Abierta 31 article 5.
12. Neuberger T. et al., “Superparamagnetic nanoparticles for biomedical applications: Possibilities and limitations of a new drug delivery system”, *J. Magn. Magn. Mater.* 2005, 293:483-496.
13. Zhao J., Y. Zhang, Z. Run, P. Li, Q. Guo, H. Pang, “Ferric Phosphate Hydroxide Microstructures Affect Their Magnetic Properties”, *Chemistry Open*, 2015, 4(3): 274-277.
14. Nedkov I., L. Slavov, R. Angelova, B. Blagoev, D. Kovacheva, M. V. Abrashev, M. Iliev, V. Groudeva, “Biogenic nanosized iron oxides obtained from cultivation of iron bacteria from the genus *Leptothrix*“, J. Biol. Phys. 2016. DOI: 10.1007/s10867-016-9426-3.