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MAGNETIC CONTAMINATION OF ENVIRONMENT – LABORATORY SIMULATION OF MIXED IRON OXIDES IMPACT ON MICROORGANISM CELLS

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Abstract

Magnetic contamination is considered more and more as a challenging issue related to biosphere pollution with magnetic materials originating in natural and artificial sources (volcanic eruptions and respectively industrial activities that contributed to iron and other metal compounds spreading in air, water and soil). Aiming to study the impact of magnetic metal ions such as iron and cobalt on the metabolism of some environmental microorganisms, in this paper an experimental simulation of magnetic contamination was carried out based on mixed iron/cobalt oxides as source of ions. Magnetic nanoparticles were prepared following chemical route with appropriately adjusting of their surface to ensure uniform dispersion in water. Typical crystalline structure of studied nanoparticles was evidenced with X-ray diffraction, while microstructural and magnetic properties were investigated by scanning electron microscopy and respectively vibrating sample magnetometry. Increased level of peroxidase activity in a fungus mycelium has suggested microorganism adaptation to higher levels of reactive oxygen species following the supply with magnetic nanoparticles suspensions (0-10-20-30-35 mg/L, comparable with detected levels of iron in the living organism). Lipid peroxidation was evidenced also; being assigned to the increased level of hydrogen peroxide that catalase seems enable to balance - as resulted from its decreasing activity. The variations of analyzed indicators of oxidative stress were of no more than 15%, reflecting organism adaptation to environmental constraints but also possible damages of cell membrane system.

Key words: cobalt, iron, oxidative stress markers, Phanerochaete chrysosporium

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1. Introduction

Decades ago the main threats for the environment were the radioactivity impact and chemicals toxicity; more recently electromagnetic pollution and magnetic contamination came into the scientist attention. The latter is related to the large spread of magnetic materials, basically iron compounds that can be found in the Earth crust and exploited by mining or widely spread by volcanic eruptions and intense storms. Also industrial development of metal processing and especially nanotechnologies based on magnetic nanoparticles became new sources of particulate matter released in water, air, soil – that suggested the new concept of magnetic contamination.

The biomedical use of magnetic nanoparticles in diluted suspensions includes nanosized iron oxides as contrast agents in magnetic resonance imaging, in experimental cancer treatment through hyperthermia, in magnetically targeted drug delivery, magnetic separation of biomolecules and cells etc. Magnetic nanoparticles (MNPs) designed for applications in life sciences should be supplied to biological systems

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in fluid form (magnetizable nanofluids) as they are planned to circulate toward target organs or tissues through the circulating body fluids; thus, they are fabricated with a variety of surface stabilizers which provides uniform dispersion in aqueous media. First MNPs were considered as inert factors from the viewpoint of their metabolic interaction with cells (Huang et al., 2013). More recent studies were developed with focus on the MNPs toxicity. Either coated or non-coated magnetite or maghemite nanoparticles could be internalized by endocytosis in the cells where the lysosymal degrading triggers ferric and ferrous ions release (Sing et al., 2010; Wang and Pantopoulos, 2011).

One of the main mechanisms that can influence cell metabolism is the oxidative stress associated with reactive oxygen species (ROS) which can result in lipid peroxidation - as shown by specific indicators like glutathione, malondyaldehyde (MDA) (Ma et al., 2012) and others, as well as in damages of proteins, nucleic acids and polysaccharides (Halliwell and Gutteridge, 2007). Environment safety has led to experimental investigations of MNPs influence on microorganisms (Niazi and Gu, 2009) where also ROS increase was evidenced being associated with membrane system damages. Microorganisms have been even entitled as model organisms for the study of engineered nanoparticle toxicity (Kumar et al., 2012).

Few studies were dedicated to specific fungi response to MNPs impact (Navarro et al., 2008; Saucedo et al., 2011). However the uptake of metal ions with magnetic properties by environmental fungi cells was studied recently (Sepehr et al., 2014) and a mechanism of internalized metal ions impact on Phanerochaete chrysosporium cellulolytic fungi was proposed based on microscopy data (Murugavelh and Mohanty, 2014). In previous reports we evidenced possible utilization of magnetite colloidal nanoparticles in fungi biotechnology, based on the fact that enzymatic equipments appeared to be stimulated for low levels of magnetite aqueous or oily suspensions (Manoliu et al., 2005; Manoliu et al., 2002). In the present study cobalt ferrite nanoparticles were utilized considering their promising - and therefore widely spread - use in cancer therapy through hyperthermia (Mazario et al., 2013). Both elements i.e. iron and cobalt are naturally present in the living cells. Iron, the fourth abundant element in the Earth crust is present in several categories of biomolecules like cytochromes, haemoglobins, catalase enzymes etc. Cobalt is the active center of coenzymes called cobalamins, the most common example of which being the cyanocobalamine, i.e. B12 vitamin. In this study we searched for biological response of cellulolytic fungi relatively environmental pollution to with concentrations of magnetic nanoparticles corresponding to the order of magnitude of iron accumulation in human brain (Buzea et al., 2007).

The importance of these types of microorganisms is related to the fact that biosphere

recirculation circuits of carbon include decomposition of organic matter in general and cellulosic substrate in particular - cellulose appearing to be its most abundant organic component.

2. Experimental

2.1. Technology of nanoparticles yielding

Metal salt precursors were Merck chemicals at molar ratio 2:1 i.e. 10.866g FeCl₃×6H₂O and 5.648g CoSO₄×7H₂O, each dissolved in 300 mL deionized water (Kim et al., 2003). Deionized water (18.2 MΩ/cm, Barnstead EASYPureII ultrapure water system) was used in all steps of magnetic nanoparticle suspension synthesis. Cobalt ferrite coprecipitation was produced by stirring the two stock solutions at 75 °C and by slowly pouring of 2M NaOH (150 mL). To ensure ferrite particles uniform dispersion in deionized water, 12 mL perchloric acid aqueous solution (25%) was added (under continuous stirring at 75 °C - thus modifying the MNPs surface in order to prevent their agglomeration in the presence of ubiquitous gravitational and magnetic fields (Laurent et al., 2008).

2.2. Nanoparticle characterization

The final product was a magnetizable nanofluid based on electrostatic stabilization (Gazova et al., 2012) that presented good stability over time at pH close to biological one. Rheological investigation of MNPs suspension was carried out using: semianalytical balance type ADAM PW254 with 10⁻⁴g accuracy, 5 mL picnometer, Ubelhode capillary viscosimeter, ROHR B type stalagmometer and deionized water as reference fluid. Physical characterization of colloidal particles was carried out using: Shimadzu LabX XRD-6000 diffractometer with Cu-K α radiation of λ =1.54 Å, Vibrating Sample Magnetometer (VSM) MicroMag model 2900/3900 at room temperature, Scanning Electron Microscopy (SEM) device type VEGA\TESCAN (SE detector, HV: 30.00 kV).

2.3. Biotechnological procedure

2.3.1. Biological material

The white rot fungal strain *Phanerochaete chrysosporium* used in this experiment was obtained from the collection of the Faculty of Biology at "Al. I. Cuza" University Iaşi, Romania being achieved from the Institute Sciéntifique de Santé Publique, Belgium (HEM no. 5772). This species is one of the microorganisms with important role in decomposing cellulose wastes from environment. The fungus was cultivated on agarized Sabouraud medium (peptone 10 g/L, glucose 35 g/L, agar 2 g/L, distilled water up to 1.0 L (Manoliu et al., 2010) in adequate Petri dishes) and kept at 28 °C. Further 6 mm mycelial plugs taken from a 7-day-old Petri dishes culture were used as inoculum for 500 mL flasks containing

liquid Sabouraud medium. MNPs were supplied to fungi culture samples in concentration of 0-15-25-30-35 mg/L equivalent with metal ion concentrations of 0-10.2-15.3-20.3-25.5 μ g/mL.

2.3.2. Biochemical investigation

SOD (superoxide dismutase) activity assay in fungus mycelium was accomplished according to Winterbourn's method- measuring light absorbance at 560 nm wavelength (Artenie et al., 2008). CAT (catalase) activity was assayed through the method described by Sinha (1972). Malondialdehyde (MDA) assay (the end product of lipid peroxidation) was carried out using thiobarituric acid (TBA) according to Hodges et al. (1999). The results were expressed relatively to protein content (the assay of soluble protein content was carried out according to Bradford (1976). Graphical plots were drawn with average values and standard deviations resulted from five measurements of each biochemical repeated parameter.

3. Results and discussion

3.1. Magnetic nanoparticles characterization

XRD investigation revealed typical spinel structure with characteristic peaks (Fig. 1) at known positions on the scale of X-ray scattering degrees (Table 1). Average value of crystallite size (of 11.5 nm) was assessed with Scherrer's formula (Eq. 1), where: *K* is a dimensionless factor which varies with the actual shape of the crystallite (in this case K=0.89), β is the half width of the (*ijk*) diffraction peak, λ is the X-ray wavelength and θ is the Bragg angle of the peak).

$$D_{ijk} = (k\lambda) / (\beta \cos \theta) \tag{1}$$

The results from XRD diagram processing were comparable with other results reported by different authors. Mahadevan et al. (2007) reported crystalline domains for Fe₃O₄ at 5.1 nm, while Kumar et al. (2013) reported crystalline domains for CoFe₂O₄ at 9 nm.



Fig. 1. X-ray diffraction investigation of MNPs

Microstructural investigation with SEM (Fig. 2) revealed quasi-uniform particles with spherical shape that tend to form associations when deposited on the sample support of SEM device following the evaporation of the dispersion liquid; average size of physical diameter was estimated at 40 nm. Magnetic properties were estimated from the magnetization curve (Fig. 3) where saturation magnetization for CoFe₂O₄ was of M_S =58 Am²/kg corresponding to magnetic field intensity of 790 kA/m while coercive field was of 239 kA/m.

The presence of the hysteresis cycle suggests ferrophase synthesis that during both superparamagnetic and ferrimagnetic nanoparticles were precipitated that resulted in the presence of magnetic coercivity at room temperature (Kim et al., 2004). Rheological data have evidenced that CoFe₂O₄ colloidal suspension was characterized by increased average values of surface tension coefficient and viscosity coefficient comparatively with reference liquid - the deionized water: from 7.27 to 7.60 Nm⁻¹ and respectively from 1002 to 2162 NSm⁻² (from ten repeated measurements in identical ambient conditions); the results were similar with those reported for magnetite colloidal suspension stabilized with perchloric acid, according to Racuciu et al. (2010).

3.2. Biochemical assay results

Following the supply with colloidal suspension as source of magnetic metal ions for the culture medium of *P. chrysosporium* fungus, grown mycelium specimens were withdrawn and repeated measurements of biochemical parameters indicating oxidative stress action were carried out.

Miller indices	2 0 (°)	Intensity (a.u.)	β (rad)	D _{ijk} (nm)
(111)	23.9	57.6	0.0170	8.2
(220)	30.2	118.8	0.0129	11.0
(311)	35.6	301.1	0.0122	11.8
(400)	43.2	91.3	0.0118	12.4
(422)	53.6	64.3	0.0118	12.9
(511)	57.2	116.7	0.0132	11.7
(440)	62.8	165.1	0.0125	12.7

Table 1. Estimation of crystallite size from XRD raw data



Fig. 2. Characteristic image of MNP with SEM investigation

In Fig. 4, the SOD activity in fungi mycelium is presented; the tendency of SOD increasing with up to 15% at 14 days compared to the control (p<0.05) was evidenced for MNP increasing concentration - although no precise quantitative correlation could be established, at least for the 7 days situation. This could be taken as an indication on the intensified O_2^- release into the cells that further triggered the amplification of SOD biosynthesis as adaptation to the oxidative stress threatening (Eq. 2), since the enzyme neutralizes the O_2^- radical.

$$2O_{2}^{-} \cdot + 2H^{+} \xrightarrow{SOD} H_{2}O_{2} \xrightarrow{CAT} H_{2}O + O_{2} \xrightarrow{(2)} O_{2}$$

In the same time the increasing of SOD activity could lead to increased H_2O_2 level. Possibly, the hydrogen peroxide triggered peroxidasic reaction cascade and has caused the lipid peroxidation.

Indeed the indicator of final product of lipid peroxidation, namely MDA was found increased at 14 days (with about 13%, p<0.05) in fungi mycelium (Fig. 5) – although not in 7 day old samples – this result being concordant with SOD activity variation.



Fig. 3. Magnetization curve of MNPs sample



Fig. 4. SOD activity in P. chrysosporium samples

In the present investigation iron ions are predominant metal components so their role could be assumed to be the essential one.

The tests carried out on the effect of MNP surface coating, when supplied in equivalent amounts with those corresponding to colloidal suspensions, gave no discernible results. According to some reports (Huang et al., 2013; Singh et al., 2010; Wang and Pantopoulos, 2011), it was expected that magnetic nanoparticles were degraded and metabolized in the cellular endocytic organelles with release of free iron ions - ROS yielding intensification being the main cellular mechanism underlying the supposed toxicity of iron ions (Halliwell and Gutteridge, 2007; Ma et al., 2012; Niazi and Gu, 2009).

The hydrogen peroxide yielded by SOD action is subject of concurrent mechanisms decomposing it: the catalase action (Eq. 3):

$$2 \operatorname{H}_2\operatorname{O}_2 \rightarrow \operatorname{H}_2\operatorname{O} + \operatorname{O}_2 \tag{3}$$

and the iron ions Fenton reactions (Eqs. 4-5), where: H_2O_2 is transformed into hydroxyl or superoxide radicals which are highly reactive.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^-$$
(4)

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH + H^+$$
(5)

Presuming that cell adaptation capacity includes also catalase synthesis stimulation in the presence of increased hydrogen peroxide one would expect enhanced CAT activity evidence in the fungi samples.

However this biochemical parameter was found not increased but on the contrary – diminished in the samples where metal ions were supplied by means of magnetic nanoparticles in diluted suspension form. As one can see in Fig. 6 the catalase activity appears to be diminished with up to 10% (p<0.05). This could be explained also by the presence of MNP in the cellular medium.



Fig. 5. MDA level in *P. chrysosporium* samples

According to some authors (Freitas et al., 2003), following MNPs supply in living mice not only the oxidative changes (lipid peroxidation) were intensified but also liver catalase activity exhibited variations that were time and MNP-concentration dependent. Other report (Doğaç and Teke, 2012) demonstrated the CAT attachment onto the MNP surface – with probable enzyme activity blockage. Thus it was presumed that concurrent processes could be elicited by MNP impact on living cells: the stimulation of CAT synthesis on a side and its inactivation on the other side.

In the present case it seems that the second one was dominating the whole picture. Cobalt is essential element to cell metabolism but, like iron, it can be also toxic. Toxicity of cobalt ions in certain concentrations was discussed by Fleury et al. (2006) based on mammals cell cultures; Kubrak et al. (2012) reported the cobalt stimulated oxidative stress in the goldfish. Cobalt ferrite MNP toxicity was reported also by Peeples et al. (2014) and Di Guglielmo et al. (2010); Drašler et al. (2013) have discussed the damaging impact of coated MNP of cobalt ferrite on the artificial lipid membranes; the observed correlation (Horev-Azaria, 2013) between the oxidative stress, caused by the presence of nanoparticulate CoFe₂O₄, and the sensitivity of different cell types towards toxicity, suggests that oxidative stress is one possible mechanism for the toxicity of cobalt ferrite MNPs. It is assumed that Co^{2+} may enter the Fenton reactions, Co^{2+} appearing to be a better catalyst than Fe³⁺ although only limited free radical formation in the Co²⁺/H₂O₂ reaction system was shown in ESR studies (Strlič et al., 2003).

Thus, in the study presented above we might have to deal with the toxicity of both metal ion types originating in the nanoparticles delivered into the culture medium of cellulolytic fungus taken as model organism.



Fig. 6. CAT activity in P. chrysosporium samples

4. Conclusions

The increased SOD activity was assigned to ROS species yielded by metal ions supplied in *P*.

chrysosporium culture medium that led to hydrogen peroxide formation. The effect of lipid peroxidation was further evidenced by increased level of MDA in the fungus mycelium. CAT activity was found diminished which is probably due to the nanoparticulate form of the metal ion source since nanoparticles can bind to CAT molecules blocking their active site. All these results suggest that environment pollution with cobalt ferrite MNPs released from specific nanotechnologies can impede cellulolytic fungi metabolism and their ecological role of wood waste decomposing.

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