COBALT FERRITE NANOPARTICLES EFFECT ON CELLULOLYTIC FUNGUS *PHANEROCHAETE CHRYSOSPORIUM*

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Abstract. Experimental study was carried out to study simulated magnetic contamination influence on cellulolytic fungi with important role in wood waste decomposing. Cobalt ferrite nanopowder was precipitated from divalent cobalt salts and trivalent iron compounds in alkali reaction medium. Perchloric acid was used to get surface modification of fabricated magnetic nanoparticles with the aim of suspending them in water for environmental application study. Rheological properties were analyzed by standard methods considering further administration of the suspension in the agarized culture medium of fungi cells. Cellulolytic fungus *Phanerochaete chrysosporium* was grown in the presence of magnetic nanoparticle concentrations from 15 to 35 mg/L. Malonaldehyde (MDA) level in the fungus mycelium was estimated by standard biochemical method lipid peroxide assay. In seven days old cultures, non-significant changes in MDA level were found while in fourteen days old mycelium an evident increase was recorded in samples supplied with magnetic nanoparticles. Possible issue related to magnetic contamination stress exerted upon environmental fungi involved in wood waste could be formulated.

Key words: magnetite particles, cellulolytic fungus, MDA content.

INTRODUCTION

Biosphere has always been exposed to metallic nanoparticulate matter originating in natural Earth events like volcanic eruptions and transported by powerful storms at huge distances so that living species and ecosystems have adapted to protect against certain levels of nanoparticle pollution. Human activities always contributed to such nanoparticle spreading in air, water and soil; the last half century of intensive mining and industrial extensive development specifically raised the risk of magnetic nanoparticle toxicity for people and environment [2,

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14]. The balance between the benefits of magnetic nanoparticle utilization in biomedicine and the risks of the toxic bioeffects of their release into the environment represents a continuous challenge at present time [6, 10, 11, 17]. In previous reports we evidenced possible utilization of magnetite nanoparticles in fungi biotechnology, since enzymatic equipments appeared to be stimulated for low levels of magnetite aqueous or oily suspensions [8, 9].

In this paper, we report evidence of membrane system risks based on malondialdehyde increasing in fungi mycelium grown in the presence of magnetic nanoparticles.

MATERIALS AND METHODS

BIOLOGICAL MATERIAL

The cellulolytic microorganism *P. chrysosporium* fungus achieved from the Institute Sciéntifique de Santé Publique, Belgium (HEM no. 5772) was chosen for the experimental study. The fungus cells were cultivated on agarized Sabouraud medium (peptone 10 g/L, glucose 35 g/L, agar 2 g/L, distilled water up to 1.0 L [7] in adequate Petri dishes). Magnetic nanoparticle concentrations in the culture medium [8, 9] were taken from 15 to 35 mg/L equivalent with metal ions from 10 to 25 μg/mL considering data published in [2], where iron level is reported as high as 175 μg/mL in living bodies.

MDA ASSAY

The assay of malondialdehyde (MDA) (end product of lipid peroxidation) in the fungal mycelium was performed using thiobarituric acid (TBA) following the method of Hodges (1999) [5, 16]. The tissues were homogenized with 5% (w/v) trichloracetic acid (TCA); 1 mL homogenate was further mixed with 4 mL of thiobarbituric acid (TBA) reagent (0.5% of TBA in 20% TCA). The mixtures were first heated for 30 min at 95 °C in a water bath; then, they were quickly cooled on an ice bath; thereafter centrifugation at 1900 × g for 10 min was carried out; the light absorbance at 532 nm was measured for the supernatant by Shimadzu spectrophotometer type 1800 Pharmaspec with quartz cells; reference blank consisted of 1 mL of 5% (w/v) TCA mixed with 4 mL of TBA reagent in 20% (w/v) TCA. The results were expressed as nanomol of MDA per milligram of protein (nM/mg protein).

The determination of soluble protein content was done according to Bradford method [1] using 50 mM Tris–HCl buffer, pH 7, with bovine serum albumin as

standard. The assay is based on the binding of Coomassie Brilliant Blue G-250 (from Fluka) at aromatic amino acid radicals with the measure of light intensity at 595 nm. The result was expressed in mg protein per g of mycelium mass.

MAGNETIC NANOPARTICLES PREPARATION AND RHEOLOGIC PROPERTIES

Preparation

Metal salt precursors taken for cobalt ferrite yield were 10.866 g $FeCl_3 \times 6H_2O$ and 5.648 g $CoSO_4 \times 7H_2O$; 300 mL deionized water was used to solve carefully each amount of crystallized powder that resulted in two stock solutions (molar ratio 2:1) which were mixed near boiling point at 75 °C with continuous magnetic stirring for about 5 min. From the intermediate metal hydroxides products, mixed iron and cobalt oxide was precipitated in the presence of 2 M NaOH solution that was slowly poured drop by drop after heating up to the boiling point with continuous magnetic stirring. Black brownish powder decanted at the reaction flask bottom was further washed with three volumes of 500 mL deionized water to remove all impurities. 12 mL perchloric acid aqueous solution (25%) was slowly added to the wet powder and mixed for about 60 min under mechanical stirring to modify the ferrite nanoparticles surface in order to prevent their agglomeration through magnetic dipole attraction. Perchloric acid coated nanoparticles were further washed two times with 100 mL of deionized water to remove unbound products, with careful adjusting of solution pH in order to reach a final value of 5.5 (controlled with pH paper). Finally, a stable nanoparticle suspension was obtained in 125 mL of deionized water.

Rheological features

Density was measured by gravimetric method using an analytical type ADAM PW254 balance with an accuracy of 0.0001 g.

Viscosity was measured using an Ubbelohde device with distilled water as reference liquid (at 295 K; water viscosity: 1.002×10^{-3} N·s·m^{-2}; water density: 0.9982 g·cm^{-3}).

Surface tension was measured by stalagmometric method, using a ROHR B type stalagmometer, the reference liquid being also the distilled water (water surface tension – 71.97×10^{-3}N·m^{-1} at 295 K).

METHODS

Student t–test was applied to assess the statistical significance of the differences between the average values corresponding to magnetic nanoparticle samples and the control ones, by taking into consideration the measurement results from four repetitions of every experimental variant.

RESULTS AND DISCUSSION

The rheological features of cobalt ferrite suspension – as they resulted from measurements carried out with the standard methods mentioned above, are presented in Fig. 1.

The concentrations of magnetic nanoparticles in the culture medium are given in Table 1 – where also the equivalent concentrations of metal ions are calculated.

Table 1

Concentrations of ferrite in the fungi samples

Ferrite concentration (mg/L)	0	15	20	30	35
Metal ions concentration (μg/mL)	0	10.2	15.3	20.4	25.5

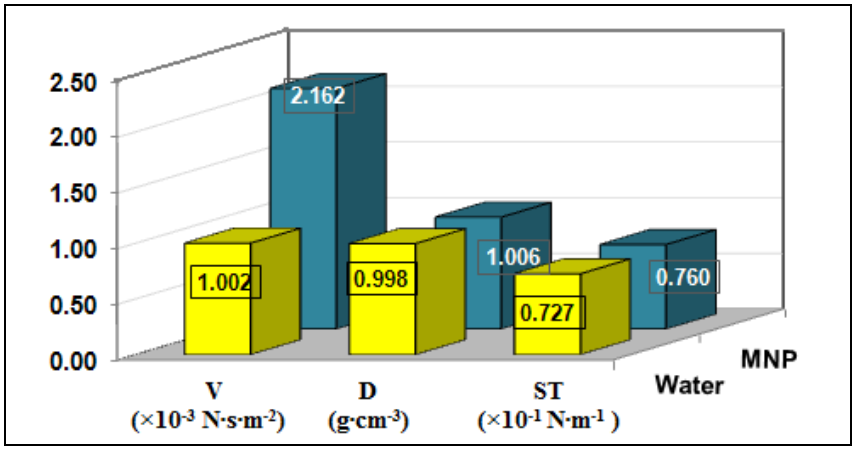

Fig. 1. Viscosity (*V*), density (*D*) and superficial tension (*ST*) of magnetic nanoparticles (MNP) compared to the suspension medium (water).

The most remarkable increase was evidenced at the level of fluid viscosity – of about twice compared to the suspension medium. The results of all three parameters characterizing magnetic nanoparticle suspension are similar with those reported for similar products in [4, 13].

The results of MDA assay in the fungi mycelium are presented in Fig. 2. As expected, the phenomenon of lipid peroxidation occurred evidently in most samples where magnetic nanoparticles were supplied. The variation of MDA in 7 days old samples is almost imperceptible among control sample and fungi samples supplied with cobalt ferrite nanoparticles regardless the concentration; standard deviation was of 6.5–7.0%. Thus in 7 days old fungi mycelium no influence of the magnetic nanoparticles could be evidenced. The peroxidation processes normally occur in living cells due to the generation of peroxide radical under the impact of

external constraints of either chemical or physical nature. When stress level is relatively low, then the enzymes of oxidative stress are able to balance the peroxides formation that contributes to cell protection. As known, the intensification of peroxidase like enzymes production is triggered when the level of free radicals of oxygen reactive species is increased and cell adaptation mechanisms are activated.

In some situations, the impact of constraints that generate oxidative stress also damages enzymes mainly at the level of their spatial configurations, where hydrogen bonds are rather sensitive especially to radiations and heat. The final result of such opposite processes could be assessed by various methods such as enzyme activity assays or the assay of peroxidation reactions products like malondialdehyde (MDA) – indicator of lipid peroxidation. We have chosen to measure the content of MDA considering that it better describes the total effect of peroxidation processes than the assay of each peroxidase like enzyme that would refer only to part of the whole picture.

In 14 days old samples (Fig. 2), the increased level of MDA is visible in all samples compared to the control; however, the statistical significance ($p < 0.05$) was ensured only for the samples corresponding to magnetic nanoparticle concentrations of 15 mg/L and respectively 30 mg/L, where about 12–15% increase was evidenced (standard deviation of 7.5%).

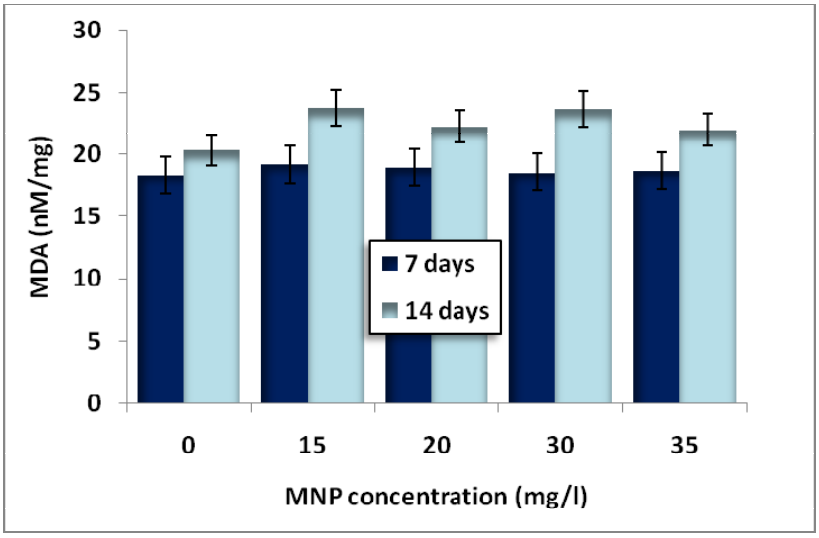

Fig. 2. MDA content in fungi samples following magnetic nanoparticle (MNP) supply.

This result is concordant with the other reports where various, different, biological materials were used; in [15] magnetic nanoparticles (300 mg/kg body) were supplied to mice inducing MDA increasing (with 30%) – as well as the activities of peroxidasic enzymes. Also similar changes in MDA (12% increase)

and in other biochemical parameters were reported in [3] following the administration of nanosized magnetite (100 mg/L) to aquatic photosynthetic species.

Lipid peroxidation was found increased in cell cultures supplied with nanometric magnetite (100 mg/L) but only in macrophages, not in liver ones [12], suggesting that different biological systems could respond differently from the perspective of oxidative stress (12% MDA increase) induced by magnetic nanoparticles.

The mechanisms of magnetic nanoparticles interaction with living cells may be related to the chemical aspects – or physical ones with further triggering of biochemical and biological changes [15]. The oxidative stress, basically the formation of like superoxide anions, hydrogen peroxides and hydroxyl radicals (reactive oxygen species – ROS) is accepted as one of the leading mechanisms of nanoparticles toxicity. Normally, the yielding of such chemical species in the mitochondria, where the respiratory chain of cell is located, elicits synergic mechanisms involving activation of various antioxidant enzymes, able to decompose peroxides before they attack important cell structures such as the membrane system.

Thus, in the studied fungi, it seems that the activity of antioxidant defense system almost totally compensates lipid peroxidation at 7 days after magnetic nanoparticle administration, so that the corresponding indicator, the MDA level was almost not increased. Later, the persistence of uptaken nanoparticles in the fungus cells led to higher, significant intensification of lipid peroxidation despite of defense mechanisms. Anyway, the variation recorded in biochemical parameters of the studied cellulolytic fungus is much smaller than in the case of other cellulolytic fungi studied in similar conditions, so that the interpretation has to take into consideration possible differences in fungi sensitivity depending on the species. The above discussed results could be considered rather significant relatively to environmental pollution with ferrite nanoparticles since the accumulation in human brain reaches several times more iron than the largest dose tested in the present experiment [2].

CONCLUSION

The study of magnetic contamination with cobalt ferrite nanoparticles administered to fungi cells has led to encouraging results for environmental issues. Only a small increase of lipid peroxidation end product was found by applying the assay of malondialdehyde. Consequently, the cell membrane system including lipid bilayer is not significantly threatened by magnetic nanoparticles in relatively low concentrations. At the same time, the necessity of a new biochemical parameter investigation has resulted which represents the next project of our multidisciplinary study.

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