

PRODUCTION OF MAGNETICALLY CONTROLLED BIOSORBENTS BASED ON FUNGI *Agaricus bisporus* AND *Lentinula edodes*

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The aim of the study was to produce magnetically controlled biosorbent based on fungi of champignon and shiitake, to determine the proportion of the magnetically controlled phase of the biomass of fungi when the magnetic fluid (MF) was added to the substrate and to explore the efficiency of extraction Fe³⁺ ions by shredded biomass of the fungus. The object of the study was mushrooms champignon *Agaricus bisporus* and shiitake *Lentinula edodes* grown in the laboratory. An effective and cheap way to remove waste biosorbent from the working environment is a high-gradient magnetic separation (HGMS), which takes place in high-speed mode. The separation of the magnetically controlled phase of fungi biomass *A. bisporus* and *L. edodes* was carried out by HGMS methods. It was investigated that when using the biomass of champignon grown on MF, the properties of the sorbent were significantly improved, the full saturation was 6 times faster in comparison with the biosorbent based on the biomass of the fungus grown without MF.

Key words: biogenic magnetic nanoparticles, magnetite, magnetically controlled biosorbent, champignon *Agaricus bisporus*, shiitake *Lentinula edodes*.

The interest in the biosynthesis of iron-containing biogenic magnetic nanoparticles (BMNs) is associated with their ferrimagnetic properties. BMNs are the subject of intense research when they were first discovered in magnetotactic bacteria (MTB) [1]. BMNs are found in organisms that belong to all three domains: prokaryotes, archaea and eukaryotes [1–11]. BMNs has been experimentally detected in algae and protozoa [12], worms [7], chitons [9], snails [13], ant and butterflies [14–16], honey bees [5, 15], termites [6], lobsters [17], tritons [18], migratory and non-migratory fish [8, 19–24], turtles [10, 25], birds [26–29], bats [30], dolphins and whales [31], pig [32], humans [33–35], plants [36, 37] and mushrooms [37–39].

It was found that the mechanism of biomineralization of BMN is the same for all living organisms [40–42].

All species are potential producers of BMNs among investigated representatives of the divisions of fungi the Ascomycetes (Ascomycota) and Basidiomycetes (Basidiomycota), which decoded the genomes of more than 50% in the database GenBank NCBI as it was proved by the methods of comparative genomics. At the same time, experimental studies of BMNs in fungi samples *A. bisporus* and *L. edodes* by means of methods atomic force microscopy (AFM) and magnetic force microscopy (MFM) showed that the BMNs in the fungi form chains that are localized on the walls of hyphae of samples of fungi. In recent decades, the search for sorbents of biological origin has become one of the most promising areas of problem solving for combating pollution with heavy metals in the environment. Heavy metals are elements of pollution from transport and many enterprises

in various industries. These metals entering into the human body cause poisoning and lead to serious disruption of metabolic processes and vital body functions [43].

And it is known [43–45] that the fruiting bodies of macromycete fungi (*Boletus edulis* (penny bun), *Ganoderma lucidum* (lingzhi mushroom), *Calvatia excipuliformis* (handkea excipuliformis), *Paxillus involutus* (brown roll-rimy), *Tricholoma terreum* (grey knight), *Armillaria mellea* (honey fungus) are natural and safe sorbents of heavy metal ions, dyes and pesticides.

Chitin is the only polysaccharide that contains nitrogen atoms and has uniquely high sorption properties. Fungi were chosen as the main active agent for the production of magnetically controlled biosorbent, because of the high content of chitin in the cell wall [43, 44].

Fungi can accumulate high concentrations of heavy metals [46–50]. Zinc (Zn), copper (Cu), manganese (Mn), lead (Pb), chromium (Cr), mercury (Hg), cadmium (Cd), nickel (Ni) and iron (Fe) can be accumulated in the largest quantities in fruiting bodies and the mycelium of many species of fungi [43, 44, 51].

However, the problem of removing metal-saturated biosorbent from the working solution remains relevant. The known method — filtering through a filter-paper, is quite long and inefficient [52, 53]. Therefore it is important to find a more efficient way to extract metal-saturated biosorbent from the working solution. Such a cheap and effective method is high-gradient magnetic separation (HGMS) [54], which operates in a high-speed mode.

Therefore, the aim of the work is to obtain a magnetically controlled biosorbent based on champignon and shiitake, the fraction of the magnetically controlled phase of the biomass of the studied fungi when added to the substrate magnetic fluid (MF) and study the efficiency of the extraction of Fe^{3+} ions by the biomass of the fungus *A. bisporus*.

Materials and Methods

Agaricus bisporus and *Lentinula edodes* were grown according to the standard method [55, 56].

Champignons and shiitake, were grown on medium with the addition of MF (magnetite — iron oxide Fe_3O_4), using a concentration MF of 0.1 mg/ml, which is close to the content of magnetite in soils [57–59] and 1 mg/ml, to study the characteristics of sorbents from the biomass of fungi.

Preparation of fungus biomass for high gradient magnetic separation includes the following steps: drying of a fresh fungus in an oven at $t = 60\text{ }^\circ\text{C}$ to a constant mass, grinding dry biomass using an electric mill for 1–5 min, sifting the biomass of fungi through a sieve with a cell diameter of 0.5 mm.

Suspensions were prepared for HGMS by mixing dried and crushed biomass of *A. bisporus* and *L. edodes* fungi with water, so that the ratio of the mass of the biosorbent to the water mass was 1: 200 (1 g per 200 ml of water). It is optimal concentration of mushroom/water, because its increase causes the grinding of a ferromagnetic matrix.

The suspensions (200 ml) that are based on fungus biomass of fungi *A. bisporus* and *L. edodes* were separated by high-gradient ferromagnetic matrix. The value of the magnetic field flux density is 3500 G. The diameter of the coils is 41.0 cm, the size of the magnet tips is 20.0×15.0 cm and size of the cuvette is 3.5×5.0 cm. External magnetic field is homogeneous, since the size of the cuvette with a ferromagnetic matrix of low carbon steel (according to the Ukrainian standards 380–2005 and 1050–90, composition: C — 0.25%, Si — 0.35%, Mn — 0.8%, S — 0.06%, P — 0.08%) is much less than the size of the pole tips of the electromagnet (dimensions are shown in Fig. 1).

The working fluid laminar flows through a high-gradient magnetic separator. The particles retained on the ferromagnetic matrix in the filter are washed with a small amount of distilled water.

The experimental setup for separating the magnetic phase of fungi from the nonmagnetic is shown in Fig. 1 [60].

Dry and crushed, using a laboratory mill, the biomass of the fungus *A. bisporus* grown on substrates with addition of MF of different concentrations, was tested for sorption capacity with respect to Fe^{3+} ions.

The process of biosorption was carried out with mechanical stirring 180 rpm, sorption duration 30 min.

Concentration of Fe^{3+} ions in solution — 50 mg/l.

Biosorbent concentration — 2 g/l.

Sampling time — 5 min, 10 min, 20 min, 30 min.

After sampling, the blue ribbon filter was used to determine the residual amount of Fe^{3+} ions.

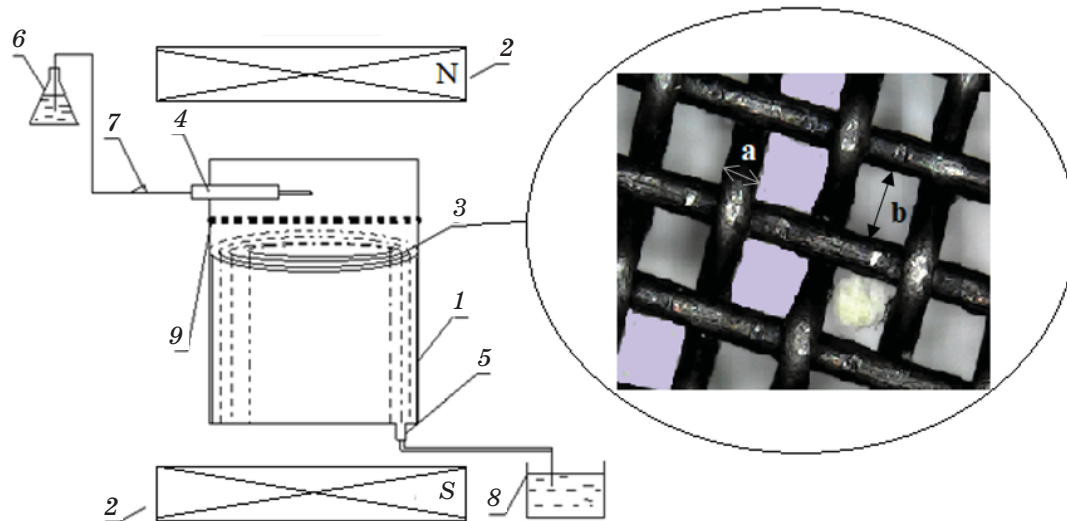


Fig. 1. The scheme of an experimental installation for HGMS:

1 — cuvette; 2 — magnetic system; 3 — a ferromagnetic matrix of low carbon steel (a is wire diameter — 0.58mm, b is cell size — 0.5×0.5 mm); 4 — an inlet pipe; 5 — outlet pipe; 6 — container for the working fluid; 7 — the speed control fluid; 8 — container for resetting the nonmagnetic phase; 9 — perforated plate for distributing the flow of liquid

Table 1. Cluster size before and after HGMS, percentage of separated parts of dry crushed biomass of shiitake (*L. edodes*), penny bun (*B. edulis*) and champignons (*A. bisporus*) depending on grinding, time

Fungi	Characteristic	The time of grinding dry mushroom, min				
		1 min	2 min	3 min	4 min	5 min
<i>L. edodes</i>	Size of the clusters before HGMS, μm	1.74±1.03	1.7±1.19	1.68±0.78	1.52±1.03	1.49±0.85
	Size of the clusters after HGMS, μm	3.6±1.2	3.93±0.51	4.1±1.05	4.4±0.45	4.3±0.78
	% magnetic phase	3.3	3.7	4.38	4.37	4.34
<i>B. edulis</i>	Size of the clusters before HGMS, μm	1.83±1.19	1.76±1.14	1.5±0.32	1.36±0.24	1.3±0.47
	Size of the clusters after HGMS, μm	1.93±1.01	2.1±1.07	2.38±1.51	2.27±0.61	2.25±0.68
	% magnetic phase	1.62	1.94	2.23	2.1	2.15
<i>A. bisporus</i>	Size of the clusters before HGMS, μm	2.44±1.1	2.23±0.93	1.73±0.56	1.66±1.21	1.58±0.85
	Size of the clusters after HGMS, μm	2.96±0.63	3.61±0.78	3.18±1.03	3.58±1.05	3.97±0.68
	% magnetic phase	1.01	1.01	1.05	1.44	1.17

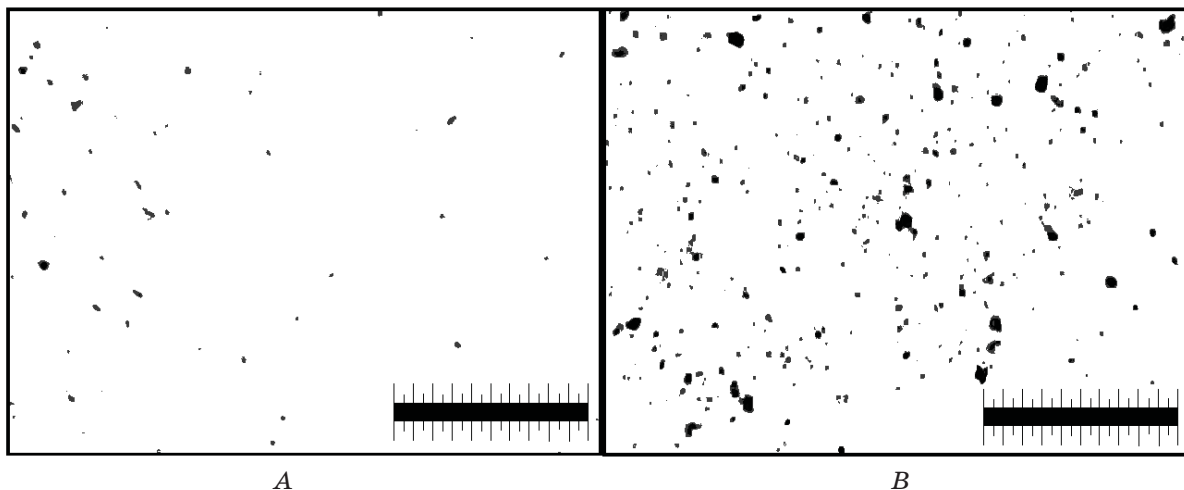


Fig. 2. Optical microscopy of dry biomass champignons before (A) and after separation (B) (scale bar 100 μm)

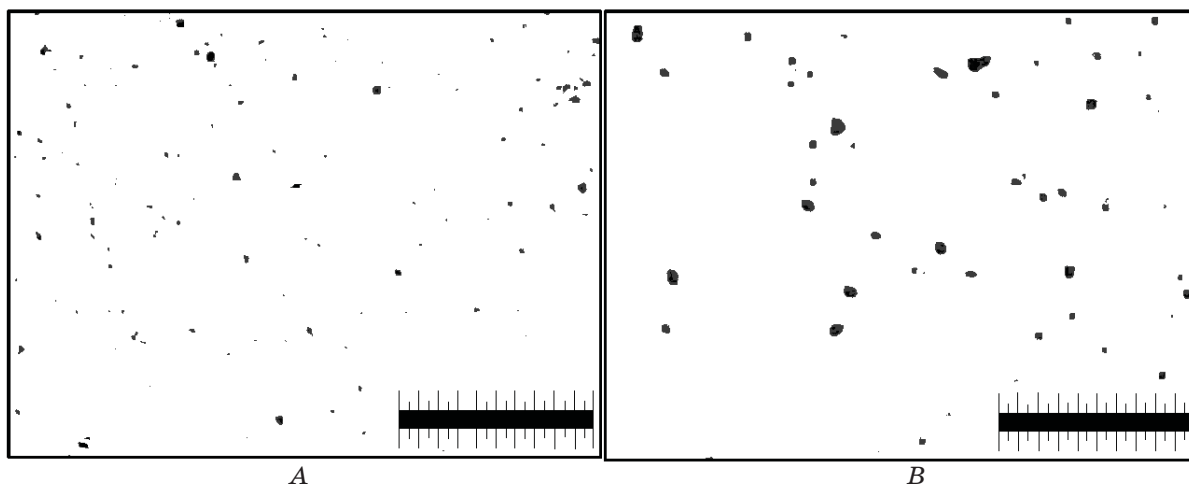


Fig. 3. Optical microscopy of dry biomass shiitake before (A) and after separation (B) (scale bar 100 μm)

Results and Discussion

The separation of the magnetized magnetic phase of the biomass of fungi was carried out with the installation for the HGMS. The average size of clusters was calculated using “Gwyddion” software. The results are shown in Table 1.

The results (Fig. 2, 3) show that after the HGMS the size of clusters and % of separated magnetically controlled phase increased significantly. The optimal time of grinding the dry biomass of the fungus is 3–4 min, so as further grinding does not reduce the size of the clusters and does not increase the number of magnetically controlled phase.

It is investigated (Table 2) that after HGMS the size of clusters of fungi biomass, grown on substrates with the addition of concentrated magnetite, increased almost 4 times, compared to the size before separation for shiitake and 3.3 times for champignons. This can be explained by the coagulation of clusters, containing magnetic particles, in the external magnetic field of the separator. Shiitake contains more magnetically controlled phase (3.3–4.8%) compared to champignon (1.05–1.8%).

Biosorbent based on the biomass of mushrooms was prepared as follows to

Table 2. Comparison of the results of HGMS of fungi *A. bisporus* and *L. edodes* grown on the substrate with the addition of MF of different concentrations

Fungi	Characteristic	Control	MF 0.1 mg/ml	MF 1 mg/ml
<i>L. edodes</i>	Size of the clusters before HGMS, μm	1.52 \pm 0.05	1.56 \pm 0.08	1.61 \pm 0.2
	Size of the clusters after HGMS, μm	4.4 \pm 1.1 (189%)*	5.1 \pm 1.1 (227%)*	5.8 \pm 1.4 (260%)*
	% magnetic phase	3.3%	3.6%	4.8%
<i>A. bisporus</i>	Size of the clusters before HGMS, μm	1.73 \pm 0.1	1.52 \pm 0.56	1.59 \pm 0.05
	Size of the clusters after HGMS, μm	3.18 \pm 1.1 (83%)*	4.1 \pm 1.3 (170%)*	4.9 \pm 0.9 (208%)*
	% magnetic phase	1.05%	1.5%	1.8%

* $P < 0.05$ compared with the size of clusters before HGMS.

Table 3. The efficiency of sorption of Fe^{3+} ions

Sorption time, min	Efficiency of remote ions Fe^{3+} on the basis of biomass <i>A. bisporus</i> , %	Efficiency of remote ions Fe^{3+} on the basis of biomass <i>A. bisporus</i> (MF 0.1 mg/ml), %	Efficiency of remote ions Fe^{3+} on the basis of biomass <i>A. bisporus</i> (MF 1 mg/ml), %
5	43 \pm 2	91.5 \pm 0.5	94 \pm 0.5
10	67 \pm 2	93.5 \pm 0.5	95 \pm 0.5
20	80 \pm 2	94.0 \pm 0.5	96 \pm 0.5
30	90 \pm 2	94.5 \pm 0.5	99.5 \pm 0.5

determine the sorption capacity: fresh biomass of the fungus *A. bisporus* were dried to constant weight in a drying cabinet at $t = 60^\circ\text{C}$ to a constant mass; grinding dry biomass using an electric mill for 1 min, sifting the biomass of fungi through a sieve with a cell diameter of 0.5 mm. Carried out the sorption of iron ions Fe^{3+} and carried out the determination of residual amount of iron in the solution after mixing. The results of the experiments are presented in Table 3 and Fig. 4.

Thus, dry biosorbent based on mushroom biomass champignons has a high sorption

capacity with respect to Fe^{3+} ions since the efficiency of extraction of iron (III) ions at 30 min of sorption in all samples is more than 90%. It is proved that the full saturation is 6 times faster, that is, 5 min, compared with 30 min for biosorbent based on the biomass of the fungus grown without MF.

Conclusions

The HGMS divided the magnetically controlled phase of fungi biomass *A. bisporus* and *L. edodes*. It was investigated that after HGMS the particle size for *A. bisporus* increased by 1.8–3 times, for *L. edodes* by 2.8–

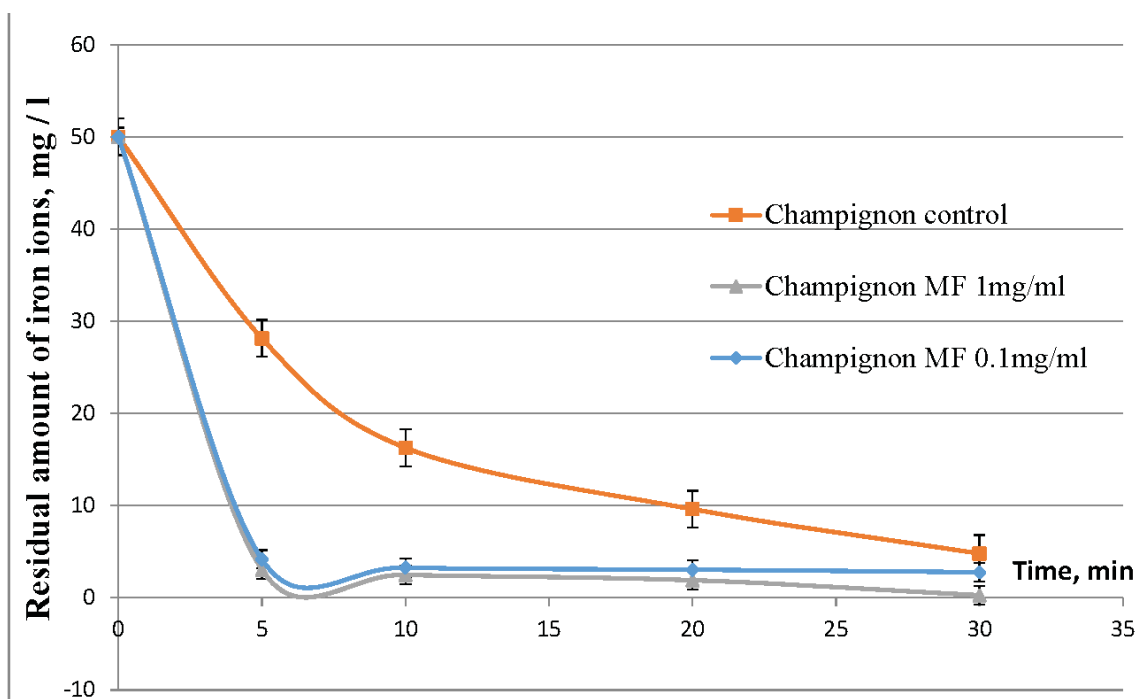


Fig. 4. Effect of germanium coordination compounds on activity of *P. tardum* α -L-rhamnosidase

3.6 times. Shiitake contains more magnetically controlled phase (3.3–4.8%) compared to champignon (1.05–1.8%).

The sorption properties of the fungus *A. bisporus* were investigated, when using the biomass of champignons grown on MF, the properties of the sorbent are significantly improved, the full saturation is 6 times faster,

that is, 5 min, compared with 30 min for the biosorbent based on the biomass of the fungus grown on a conventional substrate.

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**ОТРИМАННЯ МАГНІТОКЕРОВАНОГО
БІОСОРБЕНТУ НА ОСНОВІ
ГРИБІВ *Agaricus bisporus*
ТА *Lentinula edodes***

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Метою роботи було отримати магнітокерований біосорбент на основі грибів печериці та шиїтаке, визначити частку магнітокерованої фази біомаси грибів за додавання до субстрату магнітної рідини (МР) і дослідити ефективність вилучення іонів Fe^{3+} подрібненою біомасою гриба печериці. Об'єктом дослідження були гриби печериці *Agaricus bisporus* і шиїтаке *Lentinula edodes*, вирощені в лабораторії. Ефективним та дешевим способом вилучення відпрацьованого біосорбенту з робочого середовища є високоградієнтна магнітна сепарація (ВГМС), яка проходить у швидкісному режимі. Методами ВГМС здійснено відділення магнітокерованої фази біомаси грибів *A. bisporus* та *L. edodes*. Установлено, що в разі використання біомаси печериці, вирощеної на МР, значно поліпшуються властивості сорбенту, повне насичення відбувається в 6 разів швидше порівняно з біосорбентом на основі біомаси гриба, вирощеного без МР.

Ключові слова: біогенні магнітні наночастинки, магнетит, магнітокерований біосорбент, печериця *Agaricus bisporus*, шиїтаке *Lentinula edodes*.

**ПОЛУЧЕНИЕ
МАГНИТОУПРАВЛЯЕМОГО
БИОСОРБЕНТА НА ОСНОВЕ ГРИБОВ
Agaricus bisporus И *Lentinula edodes***

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Целью исследования было получить магнитоуправляемый биосорбент на основе грибов шампиньона и шиитаке, определить долю магнитоуправляемой фазы биомассы грибов при добавлении к субстрату магнитной жидкости (МЖ) и исследовать эффективность извлечения ионов Fe^{3+} измельченной биомассой гриба шампиньона. Объектом исследования были грибы шампиньоны *Agaricus bisporus* и шиитаке *Lentinula edodes*, выращенные в лаборатории. Эффективным и дешевым способом удаления отработанного биосорбента из рабочей среды является высокоградієнтная магнітна сепарація (ВГМС), которая проходит в скоростном режиме. Методами ВГМС осуществлено отделение магнитоуправляемой фазы биомассы грибов *A. bisporus* и *L. edodes*. Установлено, что при использовании биомассы шампиньона, выращенной на МЖ, значительно улучшаются свойства сорбента, полное насыщение происходит в 6 раз быстрее по сравнению с биосорбентом на основе биомассы гриба, выращенного без МЖ.

Ключевые слова: биогенные магнитные наночастицы, магнетит, магнитоуправляемый биосорбент, шампиньон *Agaricus bisporus*, шиитаке *Lentinula edodes*.