CZASOPISMO TECHNICZNE Ch
WYDAWNICTWO POLITECHNIKI KRAKOWSKIEJ

z. 2-Ch/2008
ISSN 0011-4561
ISSN 1897-6298

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USE OF MITOSPORIC FUNGI FOR HEAVY METAL REMOVAL FROM EXPERIMENTAL WATER SOLUTIONS

WYKORZYSTANIE GRZYBÓW MITOSPOROWYCH DO USUWANIA METALI CIĘŻKICH Z WODNYCH ROZTWORÓW MODELOWYCH

A b s t r a c t

Together 8 species (*Aspergillus niger* – An, *Aspergillus clavatus* – Ac, *Penicillium glabrum* – Pg, *Neosartorya fischeri* – Nf, *Trichoderma viride* – Tv, *Talaromyces flavus* – Tf, *Talaromyces wortmanii* – Tw, *Eupenicillium cinnamopurpureum* – Ec) and 3 strains of various mitosporic fungi (An 1–3, Nf 1–2) isolated from contaminated and non-contaminated environments were used for heavy metal removal from experimental solutions. Based on comparison of heavy metals ions used it was found that bioaccumulation decreased from Al (100%) > Cd (64–100%) > Zn (70%) > Pb (25–67%) > Cu (57%) > Cr(VI) (57%) > Ni (54%) > Cr(III) (24.7) > As (12–22.5%) to Hg (3–8%).

Keywords: heavy metals, removal, experimental solutions, mitosporic fungi, biomass

S t r e s z c z e n i e

W badaniach nad usuwaniem metali ciężkich z roztworów modelowych wykorzystano wspólnie 8 gatunków grzybów (*Aspergillus niger* – An, *Aspergillus clavatus* – Ac, *Penicillium glabrum* – Pg, *Neosartorya fischeri* – Nf, *Trichoderma viride* – Tv, *Talaromyces flavus* – Tf, *Talaromyces wortmanii* – Tw, *Eupenicillium cinnamopurpureum* – Ec) oraz 3 odmiany grzybów mitosporowych (An 1–3, Nf 1–2) wydzielonych zarówno ze środowiska skażonego, jak i nieskażonego. Na podstawie porównania badanych jonów stwierdzono, że bioakumulacja badanych metali ulega obniżeniu od Al (100%) > Cd (64–100%) > Zn (70%) > Pb (25–67%) > Cu (57%) > Cr(VI) (57%) > Ni (54%) > Cr(III) (24.7) > As (12-22.5%) do Hg (3–8%).

Słowa kluczowe: metale ciężkie, usuwanie, roztwory modelowe, grzyby mitosporowe, biomasa

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BIBLIOTEKA CYFROWA POLITECHNIKI KRAKOWSKIEJ

1. Introduction

Contamination of the environment is one of the most significant global problems at present time. A lot of heavy metals (Pb, Cd, Cu, Hg, As, Cr etc.) are discharged into the environment as industrial wastes causing serious soil, water and groundwater pollution. Many of them tend to accumulate in organisms and cause numerous diseases.

There is a possibility to remove dissolved heavy metals and toxic elements from waste or contaminated water employing physical and/or chemical methods as activated carbon adsorption, filtration, ion exchange, precipitation, ultrafiltration, reverse osmosis and electrodialysis [4]. In biological removal of dissolved heavy metals several organisms can be used:

– from procaryotic organisms sulphat-reducing bacteria *Desulphovibrio desulphuricans* [10], *Acidotiobacillus tiooxidans*, *A. ferrooxidans* and *Leptospirillum* [5, 9], *Bacillus subtilis* [20],
– from eucaryotic organisms many species of plants – phytoextraction and a wide range of species of mitosporic fungi as *Aspergillus niger*, *A. awamori*, *A. oryzae*, *A. flavus*, *Penicillium* sp., *Fusarium* sp., *Mucor* sp., *Rhizomucor* sp., *Rhizopus* sp. [2, 12, 14], *Cladosporium cladosporioides*, *Penicillium digitatum* [3, 6, 11, 22].

Altogether 8 species and 3 strains of various mitosporic fungi isolated from contaminated and non-contaminated environments were used for heavy metals (Al, As, Cd, Cr, Cu, Ni, Hg, Pb and Zn) removal from experimental solutions.

2. Material and methods

2.1. Microscopic fungi

The species of *Aspergillus niger* (An) – strain An 1 and *Aspergillus clavatus* (Ac) both isolated from drinking water from individual wells and municipal distribution systems. Species *Penicillium glabrum* (Pg) was isolated from a wet part in a water treatment plant [7]. Cosmopolitan species *Trichoderma viride* (Tv) and *Aspergillus niger* (strain An 2) were isolated from Eutric Fluvisols (pH H_2O/KCl = 7.7/7.4) in Gabčíkovo [13, 15]. Species *Aspergillus niger* (strain An 3) and heat-resistant species *Talaromyces wortmanii* (Tw), *Talaromyces flavus* (Tf), *Eupenicillium cinnamopurpureum* (Ec) and *Neosarorya fischeri* (strain Nf 1) were isolated from stream sediment of the river Blatina (pH H_2O/KCl = 5.3/4.8) in the mining region Pezinok-Kolársky vrch with the natural high content of As (363 mg/kg) and Sb (93 mg/kg). The heat-resistant species *Neosartorya fischeri*, strain Nf 4 (CCF Prague, No 1734) was used as the reference one. In all experiments with mitosporic fungi the strains were cultivated for 14 days on Sabouraud agar (SAB, HiMedia, Bombay, India). For the preparation of mycelia biomass 5 ml of conidia suspension in destilled water added to 45 ml of SAB liquid medium were used. After 10 days the mycelium was filtred out, washed in de-ionized water (Water Pro LS, Labconco, USA) and brought in 50 ml of experimental solution with the tested heavy metal. The removal of heavy metal was allowed at the stationary culture at 25°C for 24 hours. Then the mycelium was filtred, washed with de-ionized water and dried at 40°C to solid mass. The removal of heavy

BIBLIOTEKA CYFROWA POLITECHNIKI KRAKOWSKIEJ

metals from all experimental water solutions is expressed in %. All experiments were repeteated three times and the average values are mentioned.

2.2. Experimental water solutions

Experimental solutions to study the removal of heavy metals were prepared in the laboratory. Al as the solution $AlCl_3$ and $Al_2(SO_4)_3$ with concentration 65, 125, 250, 500 and 1000 ppm; Cd, Pb, Hg as $CdSO_4$, $HgCl_2$ and $PbCl_2$ (concentration 50, 100 and 200 ppm); As in concentration of 5 and 20 ppm in the solution. Removing of Cd, Cr, Cu, Ni, Pb and Zn was studied from two different types of solutions. Solution one, the heavy metal mixture, Cd [2 mg/l], Cr(VI) [5 mg/l], Cu [5 mg/l], Ni [4 mg/l], Pb [10 mg/l], Zn [1 mg/l]. In solution two, each heavy metal was dissolved individually to the concentrations: Cd [1 mg/l], Cr(III) [5 mg/l], Cr(VI) [5 mg/l], Cu [2 mg/l], Ni [2 mg/l], Pb [2 mg/l], Zn [1 mg/l].

The removal of tested heavy metals was determined using atomic absorption spectrometry (AAS) [7, 8, 16–19].

3. Results and discussion

Aluminium belongs to the most widespread elements. In waterworks it is used for coagulation in drinking water treatment. Subsequent removal of Al from $AlCl_3$ and $Al_2(SO_4)_3$ was noticed by *Penicillium glabrum* (100%) > *Aspergillus niger* strain An 1 (43–57%) and *Aspergillus clavatus* (45–48%). The biomass of *Penicillium glabrum* and *Aspergillus niger* strain An1 was not reduced in the system with Al. Production of *Aspergillus clavatus* biomass was by Al weakly reduced (Tab. 1).

The removal of Cd as $CdSO_4$, Pb as $PbCl_2$ and Hg as $HgCl_2$ in concentration 50, 100 and 200 ppm varied from Cd > Pb > Hg. Despite of the very high removal of Cd (from 64.6 to 100%) the mycelium of *Trichoderma viride* was reduced, thin and sporulation was very weak. The removal of Pb ranged from 25 to 57%. No mycelial reduction was observed. The removal of Hg was very low, from 3 to 8% (Tab. 1). A volatile form of Hg markedly damaged mycelium of *Trichoderma viride*. It was obviously reduced, very thin with minimal sporulation [18].

Table 1

The removal of heavy metals [%] from the experimental solutions by mitosporic fungi, connected with reduction of biomass

Heavy metal	Removal in [%]	Genus (strain) of mitosporic fungi	Reduction of biomass
Al	100	*Penicillium glabrum*	none
	43–57	*Aspergillus niger* strain An 1	none
	45–48	*Aspergillus clavatus*	weakly reduced
Cd	64–100	*Trichoderma viride*	reduced, thin mycelium, weak sporulation
Hg	3–8	*Trichoderma viride*	obviously reduced, very thin mycelium, minimal sporulation
Pb	25–57	*Trichoderma viride*	none

BIBLIOTEKA CYFROWA POLITECHNIKI KRAKOWSKIEJ

The removal of As in concentration of 5 and 20 ppm by heat-resistant species of mitosporic fungi *Talaromyces wortmanii* (Tw), *Talaromyces flavus* (Tf), *Eupenicillium cinnamopurpureum* (Ec) and *Neosarorya fischeri* strain Nf 1 was low, from 22.5% by Tw, 22% by Tf, 16.5% by Ec to 12% by Nf strain 1. Biovolatilization of As was apparent only when *Neosartorya fischeri* Nf 1 was cultivated in the systems [17]. The biovolatilization of As is an enzymatic conversion of organic or inorganic compounds of metaloids into their volatile derivates and we noticed this process in bioaccumulation of As(V) + As(III) and As(III) by species *Aspergillus niger* strain An 2 and strain An 3 [19].

The removal of Cd, Cr(III), Cr(VI), Cu, Ni, Pb and Zn from two types of solutions by *Aspergillus niger* strain An 2 (isolated from Gabčíkovo – the control site), *Aspergillus niger* strain An 3, *Neosartorya fischeri* strain Nf 1 (isolated from the stream sediment naturally amended with As and Sb) and *Neosartorya fischeri* strain Nf 4 (the control strain – CCF Prague) is documented in Tables 2 and 3.

The *Aspergillus niger's* removal of the metals from their mixture solution (solution one) was subsequently Cr(VI) > Pb > Zn > Cu > Cd > Ni, while the *Neosartorya fischeri's* one Cu > Cr(VI) > Pb > Cd > Ni > Zn. The species *Neosartorya fischeri* strain Nf 4 (the control strain – CCF Prague) dominated in the removal of Cr(VI) (53%), Cd (12.8%) and Ni (11.2%), (Tab. 2).

Table 2

Removal of heavy metals [%] from their mixture solution by *Aspergillus niger* strain An 2, An 3 and *Neosartorya fischeri* strain Nf 1, Nf 4

Strain of mitosporic fungi	Cd [2 mg/l]	Cr(VI) [5 mg/l]	Cu [5 mg/l]	Ni [4 mg/l]	Pb [10 mg/l]	Zn [1 mg/l]
An 2	11.5	39.9	29.6	5.2	36.3	19.7
An 3	7.4	39.9	14.3	10.4	14.5	29.3
mean	**9.4**	**39.9**	**21.9**	**7.8**	**25.4**	**24.5**
Nf 1	6.2	34.3	47.1	5.2	18	4.5
Nf 4	12.7	53	46.3	11.2	19	9.4
mean	**9.4**	**37.1**	**46.7**	**8.2**	**18.5**	**6.9**

The *Aspergillus niger's* removal of each heavy metal from its individual solution (solution two) was subsequently Cd > Zn > Pb > Ni > Cu > Cr(VI) > Cr(III) and the *Neosartorya fischeri's* one Cd > Cr (V) > Cu > Zn > Pb > Ni > Cr(III) (Tab. 3). In this case the species of *Neosartorya fischeri* strain Nf 4 (CCF Prague) was very active again. We noticed removing of Cd (96.5%), Zn (93%), Cu (86.5%) and Cr(VI) (71.1%) on a very high level (Tab. 3).

Based on comaprison of heavy metals ions used, it was found that the bioaccumulation decreased from Al (100%) > Cd (64–100%) > Zn (70%) > Pb (25–67%) > Cu (57%) > Cr(VI) (57%) > Ni (54%) > Cr(III) (24.7) > As (12–22.5%) As (12–22.5%) to Hg (3–8%). The removal of Hg was very low because of the volatile form of Hg which markedly reduced and damaged mycelium of the tested strain of mitosporic fungi (*Trichoderma viride*).

Many microorganisms, including mitosporic fungi, demonstrate resistance to heavy metals in soil, water or industrial waste. Some metals as Co, Cu or Ni serve as micronutrients. But most metals are nonessential, have no nutrient value, and are potentially

BIBLIOTEKA CYFROWA POLITECHNIKI KRAKOWSKIEJ

toxic to microorganisms. These toxic metals interact with essential cellular components through covalent and ionic bonding. Microorganisms, including mitosporic fungi, have adapted to the presence of both essential and nonessential metals by developing a variety of resistance mechanisms [1, 21]. Dark brown or black pigmented mitosporic fungi as *Cladosporium* sp., *Alternaria* sp., *Aureobasidium* sp. and others accumulate from 2.5 to 4 times more Ni, Zn, Cd and Pb in comparison with fungi without melanins. A heavy metal desorption is significantly lower from the pigmented species, too [6].

Table 3

Removal of heavy metals [%] from their individual solution by *Aspergillus niger* strain An 2, An 3 and *Neosartorya fischeri* strain Nf 1, Nf 4

Strain of mitosporic fungi	Cd [1 mg/l]	Cr(III) [5 mg/l]	Cr(VI) [5 mg/l]	Cu [2 mg/l]	Ni [2 mg/l]	Pb [2 mg/l]	Zn [1 mg/l]
An 2	72.6	24	32.9	27	47	53.9	54.5
An 3	92.2	32.2	32.2	55.5	65.5	83	84.2
mean	**82.4**	**28.1**	**32.5**	**41.2**	**56.2**	**68.4**	**69.3**
Nf 1	67.9	19.9	90.6	58.5	51.5	63	50.8
Nf 4	96.5	22.8	71.1	86.5	53.5	76	93
mean	**82.2**	**21.3**	**80.8**	**72.5**	**52.5**	**69.5**	**71.9**

The utilization of biomass of mitosporic fungi for the heavy metal removal from contaminated wastewater or water influenced with toxic heavy metals is one of alternative ways of decontamination of water and the environment. There is a great possibility to use mitosporic fungi as a bio-source of the accumulation of metals in various natural systems.

Present work has been supported by grant VEGA 1/0159/08.

References

[1] Bruins M.R., Kapil S., Oehme F.W., *Microbial resistance to metals in the environment*, Ecotoxicol. Environ. Saf., 45, 3, 2000, 198-207.
[2] Coulibaly L., Gourene G., Agathos N.S., *Utilization of fungi for biotreatment of raw wastewaters*, Afr. J. Biotechnol., 2, 12, 2003, 620-630.
[3] Edvantoro B.B., Naidu R., Megharaj M., Merrington G., Singleton I., *Microbial formation of volatile arsenic in cattle dip site soils contaminated with arsenic and DDT*, Appl. Soil Ecol. 25, 2004, 207-217.
[4] Erdem E., Karapinar N., Donat R., *The removal of heavy metal cations by natural zeolites*, J. of Colloid and Interface Sc. 280, 2004, 309-314.
[5] Fečko P., Kušnierová M., Čablík V., Pečtová I., *Environmentální biotechnologie*, Ediční středisko VŠB-TU, Ostrava 2004.
[6] Fogarty R.V., Tobin J.M., *Fungal melanins and their interactions with metals*, Enzyme Microb. Technol., 19, 4, 1996, 311-317.
[7] Franková E., Fabová M., Šimonovičová, A., Kopcová, A., Dlapa P., *Vplyv mycetických nárastov v akumulácii na kvalitu pitnej vody*, Ambrožová J.

BIBLIOTEKA CYFROWA POLITECHNIKI KRAKOWSKIEJ

(ed): 19. seminář Aktuální otázky vodárenské biologie, Praha 5-6. února 2003, 124-128.

[8] Hagarová I., Žemberyová M., Shearman, A., Chromá O., *Stanovenie a špeciácia arzénu vo vodách metódou HG AAS*, Hučko P. (ed): Zborník prednášok z XXXVI. ročníka konferencie s medzinárodnou účasťou „Nové analytické metódy v chémii vody" HYDROCHÉMIA, Bratislava 2004, 88-96.

[9] Kušnierová M., Fečko P., *Minerálne biotechnológie I. v ťažbe a úprave sulfidických ložísk*, Ediční středisko VŠB-TU, Ostrava, 2001.

[10] Luptáková, A., Kušnierová, M., *Odstraňovanie ťažkých kovov a sulfátov z odpadových vôd pomocou sulfát-redukujúcich baktérií*, Acta Montanistica Slovaca, 5, 3, 2000, 289-293.

[11] Pethkar A.V., Kulkarni S.K., Paknikar K.M., *Comparative studies on metal biosorption by two strains of Cladosporium cladosporioides*, Biosour. Technol., 80, 3, 2004, 211-215.

[12] Siegel S.M., Galun M., Siegel B.Z., *Filamentous fungi as metal biosorbents: A review*, Water, Air and Soil Poll., 53, 1990, 335-344.

[13] Šimonovičová A., *Soil microscopic fungi of Slovakia and their occurrence in environment. 1. Order Eurotiales, family Trichocomaceae*, Phytopedon, Bratislava, 3, 2, 1-53, 2004.

[14] Šimonovičová A., *Využitie biomasy mikroskopických húb na zníženie obsahu iónov ťažkých kovov a toxických prvkov z vodného prostredia*, Prokšová M., Seman, M. (eds): Mikrobiológia vody a prostredia 2006. Zborník prednášok a posterov, Poprad 20-22. sept. 2006, 78-80.

[15] Šimonovičová A., Ďugová O., *Mycocoenoses of selected localities of floodplain forest in region of Gabčíkovo*, Acta Environ., in press, 2008.

[16] Šimonovičová A., Hlinková, E., *Accumulation of different arsenic ions by heterothrophic organisms*, Fečko, P., Čablík, V. (eds): 10th Conf on Environ and Mineral Processing-Part III. VŠB-TU, Ostrava 22-24.6.2006, 305-309.

[17] Šimonovičová A., Piecková E., Ševc J., *Termorezistentné mikroskiopické vláknité huby ako biosorbenty As*, Prokšová M., Seman M. (eds): Mikrobiológia vody 2003. Zborn prednášok a posterov. Poprad 8.-10.10.2003, 97-101.

[18] Šimonovičová A., Ševc J., Iró S., *Trichoderma viride Pers. ex Gray as biosorbent of heavy metals (Pb, Hg and Cd)*, Ekológia, Bratislava, 21, 3, 298-306, 2002.

[19] Šimonovičová A., Žemberyová M., Hagarová I., *Biotransformácia anorganických špécií arzénu druhom Aspergillus niger*, Činka J., Antonická B. (eds): Odpady 2005. Zborn. prednášok z medzinár. konf. Spišská Nová Ves 3-4.11.2005, Copycenter s r.o., Košice, 160-163.

[20] Vasudevan P., Padmavathy V., Tewari N., Dhingra S.C., *Biosorption of heavy metal ions*, J. of Scien. & Industr. Res., 60, 2, 2001, 112-120.

[21] Vig K., Megharaj M., Sethunanthan N., Naidu R., *Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review*, Advances in Environ. Res. 8, 2003, 121-135.

[22] Wales D.S., Sagar B.F., *Recovery of metal ions by microfungal filters*, J. Chem. Technol. Biotechnol., 49, 4, 1990, 345-355.

BIBLIOTEKA CYFROWA POLITECHNIKI KRAKOWSKIEJ