

**THE CORRELATION BETWEEN BIOSORPTION WITH
AUREOBASIDIUM PULLULANS STRAIN CH-1 AND POLYMER
SORPTION ON POLY(GMA-CO-EGMA)-EN OF HEAVY METALS
FROM WASTE WATERS**

Milanka Dj. RADULOVIĆ^{a*}, Aleksandra B. NASTASOVIĆ^a, Olga G.
CVETKOVIĆ^a, Dragana S. DJORDJEVIĆ^a, Snežana D. NIKOLIĆ^c and Nebojša
D. NIKOLIĆ^b

^aCenter of Chemistry, IChTM, Njegoševa 12, P.O.Box 473, Belgrade,

^bCenter of Electrochemistry, IChTM, Njegoševa 12, P.O.Box 473, Belgrade,

^cFaculty of Chemistry, University of Belgrade, Studentski trg 16, P.O. Box 158,
Belgrade, Serbia

Radulović Dj. Milanka, Aleksandra B. Nastasović, Olga G.
Cvetković, Dragana S. Djordjević, Snežana D. Nikolić and Nebojša D.
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The intact microbial cells as well as derived microbial products can be highly efficient bioaccumulators of both soluble and particulate forms of metals, especially from dilute external concentrations (1, 2). The reason for testing *Aureobasidium pullulans* as a biosorbent is the fact that this fungus presents a ubiquitous saprophyte that occurs commonly in the phytoshere (3). Therefore, it has enabled the use of carbohydrate wastes as substrates for the production of pullulan such as the peat hydrolysate.

* Author for correspondence. E-mail: milan@afrodita.rcub.bg.ac.yu

The metal removal and recovery processes include: precipitation, adsorption and biosorption, electrowinning and electrocoagulation, cementation, membrane separation, solvent extraction and ion exchange (4). Galvanic waste effluent contain heavy metals ions, such as Cu^{2+} , Cr^{3+} , Cr^{6+} , Cd^{2+} , Ni^{2+} , etc., which present environmental hazard. Having all this in mind, the aim of this paper is to examine the possibility of applying *Aureobasidium pullulans* CH-1 (Collection of Microorganisms, IChTM) as a biosorbent of metals and biosynthesis of pullulan at the same time on peat hydrolysate, distribution of sorbed metals between biomass and pullulan, as well as the correlation between biosorption *Aureobasidium pullulans* CH-1 and polymer sorption on poly(GMA-co-EGDMA)-en of heavy metals from galvanic waste effluent.

Key words: heavy metals, biosorption, polymer sorption, *Aureobasidium pullulans*, poly(GMA-co-EGDMA)-en

INTRODUCTION

The uptake and accumulation of heavy metals by microbial biomass have increased attention in a biotechnological context since microbe-based technologies may provide an alternative or adjunct to conventional techniques of metal removal/recovery from polluted effluents and waste waters. Microorganisms, including actinomycetes, cyanobacteriae, fungi and yeast have the ability to accumulate heavy metals from their external environment (MANN *et al.*, 1988). Accumulated amounts of the heavy metals can be large, and variety of mechanisms may be involved ranging from physico-chemical interactions like adsorption and deposition to processes dependent on cell metabolism, e.g. transport. Both living and dead cells are capable of uptake and accumulation, as well as their products produced or derived by microbial cells, such as excreted metabolites, polysaccharides, and cell wall constituent (BRIERLEY *et al.*, 1986). Many heavy metals are essential for microbial growth and metabolism in low concentrations, e.g., Cu, Zn, Mn, whereas some of them have no known biological function, e.g., Au, Ag, Pb, Cd. A feature of all these and related elements is that they can be highly toxic towards living cells (GADD and GRIFFITHS). Thus, if the use of living cells envisaged in a metal removal system, toxicity may lead to poisoning and inactivation (SHUMATEAND *et al.*, 1985). The use of dead biomass or derived products eliminates the problem of toxicity, not only from dissolved metals but also from adverse operating conditions, and the economic component of maintenance including nutrient supply. However, living cells may exhibit a wider variety of mechanisms for metal accumulation, such as transport, extracellular complex formation and precipitation. In addition, tolerance and resistance are widely found properties of microorganisms of all the major groups (BELLIVEAU *et al.*, 1987).

We reported that *Aureobasidium pullulans* strain CH-1 has capability for accumulation of heavy metals and biosynthesis of pullulan at the same time (RADULOVIĆ *et al.*, 2001). The reason for using *Aureobasidium pullulans* as a biosorbent is of industrial importance, thanks to its capability of producing pullulan on peat hydrolysate. Pullulan is homoglycan which on account of its nontoxicity causes much interest in medicine, food, pharmaceutical and chemical industries, both in native and chemical modified forms, such as esters, ethers, reduced and oxidized derivatives (LACHKE *et al.*, 1995). Additional reason for testing *Aureobasidium pullulans* as a biosorbent is the fact that this fungus can grow and synthesize pullulan from a wide range of carbohydrate substrates including hexoses and pentoses. The peat hydrolysate from the briquetting processes with wet carbonization as a pretreatment method is the polluted industrial effluent, which must be treated because it contains significant amount of heavy metals (MENSINGER 1980). Some parameters that control the biosynthesis of pullulan are temperature, initial pH of the medium, the oxygen supply, the nitrogen concentrations, the carbon source, the culture conditions and the presence of certain trace elements.

For the sake of comparison, the sorption of heavy metals from galvanic effluent was carried out on the macroporous crosslinked poly(glycidyl methacrylate-co-ethylene glycol dimethacrylate) with attached ethylene diamine as a ligand, poly(GMA-co-EGMA)-en. The advantage of this macroporous hydrophilic copolymer over classical ion exchange resins lies in its high capacity and good selectivity for the heavy metal ions, combined with chemical and mechanical stability (SHERRINGTON *et al.*, 1988).

MATERIALS AND METHODS

Microorganism

Aureobasidium pullulans CH-1 (Collection of Microorganisms, Center of Chemistry - IChTM).

Grow conditions

Aureobasidium pullulans strain CH-1 was maintained on agar slants which consist of (g): agar 20.0; KH_2PO_4 1.0; $\text{MgSO}_4 \times \text{H}_2\text{O}$ 0.2; NaNO_3 5.0; barley malt extract 25, and distilled water up to 1000 cm^3 .

Fermentation on peat hydrolysate

Peat hydrolysate is the liquid phase resulting from the heat treatment of raw peat in acid solution. The peat hydrolysate was prepared as follows. Portions (10 g) of peat were mixed with 150 cm^3 of distilled water containing 1.5% (vol/vol) H_2SO_4 . The acid-moistened peat was then hydrolysed in autoclave at

140±10°C for 2h. Before preparation of the culture medium, the peat hydrolysate was filtered and dissolved with distilled water up to 800 cm³.

For the preparation of the inoculum for the peat hydrolysate fermentation, the microorganisms were transferred from the stock culture to a medium of composition (in %): sucrose 5, (NH₄)₂SO₄ 0.3; yeast extract 0.05; KH₂PO₄ 0.3; MgSO₄×7H₂O 0.15. The pH of the medium was adjusted to 6.0 prior to autoclaving. The inoculum was developed in Erlenmeyer flasks containing 200 cm³ of medium on a rotary shaker at 200 rpm at 26°C. Two-day-old inoculum was used to seed the culture medium for all experiments at a ratio of 10% (by volume).

The experiments were performed under batch conditions in Erlenmeyer flasks containing 100 cm³ of culture medium on a rotary shaker at 200 rpm at 26°C, during 148 h. The used medium contained (in %): (NH₄)₂SO₄ 0.3; yeast extract 0.05; KH₂PO₄ 0.3; MgSO₄×7H₂O 0.15 and acid hydrolysate of peat from Vlasina Lake. The pH of the medium was adjusted to 6.0 prior to autoclaving. The experiments were conducted in Erlenmeyer flasks containing 100 cm³ of culture medium on a rotary shaker at 200 rpm at 26°C, initial pH of 6.0, during 148h.

The biosorption and polymer sorption of heavy metals from galvanic effluent

The batch experiments of biosorption and polymer sorption of heavy metals from galvanic effluent were conducted separately under batch conditions in Erlenmeyer flasks containing 100 cm³ of galvanic effluent on a rotary shaker at 200 rpm at 26°C, initial pH 6, during 72h. The pH of the galvanic effluent was adjusted to 6.0 with NaOH and filtered through Whatmann filter paper. For biosorption, in galvanic effluent were added in (%): (NH₄)₂SO₃; KH₂PO₄; Mg SO₄ × 7H₂O 0.2; NaNO₃ 5.0; yeast extract 25; and distilled water up to 1000 cm³. After the experiment for biosorption was finished, the fermentation broth was centrifuged at 8000 min⁻¹. The precipitated biomass was then dried to constant weight at 105°C.

Metal-uptake capacities of poly(GMA-co-EGMA)-en for Cu²⁺ and Zn²⁺ under competitive conditions were determined by contacting 1.0 g of modified copolymer with 100cm³ of galvanic effluent. At appropriate times (0.5; 1; 24; 48 and 72h), 2.0 cm³ of aliquots were removed and used to determine amounts of metals.

Polymer synthesis and modification

Macroporous poly(GMA-co-EGDMA) copolymer was prepared by a radical suspension copolymerization (14). For further investigations, the fraction with particle size of 150-500 μm was used. Modification of synthesized copolymer with ethylene diamine was carried out in toluene at 70°C for 7h, with tenfold excess of diamine.

Analytical methods

The chemical investigations were carried out on two samples of the peat from Vlasina Lake. Standard analytical methods were used for organic matter and ash determination. The biotechnological process was monitored by determination of pH value, the biomass and the pullulan yield and metals content changes in fermentation broth. After the pH had been measured, the fermentation broth was centrifuged at 8000 min^{-1} . The precipitated biomass was then dried to constant weight at 105°C . Crude pullulan was precipitated from the supernatant with addition of two volumes of ethanol. The precipitate was centrifuged at 4000 min^{-1} . Pullulan was dried to constant weight at 105°C . The supernatant, dried pullulan and biomass were used to determine metal content.

The amounts of the metal ions in filtrate solution at the start of the experiments and after desired treatments periods were measured by atomic absorption spectrophotometry, AAS (SpectrAA 55, Varian).

RESULTS AND DISCUSSION

The experiments carried out with two samples from the Vlasina lake floating peat isles. They originated from different locations and various depths.

The peat hydrolysate prepared in this work is similar to that obtained from the existing industrial process called the wet carbonization process for peat dewatering for fuels. In this process, the peat hydrolysate is the polluted industrial effluent, which must be treated. In order to examine applicability *Aureobasidium pullulans* strain CH-1 as biosorbent and producer pullulan at the same time, it was necessary to determine metal and organic matter content. The obtained results concerned with organic matter content and elemental analysis in the peat samples, are shown in Table 1. The ash content and metal analysis in the samples are presented in Table 2.

The changes in the metal content, pH of the fermentation broth, yield of biomass and crude pullulan and their metal contents during fermentations with *Aureobasidium pullulans* strain CH-1, for the peat samples 1 and 2 are shown in Table 3. The results indicate significant decrease of the metals contents in fermentation broth at the end of the experiments. From the practical standpoint, the fact that heavy metals accumulate on the biomass represents very valuable result.

Namely, the accumulation of the heavy metals on the pullulan would be unfavorable due to necessity of additional treatment of this commercial product.

Table 1.-The organic matter content in peat and its elemental analysis (%)

SAMPLES	1	2
Content of organic matter	87.57	85.46
C	47.37	48.80
H	4.97	4.78
N	2.10	2.39
S	0.18	0.18

As can be seen, that the peat from the Vlasina Lake has the abundance of the organic matter.

Table 2.-The ash content (%) and metals analysis (ppm) in the peat samples

SAMPLES	1	2
Content of ash	12.26	16.36
Cu	14.68	13.23
Fe	10488	10420.2
Zn	6.55	4.96
Mn	589.95	529.28
Pb	<1.31	<1.65
Cd	<0.26	<0.33
Ni	<7.87	<10.92
Cr	14.42	11.58

Table 3.-The changes metals content (ppm) and pH, yields of biomass (gdm^{-3}) and crude pullulan (gdm^{-3}) during fermentation with *Aureobasidium pullulans* strain CH-1 on the peat samples (1 and 2)

Content of metals	Samples			
	1		2	
	0h	148h	0h	148h
Cu	0.048	0.024	0.050	0.022
Fe	0.33	0.04	0.78	0.04
Zn	0.03	<0.05	0.02	<0.005
Mn	0.52	0.01	3.59	0.27
Pb	0.002	<0.001	0.066	<0.001
Cd	0.002	<0.001	<0.001	<0.01
Ni	0.087	<0.03	0.037	<0.03
Cr	0.008	0.004	0.020	<0.03
pH	6.0	5.88	6.0	5.42
Biomass	0.35	4.76	0.35	4.35
Crude pullulan	-	3.38	-	2.75

The results of the change metal content in the filtrate solutions after biosorption with *Aureobasidium pullulans* strain CH-1 and during sorption on poly (GMA-co-EGDMA) are shown in Fig. 1 and 2.

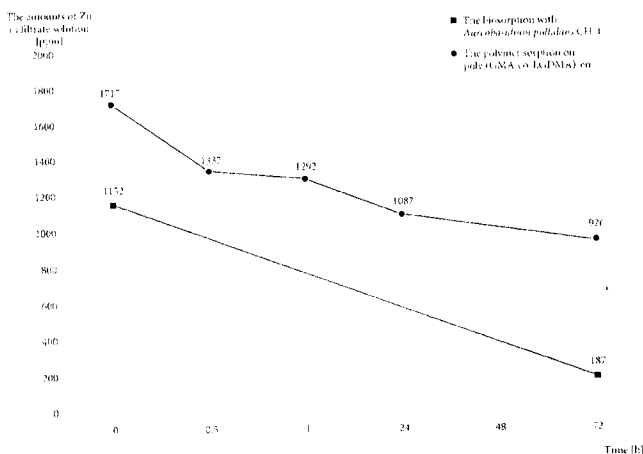


Fig 1 The changes of Zn amount in filtrate solution due to biosorption and polymer sorption

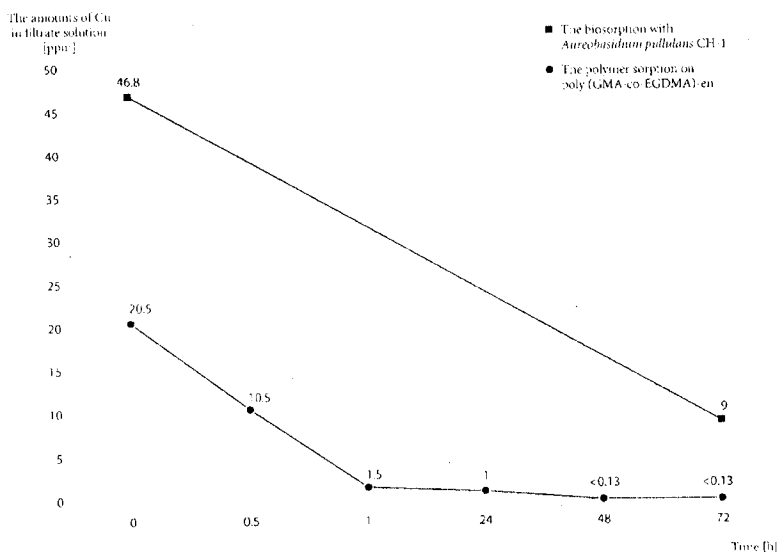


Fig 2 The changes of Cu amount in filtrate solution due to biosorption and polymer sorption

The yield of biomass was 5.1 gdm^{-3} . For the sake of the correlation of the data obtained during both processes (biosorption and polymer sorption), amounts of metals in biosorption were calculated per 1g of biomass.

The biosorption with *Aureobasidium pullulans* strain CH-1 is more successful in removing Zn than polymer sorption on poly(GMA-co-EGDMA). The amounts of absorbed Cu ions were (%) 67.53 and 46.07, respectively.

The polymer sorption on poly(GMA-co-EGDMA) is more successful in removing Cu than biosorption with *Aureobasidium pullulans* strain CH-1. The amounts of absorbed Cu ions on poly(GMA-co-EGDMA)-en were (%) 99.37 and 80.80, respectively.

CONCLUSION

The biosorption of heavy metals and biosynthesis of pullulan with *Aureobasidium pullulans* offers an alternative solution for the reduction of water pollution. Having all this in mind, the aim of this paper is to examine the possible application of *Aureobasidium pullulans* CH-1 as a biosorbent of metals and biosynthesis of pullulan at the same time on peat hydrolysate. The obtained results show satisfactory production of the pullulan comparing with usually used mediums and significant decrease of the metals contents in fermentation broth.

The obtained results show that both processes, biosorption and sorption on the poly(GMA-co-EGDMA)-en, may effectively be used for metal removal and/or recovery from galvanic effluents. The biosorption with *Aureobasidium pullulans* strain CH-1 is more successful for Zn removal, than poly(GMA-co-EGDMA)-en. Conversely, under experimental conditions, poly(GMA-co-EGDMA)-en is more efficient in Cu removal, than biosorption with *Aureobasidium pullulans* strain CH-1.

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POREDJENJE BIOSORPCIJE SA *AUREOBASIDIUM PULLULANS* SOJ CH-1 I POLIMER SORPCIJE NA POLI(GMA-co-EGMA) TEŠKIH METALA IZ OTPADNIH VODA

Milanka Dj. RADULOVIĆ^a, Aleksandra B. NASTASOVIĆ^a,
Olga G. CVETKOVIĆ^a, Dragana S. DJORDJEVIĆ^a, Snežana D. NIKOLIĆ^c,
Nebojša D. NIKOLIĆ^b

^aCentar za hemiju, IHTM, Njegoševa 12, P.O. Box 473, Beograd,

^bCentar za elektrohemiju, IHTM, Njegoševa 12, P.O. Box 473, Beograd.

^cHemijski fakultet, Studentski trg 16, P.O. Box 815, Beograd, Srbija

I z v o d

Ispitivanje *Aureobasidium pullulans*, soj CH-1 kao biosorbenta je uradjeno na kiselom hidrolizatu treseta sa lokaliteta Vlasinskog jezera. Kiseli hidrolizat treseta je dobijen na isti način kao i otpadne vode pri industrijskoj obradi treseta. Rezultati su pokazali da *Aureobasidium pullulans*, soj CH-1 može istovremeno da vrši biosorpciju metala i biosintezu pululana pri fermentacijama na kiselom hidrolizatu treseta.

U cilju poredjenja, za sorpciju teških metala iz galvanskih otpadnih voda, osim *Aureobasidium pullulans* soj CH-1 korišćen je i makroporozni kopolimer, poli(gicidilmetakrilat-co-etilenglikoldimetakrilat) modifikovan sa etilendiaminom, poli(GMA-co-EGDMA)-en. Rezultati uporednih eksperimenata biosorpcije na *Aureobasidium pullulans* soj CH-1 i sorpcije na polimernom sorbentu su pokazali da se oba postupka mogu uspesno koristiti za uklanjanje teških metala iz galvanskih otpadnih voda. Pokazalo se da je pri datim eksperimentalnim uslovima, za uklanjanje cinka uspešniji postupak biosorpcija, dok je poli(GMA-co-EGDMA)-en znatno efikasniji za uklanjanje bakra iz galvanskih otpadnih voda.

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