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ACCUMULATION OF SOME METALS IN THE BIOMASS OF MICROORGANISMS

Amongst the investigated bacteria, actinomycetes and fungi, biomass of the actinomycetes is characterized by extremely high abilities of Pb, Cu, Ni, Ca and Zn accumulation. Biomass of the actinomycetes can uptake 73 mg of lead per 1 g of dry matter. Biosorption of lead by these microorganisms is of selective character. The abilities of the bacteria and actinomycetes to accumulate metals can be presented according to the following order: Pb > Cu > Ni > Co > Zn. Such an order was not stated in the case of fungi.

1. INTRODUCTION

Microorganisms are capable of concentrating surprisingly high amounts of metals in their biomass. It has been found that some fungi, such as *Saccharomyces cerevisiae* or *Penicillium digitatum*, can accumulate as much as several percent of uranium in their dry mass [12], and, for instance, *Rhizopus arrhizus*, 18.5% [14]. Whithin the population of *Pseudomonas aeruginosa* there are cells accumulating uranium up to 50% of their dry mass [12]. This capability of living organism significantly exceeds that of some popular ionic exchange resins [13].

The metal accumulation by microorganism biomass occurs as a result of both the intracell metal storing and binding them on the surface, as well as in the region of the cell envelope [4]. Concentration and removal of metals from diluted solutions using biological methods can be the alternative to conventional chemical procedures such as: precipitation, oxidation and reduction, ion exchange, electrolysis and dissolvent extraction. The above mentioned methods are extremely expensive, and sometimes ineffective when the initial metal concentrations range from 10 to 100 g/m^3 providing that the final concentration cannot exceed 1 g/m^3.

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Removal of metallic wastes from waters is of great importance in the protection of natural environment in the time of dynamic mine development and when nuclear fuels are being brought in.

The above work presents accumulation of copper, nickel, cobalt, lead and zinc in resting cells of various microorganisms isolated from the metal pollutants of environment and in cells of museum strains.

2. MATERIALS AND METHODS

2.1. MICROORGANISMS

The strains used for investigations were of two origins. Most of them were isolated from the sludge pool in the Legnica Copper Mining Enterprise that contains wastes from a copper mine. The reaction of pool water was almost neutral (pH = 6.73) and the concentrations of metals were rather small: Cu – 0.06 mg/dm^3, Pb – 0.10 mg/dm^3, Ni – 0.20 mg/dm^3, Co – 0.08 mg/dm^3, Zn – 0.48 mg/dm^3. The microorganisms isolated from water and sludge were transferred onto enriched agar (bacteria and actinomycetes) and onto Czapek–Dox agar (fungi). The bacteria identification was conducted according to BERGEY [3] and COVAN [5] keys. The fungi were determined according to FASSATIOVA report [6], and *Streptomyces* sp. strains were described on the basis of their growth on some diagnostic Difco media according to International Streptomyces Project [10]. The other bacteria and actinomycetes strains, namely *Bacillus licheniformis* IAW 59, *Pseudomonas putida* PCM 2124, *Streptomyces purpurescens* IAW 113, *S. griseocarneus* IAW 87, *S. pilosus* RIA 1078 came from the collection of microorganisms of the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław.

2.2. CULTURES

Bacteria were cultured on sugar broth, actinomycetes on the medium consisting of: peptone (10 g), casein hydrolizate (2 g), yeast extract (2 g), NaCl (2 g), distilled water (1000 cm^3) and of pH ranging within 7.0–7.4, and fungi on the Czapek medium. The cultures were grown in the shaker (160 rpm) at the temperature of 28°C for 48 h (bacteria) and 78 h (actinomycetes and fungi). The microorganism biomass obtained as result of centrifugation after twofold washing with redistilled water was used in the experiments on metal accumulation.

2.3. INVESTIGATIONS OF BIOACCUMULATION

Microorganism biomass, in the amount equivalent to 10 mg of dry mass, was suspended in 10 cm^3 of solutions of pH equal to 5.0 containing 10^{-4} M of lead,

copper, nickel, cobalt and zinc. Chlorides ($CuCl_2$, $NiCl_2$ and $ZnCl_2$) and nitrate ($Pb(NO_3)_2$) were used to prepare the solutions of pH equal to 4.5. pH of solutions was measured using pH-meter N-512.

In the case of examining the accumulation selectivity, microorganism biomass (to 50 mg of dry mass) was suspended in 50 cm^3 of the above mentioned solutions mixed in equal volumes. All the suspensions were being shaken up at the temperature of 30°C for 1 h. Thereupon the biomass was separated from solutions by means of membrane filters of 0.45 μm pore diameter. The concentrations of metals absorbed in the biomass were determined basing on the measurement of the metal content in the filtrates. The measurements were taken using nuclear absorption spectrophotometer Perkin Elmer-403.

3. DISCUSSION OF RESULTS

Fourteen bacteria, two actinomycetes and two fungi strains were isolated from the samples taken from sludge pool. Apart from museum strains, two strains of each kind of isolated bacteria and both fungi and actinomycetes strains were selected and their metal accumulation abilities were investigated (tables 1–3).

Table 1

Accumulation of metals by bacteria

Species	Concentration of metals absorbed from solutions [mg/g of dry matter]				
	Pb	Cu	Ni	Co	Zn
Bacilius sp. 12	39.9	4.0	3.5	3.1	0.9
Bacillus sp. 16	42.0	4.5	3.7	2.5	1.4
Bacillus licheniformis IAW 59	40.7	4.4	3.7	2.8	1.1
Aeromonas sp. 1	30.0	3.7	2.7	3.2	0.3
Aeromonas sp. 8	36.0	3.7	2.9	2.1	0.6
Enterobacter sp. 2	30.4	3.8	2.5	2.2	0.5
Enterobacter sp. 32	27.9	3.6	3.0	3.1	0.5
Plesiomonas sp. 5	38.0	3.9	3.3	3.7	0.8
Plesiomonas sp. 21	40.8	4.8	3.4	3.8	1.2
Alcaligenes sp. 3	36.2	4.3	3.7	3.0	0.8
Alcaligenes sp. 10	37.3	4.0	2.7	2.4	0.7
Pseudomonas putida PCM 2124	38.0	4.2	3.1	2.5	0.5

Comparison of the tables proves that the highest concentrations of metals in question are accumulated in the biomass of actinomycetes, and the lowest in fungi. It becomes clearer when we compare the concentrations of lead bound by these microorganisms. The similar results were obtained by the Japanese who were dealing with uranium and cobalt accumulation [7], [9].

Table 2

Accumulation of metals by actinomycetes

Species	Concentration of metals absorbed from solutions [mg/g of dry matter]				
	Pb	Cu	Ni	Co	Zn
Streptomyces sp. 1	73.9	7.0	5.0	5.3	1.6
Streptomyces sp. 5	72.9	4.6	5.8	6.3	2.0
Streptomyces sp. 7	61.0	6.2	2.5	4.8	1.2
Streptomyces sp. 2	52.8	5.9	6.6	7.3	1.2
Streptomyces purpurescens 113	56.9	5.8	4.5	4.5	0.7
Streptomyces griseocarneus 87	42.0	4.9	4.5	4.9	1.1
Streptomyces pilosus 1087	66.8	4.7	3.0	4.3	1.4

Table 3

Accumulation of metals by fungi

Species	Concentrations of metals absorbed from solutions [mg/g of dry matter]				
	Pb	Cu	Ni	Co	Zn
Aspergillus niger	28.9	1.1	2.6	3.2	0.1
Penicillium notatum	26.1	5.7	0.7	2.5	0.4

Of the metals used in our experiments, lead was accumulated in the greatest quantities, while zinc in the smallest quantities by all microorganisms (tables 1–3). The respective concentrations of the metals accumulated by bacteria and actinomycetes decreased according to the following order: Pb $\gg$ Cu > Ni > Co > Zn, and Pb $\gg$ Cu > Co > Ni > Zn. The authors did not manage to establish such an order for fungi.

Among the bacteria of higher capabilities of metals accumulation, the following two Gram-positive strains, i.e. *Bacillus* sp. 15 and museum strain *B. licheniformis* IAW 59, and one Gram-negative, i.e. *Plesiomonas* sp., are worth noting (table 1).

Streptomyces sp. 1 and *Streptomyces* sp. 5 were the dominating strains of actinomycetes.

Bacillus sp. 16 and *Plesiomonas* sp. 21 strains were isolated from the environment polluted with metals (a sludge pool in the copper mine). The concentrations of metals accumulated in their biomass were not higher than those of museum strains (*Bacillus licheniformis* IAW 59 or *Pseudomonas putida* PCM 2124). However, the above mentioned isolated strains of actinomycetes (*Streptomyces* sp. 1 and *Streptomyces* sp 5) were a bit more active in process discussed than the museum strains. The isolated

fungi, *Aspergillus niger* and *Penicillium notatum*, were characterized by low activity of metal accumulation. The mycelia of these kinds are known as good sorbents of the elements examined [12]. The data presented confirms the statement that the metal accumulation abilities of microorganisms isolated from environments polluted with metals are worse. The amount of lead accumulated in microorganism biomass was nearly threefold bigger than the amounts of other metals (tables 1–3). In the process in question, the most active strain, i.e. *Streptomyces* sp. 2 (table 2), accumulated 73.9 mg of lead per 1 g of dry mass. This strain accumulated also the biggest amount of copper (7 mg/g) and relatively big quantity of cobalt (5.3 mg/g). These results can hardly be compared to the literature data, because the sorption of metals in resting cells of actinomycetes, bacteria and fungi examined by other researchers refers almost exclusively to uranium [11, 12]. Similarly, there are only a few works dealing with accumulation of copper, lead, nickel and zinc in microorganism cells. The accumulation of these elements, apart from lead, was observed in living and destroyed cells of algae *Chlorella regularis* [8]. The accumulation of zinc was investigated in growing yeasts, and that of lead, in bacteria and fungi [1].

AICKIN et al. [1] reported that enrichment of growth media with organic phosphates and lead acetate resulted in increased lead accumulation, amounting to 36% and about 19% in bacteria and fungi, respectively. The concentration of this element in the resting cells of microorganisms tested by us is considerably lower. Under the most favourable conditions, the *Streptomyces* sp. 1 strain accumulated lead up to 8% of cell dry mass.

In order to establish whether microorganisms tested are capable of absorbing metals selectively, there were carried out the experiments on accumulation of lead, copper, cobalt, nickel and zinc from the solutions that contained equal quantities of these elements in the mixture. Bacteria (*Bacillus* sp. 16 and *Plesiomonas* sp. 21), actinomycetes (*Streptomyces* sp. 1 and *Streptomyces* sp. 5) and fungi (*A. niger* and *P. notatum*) were selected for experiment. The data in table 4 indicate that lead is

Table 4

Selective accumulation of lead in the biomas of selected bacteria, actinomycetes and fungi

Microorganisms	Concentrations of lead absorbed [mg/g of dry mass]	Concentrations of metal absorbed from polymetallic solutions [mg/g of dry mass]				
		Pb	Cu	Co	Ni	Zn
Streptomyces sp. 1	73.9	71.9	0.76	0.06	0.18	0.06
Streptomyces sp. 5	72.9	70.7	0.57	0.18	0.59	0.12
Bacillus sp. 16	42.0	40.3	0.57	0.35	0.12	0.00
Plesiomonas sp. 21	40.8	36.1	0.70	0.47	0.17	0.06
Aspergillus niger	28.9	26.4	0.51	0.41	0.47	0.00
Penicillium notatum	34.1	32.2	0.95	0.12	0.29	0.00

accumulated from the solutions in the selective way in the biomass of bacteria, actinomycetes and fungi. The other metals are absorbed by microorganisms in question in minute quantities, although the same kinds of microorganisms accumulate much more of copper, cobalt and nickel from other solutions containing only ions of copper, cobalt or nickel (compare tables 1–3). Thus, it can be stated that the selective lead accumulation is the result of its ion competition with other ions present in the solution. The selective uranium accumulation in the biomass of bacteria, actinomycetes and fungi from the solutions containing nine various metal cations was reported by NAKAJIMA and SAKAGUCHI [9].

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AKUMULACJA WYBRANYCH METALI W BIOMASIE MIKROORGANIZMÓW

Biomasa promieniowców w porównaniu z biomasą bakterii i grzybów miała największą zdolność akumulowania Pb, Cu, Ni, Co i Zn. Z roztworów polimetalicznych ołów był akumulowany selektywnie. Zdolność akumulowania metali przez bakterie i promieniowce układa się w szeregu: Pb > Cu > Ni > Co > Zn. Dla grzybów takiego szeregu nie ustalono. Biomasa promieniowców akumulowała ołów w ilości 73 mg/g suchej masy.

АККУМУЛЯЦИЯ ИЗБРАННЫХ МЕТАЛЛОВ В БИОМАССЕ МИКРООРГАНИЗМОВ

Биомасса актиномицетов по сравнению с биомассой бактерий и грибов имела самую большую способность аккумулировать Pb, Cu, Ni, Co и Zn. Из полиметаллических растворов свинец аккумулировался селективно. Способность аккумуляции металлов бактериями и актиномицетами составляет последовательность: Pb > Cu > Ni > Co > Zn. Для грибов такой последовательности не установили. Биомасса актиномицетов аккумулировала свинец количеством в 73 мг/г сухой массы.