

Technical Note
**Biosorption and flotation biomass, loaded
by precious or toxic metals**

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ABSTRACT

Different microorganisms have been studied, capable to accumulate metals by their cells because of their resistivity to heavy metals. The separation of loaded biomass from the liquid by the filtration, frequentative flocculation, centrifuging has low effectivity. The most perspective is so named biosorbitive flotation. As sorbents can emerge biomasses of different microorganisms: bacteria, micromycetes, fungi, yeasts (baker's, beer and forage), active silts and microalga. Scientific and practical urgency of problem consists in revealing an intercoupling between the sorption of non-ferrous and precious metals by the biomass and its floatability, determination optimal parameters of flotation biomass loaded by metals. This will allow to find basic role of polyvalent metals in hydrophobizing the surface of cells of microorganisms, and reveal new aspects of metals action at flotation separation biomasses of microorganisms, optimize technologies of extraction of metals from the dilute solutions and give concrete recommendations on bioflotation technologies of extraction of noble and non-ferrous metals. © 2002 SDU. All rights reserved.

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1. INTRODUCTION

At present studies in the field of metals biosorption covered broad circle of bacteria, fungi, yeasts and alga (Solozhenkin *et al.*, 1999; Volesky and Holan, 1999). Shown, that ability to metals sorption from solutions in some degree possessed all microorganisms.

Biosorption of metals is highly selective, since bonding of metals is realized to the account of different mechanisms (ion exchange, complexing, reducing, formation of low soluble compounds), and because various properties of different metals in solutions. Sorptive capacity of microorganisms in number of events exceeds 40% from the dry weight of biomass. For instance, maximum gold accumulation by *M. luteus* for one hour reached 45% from the weight of dry biomass.

Tracked two main practical exhibits of biosorption - cleaning of sewages and extraction of valuable elements from sewages. In gold ore industry is developing a direction of gold recovery from leaching solutions by the different biomass of microorganisms, as an alternative to activated coal sorption technology. Biomasses form complexes with gold (I) in the form of $[\text{Au}(\text{CN})_2]^-$ or gold (III) as $[\text{AuCl}_4]^-$. High level of gold recovery reached, using biomass from the fermentation departures or different alga.

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2. BIOSORPTION OF METALS

As a result of studies on sorptions metals from solutions by microorganisms revealed following:

- sorptive ability of alive cells well greater, than inactivated (Fig. 1);
- differences in sorption of different metals reach 1.5 - 2.0 orders;
- a separation of metals or their compounds occurs in the manner of forming colloidal particles on surfaces of cells;
- a capacity of cells in certain events exceeds sorptive capacity of inorganic sorbents (Ulberg *et al.*, 1990).

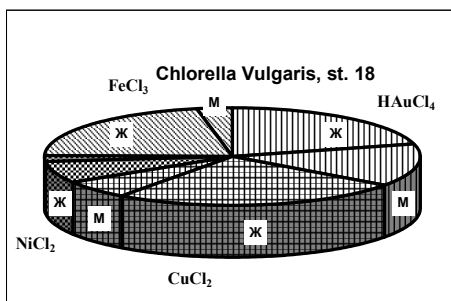


Figure 1. Sorption capacity of alive (Ж) and inactivated (М) cells of *Chlorella vulgaris*, st.18. Initial concentration in solutions - 10^{-3} mol/l, contact time - 1h (Ulberg *et al.*, 1990)

2.1. Noble metals

Biomass of microorganisms possesses great sorptive capacity, exceeding capacity of synthetic and natural sorbents. For instance, sea brown algae *Sargassum natas* sorbed gold up to 420mg/g biomasses, *Micrococcus luteus* for 30min – 177mg/g, and for 2h - 253mg/g biomasses. Granules of *AMT Bioclam*tm absorbed up to 462mg Pd and 102mg Pt per g. *Chlorella vulgaris* accumulated 120mg/g, *Chlorella pyrenoidosa* - more than 140mg/g. Sorption of gold by fungi *Aspergillus niger* formed 176mg/g and *Rhizopus arrhizus* 100 - 164mg/g. Chitosane sorbed $[AuCl_4]^-$ from solutions under pH 3.3 - 4.2, 150mg/g, and the recovery was 99.5%.

For microalga, beforehand adapted to gold ions, sorptive ability decreases in a row: Au>Fe>Cu>Ni>Mn. Kinetics to sorptions metals by cells of microorganisms show that optimal time of sorptions 20 - 60min.

The ions can transform into colloidal particles of free metal or its non soluble compounds (this are explains uncommonly great values of sorption capacity). Significant Au sorption is connected with the possibility of reducing gold ions into colloidal gold (0). Through 20, 69, 93h with the biomass of alga observed top intensity to sorption of colloidal gold (0).

It was revealed that biomass *A. niger* is an efficient sequester of silver ions from solutions with the concentration 2.5mg/l, capable to tie up to 10% of silver to the weight of dry biomass. Values pH within 5 - 7 did not change capacity to Ag biosorption. The bound silver possible completely desorb by diluted solution of nitric acid, and to regenerate biosorbent - by the washing with Ca^{2+}/Mg^{2+} salts solutions. This biosorbent is unique in the mechanism of sorptions of ions of metals and installed stoichiometric exchange with calcium and magnesium ions of biosorbent.

For the reason revealing an ability to accumulate Ag, Cd, Cu, Ni, Pb from the water solutions were practised 32 fungi from collections. Fungus *Phoma* sp., tolerate to 1mmol/l silver and selectively accumulate it. Ability of fungi to accumulate these five metals does not correlate with the ion radia of metals.

It have been found, that bioresponsible for the absorption of colloidal gold are amino acid, as well as proteins. So, for sorptions of coolloidal gold used material with the highest contents of these organic compounds. The protein - vitamine concentrate (BVK) was tested, containing 48.3% proteins and 23% amino acids. Full gold removal from colloidal solutions occur wiyh

loading BVK 1.5-2.0g/l; conditioning time - 6h. Full steady-state exchange capacity of concentrate on colloidal gold has formed 40mg/l at equilibrium concentration in dissolve 2.5mg/l. The sorption capacity BVK vastly exceeds that capacity of biomass *Aspergillus niger* because of high contents of amino acid and proteins.

2.2. Cadmium biosorption

For cadmium biosorption used a biomass of different microorganisms: fungi, yeasts, bacteria and alga (Vegliò and Beolchini, 1997; Volesky and Holan, 1999). Emphases took some fungi, as semi-products of fermentation and some brown sea alga. For instance, brown sea algae *Ascophyllum nodosum* absorbed Cd 215mg/g, and *Sargassum natans* -135 Cd mg/g. Actinomycetes type *Streptomyces griseus* (grown in the laboratory) and *Streptomyces clavuligerus* (industrial sample) sorbed Cd 0.17mol/g under initial concentrations of metal 10^{-4} mol/l.

2.3. Copper, zinc, nickel

Streptomyces rimosus (actinomycetes of tetracycline production) selectively absorb Cu cations from the mixture solutions with the concentrations, mmol/l: Zn - 0.765, Cu - 0.157, Ni - $3.408 \cdot 10^{-2}$, Ca - 2.495, Na - $4.348 \cdot 10^{-3}$ (Table 1).

Table 1
Biosorption of copper, zinc and nickel by biomass *Streptomyces* at different pH

pH	Recovery of metals cations, %		
	Copper	Nickel	Zinc
3	16.28	2.33	2.33
4	34.83	6.9	2.33
5	69.77	9.3	2.33
6	93.02	6.98	11.63
7	95.35	11.63	18.6
8	95.35	27.91	58.14
9	93.02	81.4	97.67
10	90.7	86.05	97.67
11	86.05	93.02	97.67

The copper cations were tied by the biomass under pH 4, maximal removing of copper was reached under pH=6. As contrasted with this zinc cations were precipitated only under pH above 7, and nickel cations - under pH=8. These data are indicative on abilities of biomass to the specific selective sorptions and removing Cu from the mixture of other cations in dissolve.

3. FLOTATION OF MICROORGANISMS, LOADED BY METALS

Searching efficient ways of governing surface characteristics of alive cells in different technological processes, and in flotation particularly, is one of the important and complex problem, appearing at the new biotechnological method of ore processing and removing from water ambiances of colloidal and soluble admixtures. The scientific side of problem modification the surface characteristics of microorganisms studied weakly and studies, conducted in this area, presented limited list of experimental work, not pretending on opening the main regularities for cell flotation and methods governing this process (Brierley and Vance, 1988; Pertsov *et al.*, 1991). Alive cells present itself to a considerable extent non trivial object for study their flotation from water ambiances. Difficulties, appearing at flotation microorganisms, connected with their small sizes (usually 1-3 μ m) and high, basically, hydrophilicity of their surface. So increasing efficiency of their flotation separations is usually ensured by two types of

influences: the surface hydrophobisation of cells by means of surface-active agents and aggregating cells by inorganic electrolytes and polymer flocculants. The optimal is a combination of both ways of raising efficiency. This allows to select the whole complex of cells flotation in the separate problem, requiring all-round experimental and theoretical study.

Process is concluded in the preliminary seizure of ion or molecule by particles of a carrier (due to adsorptions, absorptions, coprecipitation) with the following flotation of loaded by metal particles. As carrier can serve a biomass of microorganisms, ion-exchange resins, activated coal, the sediment, formed in dissolve. Carrier can possess by its own floatability, and be hydrophobized by collector. There are few works on flotation of biomass loaded by metals.

3.1. Green alga

Explored flotation biomasses of green alga *Chlorella vulgaris*, *Chlamydomonos* sp. loaded by Pb (II) from the water solutions. For the good flotation of chlorella with dodecilamine under pH=8 it is necessary to add polyvalent cations, such as Al (III). The best alga flotation were under pH=8 connected with the negative charge of surface of microorganism and at the addition of aluminum is observed flocculation of fine particles.

When using a sodium dodecilsulphate as collector the best flotation observed under pH 4. In presence of aluminum ions observed recharging of chlorella surfaces, and following flocculation. Maximum positive charge of chloralla was at pH from 4 to 5 and in these conditions it floated well. The role of lead ions in this system probably was similar to aluminum ions and so for the best flotation under pH 4 it is necessary presence of cations in the system.

C. vulgaris successfully floated with N-dodecil-2-aminopropionic acid, though in these conditions flocculation of particles does not occur. The surface of algae is kept both carboxilic and amine functional groups, such functional groups inhere in the structure of aminopropionic acid. Flotation of chlorella improves at the addition of iron sulphate 80g/m³ under pH 3, pH 9 and pH 9.6 without collectors. There was an unusual procedure of alga flotation completed by Chunsukatt. The gas bubbles of argon passed through solution of collector and were carried in flotation camera, where was ensured interaction between the collector, attached to argon bubbles and algae surface, i.e. it was developed so named AWDA flotation, earlier offered by Solozhenkin.

3.2. Fungi

As biomasses were used wastes from two industrial fungi production *Penicillium chrysogenum* and *Rhizopus arrhizus*. Studied non-reagent flotation biomass of fungi, beforehand saturated by the cadmium. Maximal recovery of biomasses were under pH 2. The flotation run with the high velocity: for 1-2min was recovered over 80% biomasses and cadmium without addition of flotation reagents.

Studied also removing an active silt, loaded by Cd, Zn, Ni. Biosorptive flotation with the active silt has allowed effectively remove from solution a cadmium, zinc and nickel.

3.3. Bacteria

Biomass of bacteria *Streptomyces pilosus* recovered Pb (II) from the water solutions. A sodium dodecylsulphate (DDSNa) sorbed on the biomass *S. pilosus* and in presence of Pb (II) sorption of collector increased. A flotation of biomass was low and DDSNa perfected floatability.

When loaded by Pb(II) biomass *S. pilosus* floated both in presence of, and in the absence DDSNa. The increase of flotation in the absence of the collector was from raising hydrofobicity of biomass or because of increasing aggregation of bacteria cells. When was added DDSNa flotation increased vastly? In presence of Pb sorption of collector brings about such a structure, where hydrocarbon part DDSNa was directed into dissolve and created more hydrophobic surface. The aluminium sulphate promotes floatability of *Bacillus cereus* when using laurylamine as collector.

3.4. Actinomycetes

Earlier biomass an actinomycetes *Streptomyces clavuligerus* and *Streptomyces griseus* were applied for the efficient recovery of cadmium (Solozhenkin *et al.*, 1999). Finely dispersed bubbles (10 - 120µm) generated to the account of air pressure, saturated water. Sorbent used repeatedly.

For the reason raising recovery of metals also used biomass of actinomycetes as selective biosorbent and carrier. Advantage of proposed way realised especially at low concentrations of ions, less than 50mg/l, when particles has colloidal or close to colloidal sizes and can not be effectively recovered by existing methods.

As biomasses of microorganisms used a gram - positive *Actinomycetes AK61* and *JL322* - wastes of the antibiotic production. There was low initial concentrations of cadmium 10mg/l. As collector applied CTMABr, under pH 6 - 10, and contact with the dry biomass AK-61 and J L322 was 15min. Biomass at concentrations 0.5mg/l was suspended in dissolve with the contents 5.0mg/l cadmium, pH adjusted by solutions of NaOH or HNO₃ (0.1N). Applied column flotation mashine with air disperser at the bottom with bubbles generated by size - 10-16µm. Flow of air formed 100sm³/min. Initial Cd concentration 5.0mg/l. Ethyl alcohol in the amount 0.25% v/v used as frother. For raising hydrophobicity of microorganisms biomass used cation collector CTMABr in the amount 2.5·10⁻⁵M and conditioned for 15min. The recovery of Cd 100% meant that remaining Cd contents in dissolve was below analytical sensitivity 0.03mg/l. A flotation of loaded biomass runs quickly. For 1 min recovered nearly 100% actinomycetes and over 95% cadmium under specified above conditions (Table 2).

Table 2.
 Flotation time effect on removing cadmium and biomass, loaded by cadmium, in presence of CTMABr

Time of flotation, min	Biomass J L 322 Recovery, %		Biomass AK61 Recovery, %	
	Biomass	Cadmium	Biomass	Cadmium
0	0	0	0	0
1	100	96	100	99.9
3	100	96	100	99.9
5	100	96	100	99.9
10	100	98	100	99.9
20	100	98	100	99.4

The flow of air 100cm³/min was sufficient for the efficient flotation of loaded biomass and presence CTMABr raised its recovery. In the field of pH from 6 to 10 the recovery of biomass and removing cadmium were efficient (nearly 100%), since remaining cadmium concentration was below 0.03mg/l. In the absence of biomasses a removing of cadmium was observed only under pH over 10 (Table 3).

Table 3.
 Flotation of biomass, loaded by cadmium, and removing Cd without using a biomass at different pH

pH values	Biomass J L 322 Recovery, %		Biomass AK 61 Recovery, %		Recovery Cd without biomass
	Biomass	Cadmium	Biomass	Cadmium	
3	39	42.86	100	20	0
3.6		42.86	---	---	---
4	100	64.29	100	60	---
4.5	100		100	88.1	0
5	100	90.48	100	95.25	---
6	100	95.24	100	97.62	---
7	100	97.62	100	97.62	4.77
8	100	95.24	100	97.62	4.77
9	100	95.24	100	97.62	4.77
9.5					64.29
10	100	95.24	100	97.62	95.24

Due to the internal structure of actinomycetes, their hydrophobic characteristics and abilities to coagulate/flocculate they were easily seized by air bubbles and floated better than small individual cells. Biomass comparatively easily floated (over 80%) without collectors, though adding of small dose CTMABr were useful (Table 4).

Table 4.
Influences of the collector on flotation of actinomycetes biomass, loaded by cadmium

Added CTMABr, 10^{-5} M	Biomass JK 322 Recovery, %		Biomass KA 61 Recovery, %	
	Biomass	Cadmium	Biomass	Cadmium
0	70	78.05	41.46	92.68
0.67	82.93	82.93	85.37	90.24
1	87.80	87.8	---	---
1.7	90.24	82.93	---	---
2	91.46	85.37	97.56	95.12
2.5	97.57	87.80	97.56	95.12
5	92.68	90.24	97.56	95.12

For the reason ensuring a possibility of frequentative using a biomass as a biosorbent and salvaging eluated metal were determined optimal conditions for desorption of cadmium. It was the small quantity of 1M sodium sulphate under pH below 5.2. The concentration of Cd in eluate was nearly 11 once more than in initial solution. Biomass used in circulation saved high efficiency of removing cadmium from dissolve.

In work tested samples *Chlorella vulgaris*, produced on industrial installation in the Timiryazev Institute Physiology of Plants RASci. (Moscow), and at the Institute of microbiology ASci. Republic Uzbekistan (Tashkent).

The micellyal wastes of antibiotic production were used: lincomicine (*Actinomyces roseolus*), neomicine (*Actinomyces fradiae*), riboxine (*Bacillus subtilis*), SC "Ferein" (Moscow), pharmaceutical combine (Penza), as well as penicillinum wastes - *Penicillium chrysogenum*, *Aspergillus niger* from citric acid production. Some samples of biomass were grown in laboratory conditions on known strategies.

4. CONCLUSIONS

On the grounds of theoretical and experimental data some new studies of biosorptive flotation technology for recovery of metals from dilute solutions. In the base of technology - combination of two processes: selective sorptions of metals on biological sorbents and flotation of biomass, loaded by metals.

The concentration of metals (or removal toxic inorganic ions) by microorganisms with following flotation can become industrial process both for a cleaning sewages, and for a recovery of metals. There is much reason for making broad study and further development of that field of research. Received data allow to recommend a following technological process:

- sorption of valuable component by the biosorbent from individual or complex dissolve during 30-40min at concentrations of electrolyte 10-50mmol/l under pH <7;
- flotation of biomasses, loaded by metals with or without collectors;
- desorption of metal from biomass in the manner of ions or colloidal particles;
- regeneration and washing biomass and recycling to the sorption process.

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