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REMOVAL OF HEAVY METALS FROM COAL MEDIUM WITH APPLICATION OF BIOTECHNOLOGICAL METHODS

1. Introduction

The environmental pollution in world scale is being observed from the middle of XIX century. Mainly, this is the result of the growing human population on Earth and intensive industrial development. The most important ecological problem is environmental pollution by substances which did not occur in nature before or were presented in small amounts, as: pesticides, heavy metals or non-organic compounds. These substances are slowly transformed by microorganisms what causes their long existence in natural environment influencing badly on living organisms [1].

The environmental pollution by heavy metals is caused by metals presented on Earth in mineral forms as a result of industrial works. As it occurs, the main source of metal transfers to the surrounding environment is coal combustion.

In hard coal ashes 70 elements are presented. In average, there is 200 g of lead, 400 g of uranium, 500 g of arsine, 70 g of nickel etc. in 1 Mg of ash [2].

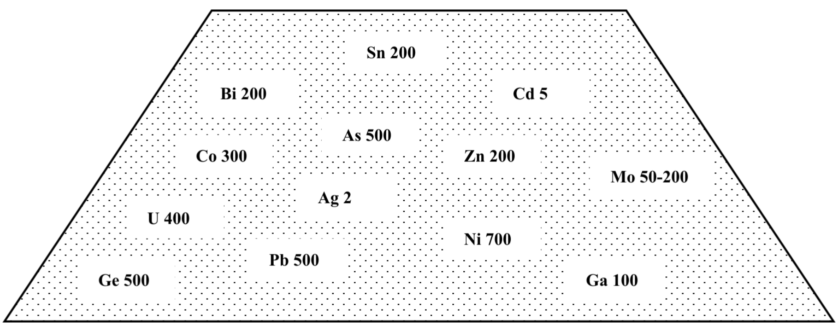

Fig. 1. Mean contents of some trace elements in 1 Mg of coal ash (g/t) [2]

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By combustion of 2,4 billion Mg of hard coal and 0,9 billion Mg of brown coal by year, 280 thousands Mg of arsine and 224 thousands Mg of uranium is spreaded together with ash, while the world production of these metals per year is 40 and 30 thousands Mg, relatively. So, only with ash of combusted coal many millions Mg of various metals are being spread to natural environment [2].

The metals being introduced to food chain circulate in it and cause a toxic effect for microorganisms presented in their various trophic stages. The heavy metals harmfully influence not only on more developed organisms, but also stop the activity of microorganisms taking part in natural compounds and xenobiotics decomposition [12, 15, 16]. However, there is a large group of microorganisms capable to grow in presence of high metal concentrations, even of several g/dm^3. Some of them can link or leach heavy metals, what may be used in practice to environmental protection and/or metal recovery [12, 16].

2. Biotechnological methods of environmental removal and recovery of heavy metals

The biotechnological methods are based mainly on natural processes occurring in nature and are based on their selective application and proper intensification of their development by their growth optimization what is connected with selection of technical parameters causing certain metabolic transformations. The most active microorganisms are autochthonic ones, isolated from natural environment heavily polluted by natural toxins.

The following microbiological processes are the most significant in removal and recovery of metals:

Biosorption

Biosorption is the group of all processes, during which alive or dead biomass removes heavy metals or other pollutants from solutions. Biosorption occurring with participation of microorganisms may conduct by surface adsorption concerning gathering of metals on cells surface and linking by extracellular biopolimers. The other way relies on metal infiltrating to the middle of cell (this term is close by meaning to intracellular accumulation).

It is often when biosorption occur as the first phase of the following intracellular accumulation. The process of surface adsorption occurring very fast — during several minutes may have dominant part in metal linking or may lead to high metal accumulation in the middle of cell in longer time.

The sorption properties of microorganisms are the result of the outer cell shield. The metals are being linked by active groups of compounds occurring in surface layers of cells. The most often this is the reaction of ion transfer between metal cations and active groups gifted with negative potential of outer cell structures. The microorganisms belonging to various systematical units feature by the presence of various chemical groups in outer structures, active in metal linking [4, 14]. The practical application of biosorption to removal or recovery of heavy metals is mainly the result of reversibility of this process. Desorption allows recovering metals (what is profitable in case of more valuable heavy metals like gold, copper, zinc) or their removal. During the desorption process of metals linked by microorganisms the solutions of weak mineral acids solutions (like 0,1 M HCl) or chelating compounds (like 10 mM EDTA) are being applied [16]. In range of pH 5–7, metal ions like: Cu^{2+}, Cr^{3+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+} are strongly linked to microorganisms biomass. Lowering of pH to the value of 2 causes liberation of metals from biosorbent. However, metal ions like Au^{2+}, Ag^{2+} stay at this pH on biosorbent.

The biosorbents can be [13]:

- biomass of microorganisms being the secondary product in sewage or pharmaceutical industry and in sewage treatment processes;
- microorganisms being cultured and proliferated on special bases indicating ability to efficient metal linking;
- sorbents of vegetable or animal origin (as nut shells, crust rich in tannines, sea plants, humus, moss peat etc.).

Bioaccumulation

The intracellular accumulation is the second phase in process of metal linking by living cells, occurring after biosorption. It conducts slower in time and it leads often to higher accumulation of metals. It is a process dependable on metabolic activity of microorganisms. The intracellular substances typical for heavy metals presence are metallothioneins being present in bacterias, fungi or algae cells and phytohelatins typical for plants — these are the proteins of low molecule mass. Phytohelatins and metallothioneins take part in detoxication process of metals by complexion and linking them in the middle of cells.

The bioaccumulation of metals may be practical and economically beneficial if it leads to high concentration of metals. This process, comparing to biosorption features by the fact that metal removal from cells and its recovery is connected with necessity of cellular structure transformation. It results in lack of possibility of biomass application in several cycles [12, 16, 18].

Many environmental bacteria species feature is the phenomenon of gathering large amounts of metals in cells, cell wall itself or in places bounded from cytoplasm. The amount of this deposit may be even 6% of dry cell mass and, taking into account in soil or water environment, this phenomenon may lead to temporary lowering of heavy metals ions concentration in environment. It allows living for other organisms being the part of eco-communion, including human beings [13].

Biotransformation

Microbiological transformation of heavy metals are reactions of oxidation, reduction, methylation and demethylation. The enzymatic systems of microorganisms take part in reactions. The practically useful may be reactions of significantly toxic or valuable metal reduction, like: bacteria gram + isolated from tannery sewers caused reduction of highly toxic chromium(VI) to less toxic chromium(III), which may be removed from environment.

Many bacteria, microscopic fungi may conduct reduction of metals ions (particularly valuable as gold or silver) to metallic form. This reaction may occur in vacuoles, on cells surface and in extracellular environment what is important from the point of view of these metals recovery [13, 16].

Bioprecipitation and biocrystallization

As the result of microorganisms activity the precipitation or crystallization of heavy metals compounds may occur, which cause transformation of metal into form sparingly soluble lowering their toxicity at the same time.

Some bioprecipitation and biocrystallization processes take part in biogeochemical cycles, like forming microfossils, deposing of iron and manganese, mineralization of silver and manganese.

Precipitation of metals on surface or inside of the cell may be the result not only of direct activity of enzymes and also the result of galactosis of secondary metabolites [16].

Bioleaching of metals

Bioleaching based on application of microorganisms, bacterial and fungi and on their metabolism products to transfer the metal contained in mineral to solution in relation to sulfide materials became the known industrial technology. The basis of this process is based on transformation of compounds of metals present in environment in form of sparingly soluble substances (most often sulfides) into forms easily soluble, where removal of metals is easy task.

Bioleaching may be conducted on the way of direct sulfides oxidation by bacteria into soluble sulfates(VI) form according to reaction:

$$\mathrm{MeS} + 2\mathrm{O_2} \rightarrow \mathrm{MeSO_4}$$

or by indirect microorganisms activity based on forming so-called leaching agents, which oxidize chemically sulfur minerals or on regenerating chemical oxidants, which are iron(III) sulfate(VI) and sulfur acid(VI) according to reaction:

$$\mathrm{MeS} + \mathrm{Fe_2}\left(\mathrm{SO_4}\right)_3 \rightarrow \mathrm{MeSO_4} + 2\mathrm{FeSO_4} + \mathrm{S^0}$$

where:

$$\mathrm{FeSO_4} + \textit{A.ferrooxidans} \rightarrow \mathrm{Fe_2}\left(\mathrm{SO_4}\right)_3$$

$$\mathrm{S^0} + \textit{A.thiooxidans}, \textit{A.ferrooxidans} \rightarrow \mathrm{H_2SO_4}$$

It was proved that the time of bacterial oxidation of Fe^{2+} ions by *A.ferrooxidans* in natural circumstances is even one million times shorter than in case of chemical oxidation.

The most important meaning in microbiological leaching processes hade chemoautotrophic, acidophilic sulfur bacteria from the sort *Acidithiobacillus* (from species *A.ferrooxidans, A.thiooxidans*).

Also the microscopic fungi are being used in research over microbiological leaching of metals. The ability of fungi to bioleaching and mobilisation of metals from indigent ores and industrial wastes is connected mainly with two processes: creation of various organic acids in living environment (as citric acid, gluconic acid, oxalic acid) and secretion of complexion agents. To such fungi the following sorts may be included: *Aspergillus sp.*, *Penicillium sp.*, *Rhizopus sp.*, *Mucom sp.*, *Alternaria sp.*, *Cladosporium sp.* and others.

The mildew fungi are the perfect alternative in metal leaching because of their biochemical abilities and relatively high resistance to bad factors as pH, temperature. There are being used mainly when it is not possible to apply classical methods of chemical or bacterial leaching with application of *A.ferrooxidans* and *A.thiooxidans*.

Biological methods of leaching are mainly used in biohydrometallurgy. The microbiological processes may be applied to metal leaching from sulfide and oxide minerals. There is a possibility to recover such metals as: arsine, antimony, bismuth, zinc, cobalt, gal, lead, copper, molybdenum, nickel, vanadium and uranium thanks to biohydrometallurgical methods. For now, the industrial application of these methods in the world is limited mainly to copper, gold and uranium leaching [1, 3, 4, 17].

Furthermore, the attempts of using this process to hard and brown coal sulfate removal, cleaning sludge, flotation wastes and dust filters from heavy metals in industry.

3. Bioleaching of metals from coal pyrites

The literature data indicate the possibility of microbiological process of metal leaching application not only from ores or ore wastes but also from wastes originated from other industrial branches. Many branches of modern industry meet several serious problems, among which the material and energetic lacks and production of huge amount of various wastes are the crucial ones. The searching for efficient and energetically economic processes, allowing their utilization caused new applications of bacterial leaching method being applied so far mainly to recovery of copper and uranium from weak ores and wastes.

Recently, the attempts of application of this method not only to metal recovery from industrial wastes as dusts, sludge, slag etc. but also to coal sulfate removal and detoxification of various sorts of water and sewers [11].

The necessity of exploitation of more and more sulfured hard coal causes that in some power plants like Siersza the certain group of wastes are being produced, which are so-called coal pyrites containing a large amount of sulfur (till 17%), mainly in form of iron sulfides. These materials are being collected on bingsteads in amount of 100 Mg per day. The influence of atmospheric oxygen and precipitation water on these bingsteads causes the oxidation of sulfides.

TABLE 1

Metal contents in solutions after 30 days of coal pyrites bioleaching in various leaching systems [6]

Metal, concentration	Contents in solution 5K before leaching	Contents in solutions after leaching				
		5K without bacteria	5K + bacteria F26 – 77 + T29 – 77 pattern	5K + autochthonic microflora	5K + autochthonic sulfide bacteria	5K + autochthonic iron bacteria
Mo, mg/dm^3	0,60±0,05	0,85±0,07	2,45±0,30	2,00±0,15	2,35±0,17	2,30±0,17
Zn, mg/dm^3	7,67±0,70	14,60±1,30	35,10±2,60	34,10±1,10	34,60±2,10	33,60±2,25
Al, g/dm^3	0,11±0,09	0,52±0,05	1,07±0,10	1,10±0,10	1,10±0,10	1,05±0,11
Mn, mg/dm^3	10,70±0,93	15,10±1,40	46,30±3,50	41,90±3,50	45,10±4,10	31,20±2,60
Cu, mg/dm^3	2,50±0,20	4,85±0,50	9,10±0,62	8,20±0,50	8,16±0,60	8,31±0,55
Pb, mg/dm^3	0,50±0,07	1,49±0,10	2,50±0,10	3,86±0,31	3,90±0,30	2,63±0,20
Fe, g/dm^3	5,70±0,32	6,11±0,52	10,60±1,10	10,30±1,00	10,40±1,10	10,40±1,10
Mg, mg/dm^3	55,80±0,50	108,00±5,10	135,00±9,50	137,00±9,60	136,00±9,65	136,00±9,60

TABLE 2

Growth of metals concentration in solutions of various initial ion Fe^{2+} contents, after 30 days of leaching of coal pyrites [5]

Metal, concentration	Mean metal leaching in solutions							
	1K		2K		5K		9K	
	without bacteria	bacteria F26 + T29	without bacteria	bacteria F26 + T29	without bacteria	bacteria F26 + T29	without bacteria	bacteria F26 + T29
Be, mg/dm^3	–*	0,8	–*	0,9	–*	1,3	–*	2,3
Ga, mg/dm^3	–*	9,6	–*	13,8	–*	19,3	–*	27,0
Co, mg/dm^3	–*	1,5	–*	1,6	–*	2,1	–*	3,2
Mn, mg/dm^3	7,1	32,8	7,1	34,7	7,2	45,6	7,2	63,0
Cu, mg/dm^3	1,0	4,7	1,5	6,0	2,9	7,1	3,9	7,8
Ni, mg/dm^3	0,05	3,5	0,05	7,3	0,25	11,5	0,25	14,2
V, mg/dm^3	0,5	25,2	0,5	50,4	2,1	109,2	2,1	117,8
Fe, g/dm^3	0,6	1,3	0,7	1,7	0,8	3,1	0,8	3,5

* The presence of element spectral lines was not indicated.

During these processes the sulfur acid is being produced what leads to strong acidification of soil and underground waters.

The other phenomenon, which is also disadvantageous is infiltration of many metals to waters and soils, including also heavy metals, which are accumulated in plants growing in neighbor areas. There is necessity then to start every activities allowing utilization of these inconvenient precipitation connected with their simultaneous detoxification.

The enlargement of the leaching method application range, with participation of microorganisms, to industrial wastes scale give better potential application of it and create possibilities to conduct environmental detoxification [5].

The investigation indicating the possibility of application of undermine waters to bacterial coal sulfide removal became an inspiration to apply autochthonic microflora of leaching material in process of metal bioextraction from coal pyrites wastes.

The microbiological analysis of researched coal pyrites indicated the presence of sulfide bacteria from the sort of *Acidithiobacillus* and iron bacteria being used in microbiological processes of metal leaching.

After 30 days of coal pyrites bioleaching in various leaching systems, the results presented in the Table 1 were given [6].

As the research proved, the leaching abilities of autochthonic sulfide bacteria and "pattern" seedlings *A.ferrooxidans* F26 – 77 and *A.thiooxidnas* T29 – 77 are nearly the same.

As a result of leaching conducted with participation of test bacteria in solution 5 K during 30 days, the leaching efficiency of about 40÷70% was given for the following elements: Be, Ga, Co, Ni, Pb, Mo, Mn, Mg, Zn, Al, Cu, V and Fe [6].

Furthermore, the influence of the initial contents of Fe^{2+} ions in leaching solution on metal extraction (Be, Ga, Co, Ni, V, Mn, Cu and Fe) from coal pyrites in presence of *A.ferrooxidans* and *A.thiooxidans* and without their participation was investigated too. It was stated that the significant better efficiency of the process occurs with higher concentration of iron ions in solution (Tab. 2) [5].

The efficiency of chemical and bacterial metal extraction from coal pyrites was examined in large laboratory scale in stoneware reactor. The material used in experiments was coal pyrite from "Siersza" power plant (marked by symbol IIa).

The material being leached contained, i.a.: 16,3% S, 14,8% Fe, 1,6% Mg, 0,03% Ni, 0,015% Pb, 0,035% Mn, 0,04% Zn, < 0,01% cu and Co (Fig. 2) [7].

4. Coal processing

The enlarging brown coal extraction in Poland causes collecting of larger amount of mantle rock. Only in region of Turoszow, this amount is about 45 million m^3 per year. The reclamation of mantle rock are difficult to conduct because of the sulfur compounds being part of it. Also, ash and slag — wastes being produced in large amounts and the most harmful ones in energy production — are the serious problem in utilization and environmental protection [9].

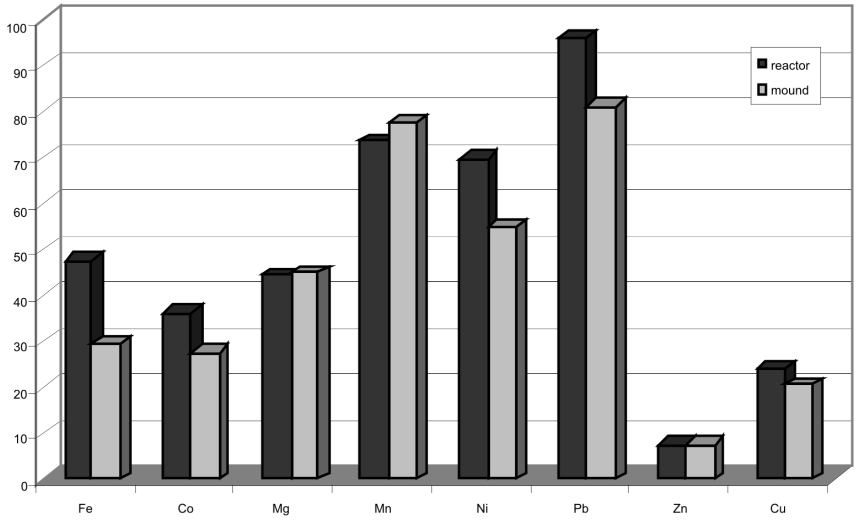

Fig. 2. Comparison of summary efficiency of metal extraction after leaching conducted in reactor and mound [7]

Thinking about the natural environment, the bingsteads are objects of reclamation, which is hardened because of their toxicity caused by the presence of large amounts of sulfur compounds, as well by aluminum, titanium, manganese ones and traces of arsenic, antimony, selenium and even mercury. It is probable that application of bacterial leaching to utilization of bingsteads surrounded open pit mine may cause lowering of sulfur compounds amounts with simultaneous leaching of less or more toxic metals.

The potential metalliferous material in bacterial leaching process may be also wastes produced by brown coal processing, as well ash and slag in power plants. The significant contents of toxic, sulfide and oxide metal compounds, as well the eventual presence of actinogenic elements like K_{40}, Ra_{226} and Th_{228} makes impossible the application of these materials, i.a. as fillers in construction engineering.

The eventual treatment of ash and slag by bacterial leaching might lower the amount of toxic and actiongenic compounds, making their application more practical. It can be said that utilization of mantle rock, ash and slag by bacterial leaching may simplify the reclamation of open pit area and become non-conventional way to recover precious metals being part of these materials [9].

Certain investigations over the possibility of bacterial leaching of mentioned wastes were already done . It was stated that the possibility of conduct bacterial leaching process of coal wastes, together with simultaneous leaching of 40–67% Ti, 45–77% Be, 70–89% Cu, 54–84% Mn, 78–84% As, 48–60% V and 62–83% Ga is real. It was proved that the utilization of wastes after brown coal processing with simultaneous treating them as metalliferous materials in bacterial leaching process is possible [9].

The results of this research are presented in Table 3.

TABLE 3

Contents of metals in mantle rock, ash and slag and efficiency of their bioextraction of leached metal [9]

Metal	Cover		Ash		Slug	
	content in material, [g/kg]	leaching efficiency, [%]	content in material, [g/kg]	leaching efficiency, [%]	content in material, [g/kg]	leaching efficiency, [%]
Ti	9,84	67,0	9,12	53,2	8,58	40,8
Cu	0,024	89,2	0,067	63,8	0,043	69,4
Mn	0,125	83,8	0,20	61,2	0,45	51,3
V	0,12	59,6	0,147	51,0	0,19	48,3
As	0,09	84,1	0,10	52,0	0,15	78,0
Be	0,02	77,0	0,013	62,2	0,016	44,7
Ga	0,006	82,8	0,005	63,2	0,003	62,0

The possibility of extraction of titanium only from mantle rock, ash and slag was researched too — after brown coal processing in process of bacterial leaching. The leaching of the mentioned materials was conducted in acid environment with co-operation of bacteria from the sort *Acidithiobacillus* and *Siderocapsa*. In process of mantle rock leaching in presence of bacteria from the sort *Acidithiobacillus*, 67% Ti were leached while during ash and slag leaching these amounts were 55% and 48%, respectively. The results of conducted research proved also the fact that the wastes slightly influence on bacteria activity [16].

5. Biological coal sulfide removal

The microbiological coal sulfide removal processes are highly connected with metal bioextraction, which is brown and hard coal bioleaching. They may be promising alternative in comparison with other technologies, which purpose is to lower sulfur dioxide emission to atmosphere which can work simultaneously with application of the same microorganisms.

This issue is a matter of research of many scientific centers in Poland and other countries. The very good effects are given. For example, the investigation conducted by J. Farbiszewską-Bajer and T. Farbiszewską indicate the possibility of bioextraction of 87% S contained in brown coal to solution during 32 days process occurring by the temperature of 30°C. [8].

6. Conclusions

The pollution of natural environment by toxic substances, including heavy metals, is highly hazardous for plants, animals and human beings. The microorganisms isolated from wastes and polluted biotic communities feature very often by high resistance to organic xenobiotics and toxic metals. This proves that they adapt easily to circumstances and they can be practically used to removal and/or recovery of heavy metals. Some of these processes as metals bioleaching from ores are being applied in industrial scale for many years. The biotechnological potential of many other processes as, i.a. biosorption with application of microorganic biomass was tested only in laboratory scale or small industrial scale. The main factor limiting application of microbiological method in larger scale are economic issues.

The good chance for commercializing of biological processes of heavy metals removal is, i.a. development and searching for new seedlings or sorts of microorganisms, application of economic beds for their proliferation and wastes biomass originating from other biotechnological processes. These issues are the subject of many researches published in recent years, what allow thinking that microorganisms will be applied in larger scale to remove and recover heavy metals [16].

In microbiological laboratory of Department of Mineral Processing and Environmental Protection, Faculty of Mining and Geoengineering, University of Science and Technology AGH, the researches over the microorganisms application to copper bioleaching from flotation and other types of wastes are being conducted from a long time. Furthermore, the investigation of uranium bioleaching from native uraniferous schists was conducted too giving good yields (about 80% in both, copper and uranium, cases). Also, the works over water and air environment, as well hard coal sulfide removal by means of biological methods give good results.

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