MICROBIAL REMOVAL OF HEAVY METALS FROM ACTIVATED SLUDGE FOR PRODUCING A HIGH-QUALITY COMPOST

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ABSTRACT. Activated sludge contaminated with heavy metals (mainly iron but also several toxic non-ferrous metals) was subjected to pretreatment for removing these metals making the pretreated sludge suitable for producing compost for soil remediation and agriculture. The most efficient removal of the heavy metals was achieved by leaching the sludge with mixed cultures of acidophilic chemolithotrophic bacteria at pH of about 1.7 – 1.9. Such leaching, however, was connected also with the considerable removal of some essential agrobiological elements (N, P, K) from the sludge. The leaching by mixed cultures of chemolihotrophic and heterotrophic microorganisms at pH in the range of about 2 – 3 proceeded at slightly lower rates but removed also considerable portions of the heavy metals and relatively small portions of the agrobiological essential elements. Such activated sludges were very suitable for preparing a high-quality compost.

Key words: Activated sludge, Heavy metals, Bioleaching

МИКРОБНО ОТСТРАНЯВАНЕ НА ТЕЖКИ МЕТАЛИ ОТ АКТИВНА УТАЙКА ЗА ПОЛУЧАВАНЕ НА ВИСОКОКАЧЕСТВЕН КОМПОСТ

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РЕЗЮМЕ. Активна утайка, замърсена с тежки метали (главно желязо, но също и редица токсични цветни метали), беше подложена на предварително третиране за отстраняване на тези метали, правейки такава утайка подходяща за получаване на компост за възстановяване на почви и земеделие. Най-ефикасното отстраняване на тежките метали беше постигнато чрез излугване на утайката със смесени култиури на ацидофилни хемолитотрофни бактерии при pH около 1.7 – 1.9. Такова излугване обаче беше свързано също със значително отстраняване на някои съществени агробиологични елементи (N, P, K) от утайката. Излугването чрез смесени култули на хемолитотрофни и хетеротрофни микроорганизми при pH около 2 – 3 протичаше при малко по-ниски скорости, но също отстраняваше значителни части от тежките метали и сравнително малки части от агробиологично съществените елементи. Такива активни утайки бяха много подходящи за приготвяне на висококачествен компост.

Ключови думи: Активна утайка, Тежки метали, Биологично излугване

Introduction

The recultivation of dumps and heaps of mining wastes, as well as of soils contaminated with heavy metals, radionuclides, toxic elements such as arsenic, and different organic contaminants (polyaromatic hydrocarbons, oil products, chlororganic pesticides, etc) is connected with the application of large amounts of biodegradable non-toxic organic compounds. Some of the above-mentioned contaminants, mainly of the inorganic type, can be removed by leaching with different chemolithotrophic bacteria. Such way of treatment in several cases is connected with the subsequent utilization of the removed components, mainly non-ferrous and precious metals, and uranium. Huge commercial-scale operations for in situ, dump, heap and reactor leaching of uranium and some non-ferrous metals (mainly copper) from low-grade ores and mining wastes since a long time are one of the most efficient and economically attractive way for treatment of toxic wastes (Spasova, 2009; Groudeva et al., 2010; Gentina and Acevedo, 2013).

The removal of organic contaminants in most cases is connected with their full or partial degradation to relatively non-toxic components or with their isolation from the environment.

At the same time, the cleaned organic matter can be used for different purposes, mainly as a source of energy and useful nutrients containing assimilable forms of nitrogen, phosphorus and other elements for microorganisms, plants, animals and in most cases indirectly – for the people.

The microbial treatment of activated sludge from the municipal operations for wastewater cleanup is one of the most typical examples for utilization of the rich-in-organic matter wastes as sources of assimilable organic compounds for the bioremediation of heavily polluted ecosystems, as well as sources of valuable metals (Bias et al., 1993; Chan et al., 2003; Groudev et al., 2013; Nicolova et al., 2011, 1012; Pathak et al., 2009).

This paper contains some data on the extraction of heavy metals from an activated sludge from the municipal water treatment operation in Plovdiv by means of biological leaching. The sludge cleaned in this way was used for preparation of compost intended for the recultivation of heaps consisting of toxic mining wastes.

Materials and Methods

Data about the chemical composition, particle size distribution and some essential physicochemical properties of the sludge are shown in Table 1.

The fresh anaerobically digested sludge contained high concentrations of microorganisms, mainly of heterotrophic anaerobic bacteria (Table 2).

Table 1.
Some essential data about the anaerobically digested sludge

Parameters	Values	Parameters	Values
Solids, %	5.3	Net neutralization potential, kg $CaCO_3$/t	67.7
pH(H_2O)	7.1	Content of heavy metals, mg/kg dry sludge:	
Eh, mV	-244	Cu	2840
Particle size, %:		Zn	264
>0.25 mm	3.0	Cd	350
0.25 – 0.05 mm	61.1	Pb	590
0.05 – 0.01 mm	4.1	Ni	590
<0.01mm	14.0	Cr	3704
Chemical composition of the dry sludge:		Mn	941
Ash content, %	57.2	Fe	27351
Organic content, %	42.8	Contents of organic pollutants:	
Organic carbon, %	21.7	Polyaromatic hydrocarbons, %	3.2
Total nitrogen, %	4.20	Oil products, %	1.9
Total phosphorus, %	2.32	Chlororganic pesticides, %	<0.5
Total sulphur, %	1.04		
Sulphidic sulphur, %	0.32		
Carbonates, %	4.64		

Table 2.
Microorganisms in the anaerobically digested activated sludge

Microorganisms	Cells/ml	Biological pollutants	Cells/100 ml
Heterotrophic anaerobic bacteria	2.10^8	Coli bacteria (*Escherichia coli, Klebsiella spp.*)	140
Sulphate-reducing bacteria	8.10^6	*Streptococcus spp. (S. faecalis, S. viridis)*	77
Denitrifying bacteria	1.10^7	Spore-forming anaerobic bacteria – *Clostridium spp. (C. perfringens, C. difficle, C. acetobutilicum, C. butiricum)*	23
Fermenting bacteria	8.10^6		
Thiobacillus denitrificans	4.10^3		
Chemolithotrophic aerobic bacteria	5.10^2		
Fungi	2.10^2	Eggs of helminths	86

The amount of chemolithotrophic bacteria was low, with the anaerobic *Thiobacillus denitrificans* as the prevalent microorganism in this group. Some pathogenic microorganisms were also present (mainly Escherichia coli and species from the genera *Klebsiella, Streptococcus* and *Clostridium*) but in relatively low concentrations. The preliminary experiments revealed that the removal of the heavy metals from the sludge proceeded at higher rates under aerobic conditions at acidic pH (< 3.0). It was found that the treatment under such conditions eliminated most of the pathogenic microorganisms. However, some spores of the pathogenic bacteria of the genus Clostridium were still viable after the treatment. For that reason, the sludge was subjected to prior autoclaving (at 121 ^oC for 30 min) to eliminate all biological pollutants. It must be noted that the chemical composition and the distribution of the heavy metals into the different mobility fractions were changed only to a negligible extent as a result of the autoclaving. The largest portion of the non-ferrous metals were present in the relevant carbonate and oxidizable mobility fractions in the form of carbonates and secondary sulphides.

The bioleaching of the pretreated sludge for removing the heavy metals was carried out by means of four different microbial cultures to compare their efficiency in this respect.

The first culture consisted of the mesophilic chemolithotrophic bacteria of the species *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*. The optimum growth and activity of this culture were at 35^{o}C. The second culture consisted of the moderate chemolithotrophic bacteria of the species Sulfobacillus thermosulphidooxidans and *Acidthiobacillus caldus* with optimum growth and activity at 55^{o}C. The third culture consisted of the facultative chemolithotrophs *Thiobacillus novellus* and *Thiobacillus intermedius*, together with some heterotrophs of the genera *Pseudomonas* and *Aeromonas*. The optimum growth and activity of this culture were at 37^{o}C at pH values within the range of 4.5 – 7.0. The fourth culture consisted of *Thiobacillus neapolitanus, T. acidophilus* and heterotrophs related to the genera *Acidiphilium* and *Pseudomonas*. The optimum growth and activity of this culture were at 37°C at pH values from 2.5 to 3.5.

These cultures were adapted to the sludge by consecutive transfers of late-log-phase cultures to sludge suspensions with increasing pulp densities.

The bioleaching was carried out in glass buffled reactors with mechanical stirring containing 500 ml leach solution, i.e. an additional nutrient medium for the microorganisms, with 1.0 g/l $(NH_4)_2SO_4$, 0.5 g/l K_2HPO_4, 0.5 g/l $MgSO_4.7H_2O$, and 0.5 g/l KCl, inoculated by one of the above-mentioned cultures, to an initial bacterial concentration of about 5.10^7 cells/ml and different concentrations of sludge solids (from 5 to 20 % on dry weight). The pH of the leach suspensions was maintained at the values shown in the different tables by addition of sulphuric acid, and distilled water was added to compensate the losses due to evaporation. The leaching was carried out at different temperatures (usually at 35 and 55°C) for 15 days. Samples were withdrawn from the reactors to determine the concentrations of dissolved metals using inductively coupled plasma spectrometry and atomic absorption spectrometry.

Removal of heavy metals from the activated sludge was tested also by means of chemical leaching. Ammonium acetate, hydrochloric and sulphuric acids were used as leachants at different pH values.(from 2.5 to 4.8).

The sludge obtained after the removal of the biological pollutants (mainly by the autoclaving) and considerable portions of the heavy metals (mainly by the bioleaching) was used for preparation of a high-quality compost. The mixture subjected to composting contained, apart from the pretreated sludge, also plant (mainly leaf) biomass, animal manure and even suitable soil (as a source of specific microorganisms intended to be essential members of the future recultivated soil). The composting was carried out in lysimeters and was characterized by the typical two phases – the aerobic (with duration of about 18 – 20 days and at a temperature gradually increasing to about 65 – 70°C) and the anaerobic (with duration of about 30 days with a temperature gradually decreasing to about 25 – 30°C).

The sludge obtained after the removal of the biological pollutants (mainly by the autoclaving) and of the heavy metals (mainly by the bioleaching) was used for preparing a high-quality compost. The mixture subjected to composting contained, apart from the pretreated sludge, also plant (mainly leaf) biomass, animal manure and even suitable soil (as a source of specific microorganisms intended to be essential members of the future recultivated soil). The composting was carried out in lysimeters and was characterized by the typical two phases – the aerobic (with duration of about 15 – 20 days and at a temperature gradually increasing from the initial 15 – 20°C to about 65 – 70°C under enhanced aeration) and the anaerobic (with duration of about 30 – 35 days at a temperature gradually decreasing to about 25 – 30°C).

Results and Discussion

The bioleaching of heavy metals from the sludge was quite efficient (Table 3). The results obtained by the mixed culture of mesophilic chemolithotrophic bacteria at 35°C, and by the mixed culture of moderate chemolithotrophic bacteria at 50°C were quite similar. This was connected with the ability of some of these bacteria, mainly of *At. ferrooxidans* at 35°C and of *Sulfobacillus thermosulfidooxidans* at 50°C to oxidize efficiently the sulphides present in the sludge. The analyses of the sulphidic sulphur in the solid residues after the leaching of the sludge by these two cultures revealed that more than 85% from the initial content of sulphides was oxidized to the soluble sulphatic form. For that reason, most of the heavy metals were dissolved in the leach solutions as the relevant sulphates. However, lead was solubilized as complexes with some of the dissolved organic components of the sludge, mainly with organic acids and exopolysaccharides. Relatively small portions of the other dissolved heavy metals were also present in the forms of such complexes.

Table 3.
Leaching of heavy metals from the activated sludge by means of acidophilic chemolithotrophic bacteria

Heavy metals	Before leaching, mg/kg	After leaching by chemolithotrophic bacteria			
		At 35°C by mesophilic chemolithotrophs		At 50°C by moderate thermophiles	
		mg/kg	Extraction, %	mg/kg	Extraction, %
Cu	2840	190	93.31	152	94.65
Zn	264	34	87.12	38	85.61
Cd	350	23	93.43	21	94.00
Pb	590	280	52.54	235	60.17
Ni	590	41	93.05	51	91.36
Cr	3704	820	77.86	790	78.67
Mn	941	82	91.28	75	92.30
Fe	27351	6417	76.54	6840	75.00

Duration of leaching – 15 days; pH of the leach suspensions was maintained at 1.7 – 1.9 by means of sulphuric acid. Initial pulp density 10%.

Sulphuric acid was generated during the leaching as a result of the bacterial oxidation of sulphides. However, the leaching was possible only by addition of sulphuric acid from outside, due to the positive net neutralization potential of the sludge. Ferrous ions were also generated during the leaching as a result of the solubilization of the iron present in the sludge (mainly as FeS and other sulphides but also as iron present in carbonates, hydroxides and in some organic components of the sludge). Some of the chemolithotrophic bacteria (*At. ferrooxidans. L. ferrooxidans* and *S. thermosulfidooxidans*) oxidized the ferrous ions to the ferric state and in this way produced in situ an efficient oxidizer of the sulphides. It must be noted that the addition of soluble ferric iron from outside accelerated the oxidation of sulphides.

The bioleaching by the mixed culture consisting of the facultative chemolithotrophs *T. novellus* and *T. intermedius* and some heterotrophic bacteria at the slightly acidic pH (from (4.5 to 6.0) was not efficient and the extractions of the heavy metals were relatively low (within the range of about 15 – 25% for the period of 15 days). The prolongation of the leaching period to 45 days resulted in the increase of the metal extraction to about 32 – 41%. The analyses of the solid residues after the leaching revealed that the residual contents of the heavy metals were present mainly in the relevant oxidizable mobility fractions, mainly in the sulphides. It is known that the facultative chemolithotrophs present in this culture are not able to oxidize sulphides but only S^0 and some soluble sulphur-bearing compounds such as thiosulphate.

The bioleaching of the sludge by the acidophilic mixed culture containing the chemolithtrophs *T. neopolitanus* and *T. acidophilus* and the heterotrophs of the genera *Acidiphilium* and *Pseudomonas* at pH 2.5 – 3.5 was also less efficient than that by the cultures containing bacteria able to oxidize directly the sulphides and ferrous iron. The extractions achieved by this culture were to some extent close to those achieved by the acidophilic heterotrophs at pH 2.5 but were obtained for a much shorter leaching time (15 days).

The bioleaching of the sludge by means of mixed cultures of acidophilic heterotrophic microorganisms strongly depended on the pH of the leach system (Table 4). The best results were achieved by means of the culture growing under the highly acidic conditions (pH 2.5) maintained by addition of sulphuric acid from outside. However, these results were considerably lower than the results achieved by the cultures of the chemolithtrophic bacteria for a shorter period of leaching (Table 3). The leaching by the heterotrophs was connected also with a more essential degradation of the organic matter of the sludge and a much higher consumption of the biodegradable organic compounds in comparison with the relevant values obtained as a result of the bioleaching with chemolithotrophic bacteria. These changes resulted in a more considerable decrease of the nutrient potential of the sludge pretreated by means of heterotrophic microorganisms.

The chemical leaching of the heavy metals from the sludge by means of different solutions at different pH values was not efficient (Table 5). Such solutions are usually used for determination of the easily soluble mobile forms of metals in the activated sludge.

Table 4.
Leaching of heavy metals from activated sludge by means of acidophilic heterotrophic bacteria

Heavy metals	Before leaching, mg/kg	After leaching by heterotrophic bacteria					
		At pH 4.5		At pH 3.5		At pH 2.5	
		mg/kg	Extraction, %	mg/kg	Extraction, %	mg/kg	Extraction, %
Cu	2840	2473	12.92	1540	45.78	1205	57.57
Zn	264	184	30.30	142	46.21	70	73.49
Cd	350	224	36.00	82	76.57	67	80.86
Pb	590	509	13.73	271	54.07	257	56.44
Ni	590	473	19.83	291	50.68	140	76.28
Cr	3704	3251	12.24	2120	43.77	1470	54.78
Mn	941	790	16.05	512	45.59	280	70.25
Fe	27351	2415	11.70	18254	33.26	14261	47.86

Duration of leaching – 45 days; pH of the leach suspensions was maintained at 1.7 – 1.9 by means of sulphuric acid. Initial pulp density – 10%. Temperature – 35 °C

Table 5.
Chemical leaching of heavy metals from the activated sludge

Heavy metals	Before leaching, mg/kg	Leach solutions					
		CH_3COONH_4 (pH 4.8)		CH_3COONH_4 + HCl (pH 3.0)		H_2SO_4 (pH 2.5)	
		Residual content, mg/kg	Extraction, %	mg/kg	Extraction, %	mg/kg	Extraction, %
Cu	2840	2762	2.75	2105	25.88	2059	27.50
Zn	264	221	16.40	214	18.94	202	23.45
Cd	350	218	37.82	161	54.00	150	57.20
Pb	590	573	2.80	505	14.41	515	12.74
Ni	590	506	14.25	404	31.53	382	35.20
Cr	3704	3634	1.90	3252	12.20	3115	15.90
Mn	941	898	4,51	842	10.52	838	10.90
Fe	27351	26771	2.12	23891	12.65	24156	11.68

Duration of the leaching 15 days, at 35 °C. Initial pulp density of 10 %.

The pretreated sludge was an essential component in the mixture containing also plant (leaf) biomass, animal manure and cinnamonic forest soil, and subjected to the process of composting. The compost obtained on the basis of this mixture contained 52 – 55% dry substance, with 55 – 60% organic content (with 28 – 31% organic carbon) and 40 – 45% ash content, 1.7 – 2.1 % total nitrogen, 0.8 – 1.0 % phosphorus and 1.5 – 1.8 % potassium, at pH of 6.8 – 7.1.

The compost contained also the other essential macroelements (S, Ca, Mg) and all essential microelements (Fe, Mn, Cu, Zn, Mo, Co, B) in the relevant acceptable concentrations. The addition of the compost to different soils in ratios in accordance with the chemical composition of the relevant soil had a highly positive effect on the growth of different plants.

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