BACTERIAL LEACHING OF ACTIVATED SLUDGE FOR RECOVERY OF NON-FERROUS METALS AND FERTILIZER FOR AGRICULTURE

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ABSTRACT. Samples of anaerobically digested sludge from operations for municipal wastewater treatment in Bulgaria were characterized by high contents of some valuable heavy metals (mainly copper and zinc) but also of some organochemical pollutants (mainly polyaromatic hydrocarbons and oil products) and some pathogenic microorganisms and eggs of helmiths. The objective of this study was not only to remove the toxic pollutants and to make the sludge acceptable for use as an amendment in agriculture but also to use it as a source for producing copper and zinc. The processing of sludge initially by autoclaving at 120 °C for 15 min and then by bioleaching with a mixed culture of moderately thermophilic chemolithotrophic bacteria at 55 °C for 7 days resulted in removal of all chemical and biological pollutants and in high extraction (>98 %) of copper and zinc. These metals were selectively recovered from the pregnant solution by means of solvent extraction plus electrowinning. The residual sludge contained nitrogen, phosphorous, potassium and some essential microelements in sufficient concentrations for application in agriculture.

БАКТЕРИАЛНО ИЗЛУГВАНЕ НА АКТИВНА УТАЙКА ЗА ДОБИВ НА ЦВЕТНИ МЕТАЛИ И НА ТОР ЗА ЗЕМЕДЕЛИЕТО

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РЕЗЮМЕ. Проби от анаеробно разградена активна утайка от операции за третиране на битови отпадни води в България се характеризираха с високи съдържания на някои ценни тежки метали (главно мед и цинк), но също и на някои органохимични замърсители (главно полиароматни въглеводороди и нефтени продукти) и някои патогенни микроорганизми и яйца на хелминти. Целта на това изследване не бе само да се премахнат токсичните замърсители и да се направи утайката приемлива за използване като добавка в земеделието, но също тя да бъде използвана като източник за получаване на мед и цинк. Преработването на утайка, първоначално чрез автоклавиране при 120 °C за 15 мин и след това чрез биологично излугване със смесена култура на умерено термофилни хемолитотрофни бактерии при 55 °C за 7 дни даде като резултат отстраняването на всички химични и биологични замърсители и във високи извличания (>98 %) на мед и цинк. Тези метали бяха селективно добити от продукционния разтвор чрез течностна екстракция плюс електролиза. Остатъчната утайка съдържаше азот, фосфор, калий и някои съществени микроелементи в достатъчни концентрации за прилагане в земеделието.

Introduction

The activated sludge from the operations for municipal wastewater treatment contains organic compounds and microelements which can be used as nutrients for the microbial and plant communities in the agricultural lands (Lombard and Garcia, 1999). However, in most cases the sludge is characterized by relatively high contents of some toxic metals and organochemical pollutants, as well as of some pathogenic microorganisms and eggs of helminths. Different methods to detoxify the sludge are known and most of them are connected with the removal of the toxic metals. Bioleaching by means of iron- and sulphur-oxidizing bacteria is regarded as an efficient and cost-acceptable method with this respect (Blais et al., 1993, Coillard, 1994, Chan et al., 2003, Pathak et al., 2009). In the anaerobically designed sludge most heavy metals are present mainly in the form of the relevant secondary sulphides. Comparative experiments revealed that the bioleaching by moderately thermophilic chemolithotrophic bacteria was the most efficient way to solubilize the metals present in such sludge (Nicolova et al., 2011).

The present paper contains data about the microbial removal of heavy metals from anaerobically digested sludge containing some valuable non-ferrous metals (mainly copper and zinc) in relatively very high concentrations which makes such type and attractive source for producing these meals. At the same time, after the bioleaching the residual sludge contains no toxic chemical and biological pollutants and bioassimilable form of nitrogen, phosphorous and potassium and some essential microelements were still present in sufficient concentrations making this sludge suitable for application as a fertilizer in agriculture.

Materials and Methods

Data about the chemical composition, particle size distribution and some essentials physiochemical properties of the sludge used in this study are shown on Table 1.

The fresh anaerobically digested sludge contained bacteria (about 10^8 cells/ml). Some heterotrophic anaerobic bacteria (mainly facultative aerobes) were also present (about 10^4 – 10^5 cells/ml). The amount of chemolithotrophic bacteria was low. The anaerobic *Thiobacillus denitrificans* was the prevalent microorganism in this group (about 10^3 cells/ml). Some fungi were also present in low concentrations (<10^2 cells/ml).

However, the fresh anaerobically digested sludge contained also some pathogenic microorganisms and eggs of helminths which made this sludge not suitable for application in agriculture. *Escherhia coli* was the prevalent species in this group (in some samples the concentrations of these bacteria was in the range of about 100 – 150 cells/100 ml) but species related to the genus *Klebsiella* were also found, although in lower concentrations (about 20 – 40 cells/100 ml). Representatives of the genus *Streptococcus* (*S. faecalis, S. viridis*) and different sporeforming anaerobic bacteria of the genus *Clostridium* (*C. perfringes, C. difficile, C. acetobutilicum, C. butiricum*) were also found but in lower concentrations. The number of the eggs of helminths in the sludge samples was in the range of 51 – 99/100 ml.

Table 1.
Some essential data about the anaerobically digested sludge

Parameters	Value	Parameters	Value
Solids, %	5.9	Total sulphur, %	1.22
pH (H_2O)	7.1	Sulphuric sulphur, %	1.12
Eh, mV	-244	Carbonates, %	2.84
Particle size, %:		Net neutralization potential, kg $CaCO_3$/t	+ 13.1
> 0.25 mm	2.3	Content of heavy metals (mg/kg dry sludge):	
0.25 – 0.05 mm	43.7		
0.05 – 0.01 mm	37.0	Cu	2840
< 0.01 mm	17.0	Zn	5140
Chemical composition of the sludge:		Ni	480
		Co	23
Ash content, %	59.0	Fe	21520
Organic content,%	41.0	Mn	910
Organic carbon, %	22.1	Cr	1070
Total nitrogen, %	4.10	Content of organic pollutants:	
P(total), %	2.24	Polyaromatic hydrocarbons, %	2.8
K, %	0.95		
Sodium, %	1.07	Oil products, %	1.9
Magnesium, %	2.12	Chlororganic pesticides, %	< 0.5
Calcium, %	1.14		

The preliminary experiments revealed that the removal of the metals from the sludge proceeded at highest rates under aerobic conditions and acidic pH. It was found that the treatment under such conditions eliminated most of the undesirable biological pollutants.

However, some spores of the pathogenic microorganisms related to the genus Clostridium were still viable after the treatment. For that reason, the sludge was subjected t prior autoclaving (at 121^{o}C, 1.2 atm, for 15 min) to kill the biological pollutants. The autoclaving was efficient in this respect and, at the same time, the chemical composition and the distribution of the heavy metals into the different mobility fractions were changed only to a negligible extent. The oxidizable mobility fraction of the Cu and Zn (consisting mainly of the relevant sulphides and, in smaller concentrations, of metals connected in the pretreated sludge. Smaller portions of these metals were present as the easily leachable mobility fractions (the exchangeable and carbonate) and in the educible mobility fraction (as metals encapsulated by iron hydroxides).

The bioleaching of the pretreated sludge was carried out by means of moderately thermophilic culture of acidophilic chemolithotrophic bacteria consisting of the species *Acidithiobacillus caldus* and two species related to the genera *Sulfobacillus*. The culture was adapted to the pretreated sludge by consecutive transfers of late-log-phase cultures to sludge suspensions with increasing pulp densities, at 55 ^{o}C, with a pH adjusted to 1.7 – 1.8 by sulphuric acid and containing initially 5 g/l Fe^{2+} ions added as $FeSO_4$.

The bioleaching was carried out in 500 ml Erlenmayer flasks containing 180 ml sludge (with 20 % solids; some variants were amended with 5 g/l Fe^{2+}) inoculated with 20 ml of active late-log-phase microbial culture adapted to the sludge. The cultivation was carried out on a rotary shaker (180 rpm) at 55 ^{o}C for 7 days. The pH of the suspension was added to compensate the losses due to evaporation. Samples were withdrawn from the flasks to determine the concentrations of dissolved metals using inductively coupled plasma spectrometry and atomic absorption spectrometry.

Copper and zinc were recovered from the pregnant solutions after bioleaching by means of solvent extraction plus electoriwinning. The solvent extraction of copper was carried out by the reagent LIX984N dissolved in kerosene. The solvent extraction of zinc was carried out by a mixture of dithiophoshinic acid and di-2-ethylhexylphosphoric acid. The electrolysis was carried out in a cell containing two lead anodes and a stainless steel cathode.

The sludge initially subjected to bioleaching then was used in experiments for assessing its effect as a soil fertilizer. These experiments were carried out in plastic vessels containing 3 kg dry weight each of a leached cinnamonic forest soil mixed with different amounts of pretreated sludge to obtain sludge concentrations from 1 to 10 % dry weight in the soil. Lucerne (*Medicago sativa*) seeds were sowed in the vessels at a shallow depth (about 10 – 15 mm) from the soil surface. The cultivation was carried out in a green house at 22 – 23 ^{o}C and a light period of 12 hours per day for 6 months. Here harvesting procedures to recover the plant biomass from the vessels were carried out after the second, fourth and sixth months.

Control experiments with soil without sludge amendments as well as experiments with soil without sludge but containing 0.20 % nitrogen, 0.15 % phosphorous and 0.10 % potassium added by a complex mineral fertilizer were also set up.

For obtaining the relatively large amounts of sludge needed for the vegetative experiments, the bioleaching of sludge was carried out in 5 l agitated bioreactors with 20 % (200 g/l) pulp density.

The isolation, identification and enumeration of microorganisms during this study were carried by the methods described elsewhere (Karavaiko et al., 1988; Hallberg and Johnson, 2001).

Result and Discussion

The bioleaching by the mixed culture of moderately thermophilic chemolithotrophic bacteria at 55 ^{o}C was very efficient (Table 2). The concentrations of copper and zinc at the end of leaching reached 556.6 and 1010.5 mg/l, respectively.

The concentration of iron was higher (2384.4 mg/l) and most of it was present in the ferric state. Most of the heavy metals were present in the leach solution as the relevant sulphate forms.

However, lead was solubilized as complexes with some of the dissolved organic components of the sludge, mainly with organic acids and exopolysaccharides. Such complexes but in much lower concentrations were established also for the other dissolved heavy metals.

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.

Furthermore, the initial acidification of the pulp to 1.9 facilitated the oxidizing activity and growth of the acidophilic bacteria which oxidized the ferrous ions to ferric state and in this way produced in situ an efficient oxidizer of the sulphides. It must be noted that ferrous ions were generated as a result of the solubilization of he iron present in the sludge (mainly as FeS but also as iron present in carbonates or connected with some organic components of he sludge). However, the addition of soluble ferric iron from outside at the start of he leaching accelerated this process.

Table 2.
Data about the bioleaching of heavy metals from anaerobically activated sludge by a mixed culture of acidophilic moderately thermophilic chemolithotrophic bacteria

Element	Content, mg/kg dry weight		Extraction, %	Permissible levels for use in agriculture, mg/kg dry
	Before leaching	After leaching		
Cu	2840	56.8	98.0	1500
Zn	5140	87.4	98.3	3000
Ni	360	42.1	88.3	300
Co	23	0.23	99.0	300
Cd	260	3.10	98.8	30
Pb	120	93.7	21.9	1000
Cr	850	173.4	79.6	500
Fe	21520	9598	55.4	-
Mn	910	143.8	84.2	-

The extraction of the other heavy metals present in the sludge was also efficient, with exception of the lead which was not soluble as $PbSO_4$.

The relatively high concentrations of the dissolved copper and zinc in the pregnant solution after leaching were suitable for obtaining these *valuable non-ferrous metals as the relevant cathode forms with a ver*y high purity (> 99.7 %) by means of solvent extraction plus electrowinning. At the same time, the residual concentrations of these metals present in the sludge were decreased below the relevant permissible levels for the sludge intended for application in agriculture (Table 2). Furthermore, the residual pretreated sludge contained no toxic chemical and biological pollutants and the bioassimilable forms of C, N, P, K and most biologically essential elements microelements were still present in sufficient concentrations making this sludge suitable for such application (Tab. 2 and 3).

Table 3.
Data about the extraction of some elements essential for the agriculture during the bioleaching of the anaerobically activated sludge by he mixed culture of acidophilic moderately thermophilic chemolithotrophic bacteria

Element	Content, mg/kg dry weight		Extraction, %
	Before leaching	After leaching	
N	41 000	20 800	49.27
P	22 400	8 200	63.39
K	6 800	2 800	58.82
Na	10 700	3 200	70.09
Ca	11 400	2 300	79.83
Mg	21 200	5 300	75.00

The experiments for assessing the effect of the pretreated sludge as a fertilizer considerably increased the contents to nitrogen and phosphorous in the leached cinnamonic forest soil used for cultivation of the lucerne (Table 4). At the same time, the content of the toxic heavy metals, even in the soil amended by 10 % sludge, were much lower than the relevant permissible levels (Table 4). The addition of pretreated sludge to the soil steadily increased the production of plant biomass, and addition of 10 % sludge was more efficient even in comparison with the data obtained by adding the complex mineral fertilizer to the soil (Table 5).

Table 4.
Contents of biologically essential elements and heavy metals in the mixtures of soil and retreated sludge used for the cultivation of lucerne (Medicago sativa)

Source of elements	Content in the soil (mg/kg dry soil)					
	N	P	K	Na	Ca	Mg
1 % sludge	208	82	28	32	23	53
2 % sludge	416	164	56	64	46	106
3 % sludge	624	246	84	96	69	159
5 % sludge	1040	410	140	160	115	265
10 % sludge	2080	820	280	320	230	530
Natural soil	1050	450	12000	7560	12800	3200

	Cu	Zn	Ni	Co	Cd	Pb	Cr
1 % sludge	1.70	2.62	1.26	0.007	0.093	2.81	5.20
2 % sludge	3.40	5.24	2.52	0.014	0.186	5.62	10.40
3 % sludge	5.10	7.86	3.78	0.021	0.279	8.43	15.60
5 % sludge	8.50	13.10	6.30	0.035	0.465	14.05	26.00
10 % sludge	17.00	26.20	12.60	0.070	0.930	28.10	52.00
Natural soil	51	71	12	2.5	1.0	18	35
Permissible level (at pH 7)	140	300	70	-	3	80	200

Table 5.
Effect of the pretreated sludge as a fertilizer at the cultivation of lucerne (Medicago sativa)

Variants	Recovery of fresh plant biomass					
	1st harvest		2nd harvest		3rd harvest	
	g/vessel	%	g/vessel	%	g/vessel	%
Control (natural soil)	8.2	100	8.8	100	9.5	100
Natural soil +:						
1 % sludge	10.2	124.4	11.1	126.1	11.8	124.2
2 % sludge	10.9	132.9	12.0	136.4	12.9	135.8
3 % sludge	12.2	148.8	14.0	159.1	15.2	160.0
5 % sludge	14.0	170.7	16.1	182.9	17.7	186.3
10 % sludge	16.7	203.6	19.4	220.4	20.8	218.9
Mineral fertilizer	14.9	181.7	17.6	200.0	18.1	190.5

Note: The values shown in the table for each of the variants are the average values from 5 vessels.

Conclusion

The data from this study revealed that the moderately thermophilic chemolithotrophic bacteria were able to solubilize efficiently considerable portions of the heavy metals present in the pretreated by autoclaving anaerobically digested sludge. The addition of iron ions and sulphuric acid from outside at the start of the leaching facilitated the growth of bacteria and the solubilization of metals. The pregnant solutions after bioleaching contained copper and zinc in concentrations which were suitable to recover these valuable non-ferrous metals as the relevant cathode forms with high purity (> 99.7 %) by means of solvent extraction plus electrowinning. At the same time, the residual concentrations of all heavy metals present in the sludge were decreased below the relevant permissible levels. The sludge after bioleaching contained no toxic chemical and biological pollutants and he bioassimilable forms of N, P, K and the essential microelements were still present in sufficient concentrations making this sludge suitable to be used as a fertilizer in agriculture. This effect was demonstrated by experiments in which the addition of treated sludge to a leached cinnamonic forest sol increased considerably the harvest of lucerne.

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