TREATMENT OF ACTIVATED SLUDGE FOR UTILIZATION IN AGRICULTURE AND FOR RECOVERY OF NON-FERROUS METALS

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ABSTRACT

A sample of anaerobically digested sludge from an operation for municipal wastewater treatment in Bulgaria was characterized by relatively high contents of some heavy metals (mainly of iron, zinc, copper, manganese and chromium) and of some organochemical pollutants (mainly polyaromatic hydrocarbons and oil products).

Some microorganisms which are indicators for the faecal pollution of the waters being treated such as *Escherihia coli, Enterococcus faecalis* and *Streptococcus faecalis*, were found in the samples, as well as some pathogenic microorganisms (related to the genus *Clostridium*) and eggs of helminths.

The objective of this study was not only to remove the toxic pollutants from the activated sludge in connection with the relevant sanitary requirements but also to use the pretreated sludge as an amendment in agriculture and, having in mind the contents and the current prices of some heavy metals present in the activated sludge before treatment, to use it as a source of these metals. Copper and zinc were the most promising in this respect.

Different treatment techniques were tested to achieve the objective mentioned above. The best results were obtained with sludge sample initially subjected to autoclaving and then leached by a mixed culture of moderately thermophilic chemolithotrophic bacteria. The addition of iron ions (mainly in the ferric state) and sulphuric acid from outside at the start of the leaching facilitated the growth of bacteria and the solubilization of metals. Leaching at 50 ^{o}C and an initial solid content of 20 % in the suspension resulted in high extractions of copper (98.8 %) and zinc (95.7 %) within 120 hours. The dissolved metals were selectively recovered from the pregnant leach solution by means of solvent extraction plus electrowining. The residual treated sludge contained no toxic chemical and biological pollutants in concentrations higher than the relevant permissible levels. At the same time, the bioassimilable forms of C, N, P, K and some essential microelements were still present in sufficient concentrations for utilization in agriculture.

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 (Lombardi 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 some pathogenic microorganisms and eggs of helminths. Different methods to detoxify the sludge are known and most of them are connected with removal of the toxic metals. Bioleaching by means of iron- and/or sulphur-oxidizing bacteria is regarded as an efficient and cost-acceptable method with this respect (Chan et al. 2003; Pathak et al., 2009). Such treatment is usually based on the activity of iron and/or sulphur oxidizing chemolithotrophic bacteria (Couillard, 1994; Blais et al., 1993; Sreekrishnan et al., 1993), some of which are natural inhabitants of the aerobically digested sludge. Most of these bacteria (such as *Thiothrix* and *Beggiatoa*) can oxidize sulphide and/or elemental sulphur to sulpuric acid and in this way are able to solubilize the heavy metals. However, the chemolithotroph *Thiobacillus thioparus* is probably the most active sulphur-oxidizer in the aerobically digested sludge. These bacteria grow at pH higher than 3.5 and can only act as promoters of the acidification of the sludge. Some acidophilic chemolithotrophic bacteria (such as *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans*) can further decrease the pH to values lower than 2.0 and in this way to enhance the leaching of metals. These bacteria, however, are not typical inhabitants of the aerobically digested sludge and practically are not present in the anaerobically digested sludge. In the anaerobically digested sludge most heavy metals are present mainly in the form of the relevant secondary sulphides. These sulphides are amenable to oxidation by ferric ions and this makes essential the participation of acidophilic iron-oxidizing bacteria in the solubilization of metals. It has been shown that the indigenous iron-oxidizing bacteria isolated from an anaerobically digested sludge was effective in removing Zn, Cu, Ni and Cr from the sludge at a high initial pH of 5 – 7 with the addition of 4 g/l Fe^{2+} as the energy source (Chan et al., 2003). It was concluded that the addition of iron reduced the preacidification requirement for bioleaching as well as the operational cost. On the other side, it has been shown that the extraction of some metals, e.g. Cu, by means of sulphur-oxidizing bacteria is higher than that by the iron-oxidizing bacteria.

The activated sludge, apart from the heavy metals, usually contains some organochemical and biological pollutants. Different methods to eliminate these pollutants are known but the information about their effect on the leaching of the metals is scarce. In any case, the removal of the pollutants is necessary due to the existing sanitary requirements.

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 very high concentrations which makes such type of sludge an attractive source for producing these metals.

MATERIALS AND METHODS

Data about some essential physicochemical properties of the sludge used in this study are shown in Table 1. Data about the microorganisms present in the initial sample of this anaerobically digested sludge are shown in Table 2.

Different microbial cultures were used to leach the sludge and to solubilize the heavy metals:

A mesophilic culture of acidophilic chemolithotrophic bacteria consisted of the species *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans, Leptospirillum ferrooxidans* and *Acidiphilium sp.* The culture was adapted to the sludge by consecutive transfers of late-log-phase cultures to sludge suspensions with increasing solid densities, at 37 ^{o}C, with a pH adjusted to 1.8 – 1.9 by sulphuric acid and containing initially 5 g/l Fe^{2+} ions added as $FeSO_4$.

A moderately thermophilic culture of acidophilic chemolithotrophic bacteria consisted of the species *Sulfobacillus thermosulphidooxidans* and *Acidithiobacillus caldus*. The culture was adapted to the sludge in the same way as the mesophilic culture but the temperature of the sludge suspensions was maintained at 50 ^{o}C.

Table 1. Some essential physicochemical properties of the anaerobically digested sludge

Parameters	Value	Parameters	Value
Solids, %	5.1	Carbonates, %	2.12
pH	7.16	Net neutralization potential, kg $CaCO_3$/t	6.0
Eh, mV	- 235	Contents of heavy metals (mg/kg dry sludge):	
Particle size, %:		Cu	3250
> 0.25 mm	2.8	Zn	6840
0.25 – 0.05 mm	41.5	Ni	510
0.05 – 0.01 mm	37.6	Pb	770
< 0.01 mm	18.1	Cd	280
Chemical composition of the dry sludge:		Cr	1310
Ash content, %	56.9	Fe	23540
Organic content, %	43.1	Contents of organic pollutants:	
Organic carbon, %	21.5	Polyaromatic hydrocarbons, %	2.6
Total nitrogen, %	4.22	Oil products, %	1.7
Total phosphorus, %	2.35	Chlororganic pesticides, %	< 0.5
K, %	0.71		
Total sulphur, %	1.02		
Sulphidic sulphur, %	0.95		

A culture of the heterotrophic bacteria producing peroxide and secreted exopolysaccharides consisted of two strains of the genus *Bacillus* and was adapted to the sludge by consecutive transfers in suspensions which, apart from the organic components of the sludge, contained also sucrose (20 g/l) as a source of carbon and energy and 5 g/l Fe^{2+}. The initial pH of this medium was adjusted to 4.0 but steadily decreased during the cultivation to about 2.5 – 3.0.

A strain of *Aspergillus niger* producing citric acid was adapted to high densities of sludge using a nutrient medium with sucrose (10 g/l) and NH_4NO_3 (1.5 g/l) at a initial pH of 2.5.

Table 2. Microorganisms in the anaerobically digested activated sludge

Microorganisms	Cells/ml	Biological pollutants	Cells/100 ml
Heterotrophic anaerobic bacteria	5.10^{7}	Coli bacteria (*Esherichia coli, Klebsiella spp.*)	170
Sulphate-reducing bacteria	3.10^{6}	*Streptococcus spp.* (*S. faecalis, S. viridis*)	82
Denitrifying bacteria	8.10^{6}		
Fermenting bacteria	5.10^{6}	Sporeforming anaerobic bacteria – *Clostridium spp. (C. perfringes, C. difficile, C. acetobutilicum, C. bytiricum*)	18
Ammonifying bacteria	2.10^{6}		
Heterotrophic aerobic bacteria	5.10^{5}		
Chemolithotrophic aerobic bacteria	3.10^{2}		
Thiobacillus denitrificans	8.10^{2}	Eggs of helminths	91
Fungi	1.10^{2}		

The bioleaching of the sludge was carried out in 500 ml Erlenmeyer flasks containing 160 ml sludge (with 20 % solids; some variants were amended with 5 g/l Fe^{2+} and/or 10 g/l sucrose) inoculated with 40 ml of active late-log-phase microbial culture and the pH was adjusted to the relevant values mentioned above. The cultivation was carried out on a rotary shaker (180 rpm) at 37 ^{o}C (or 50 ^{o}C only for the moderately thermophilic chemolithotrophic culture) for 7 days.

Control experiments without the inoculation of microorganisms and addition of Fe^{2+} or sucrose to the sludge were carried out at 37 and 50 ^{o}C without corrections of the pH. The changes in sludge pH and Eh were monitored every day and samples were withdrawn from the flasks to determine the concentrations of dissolved heavy metals using induced coupled plasma spectrometry and atomic absorption spectrometry.

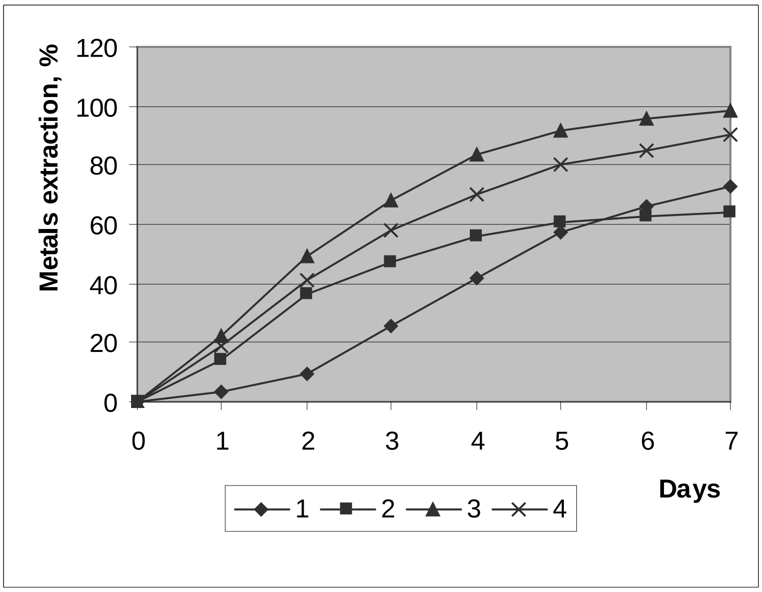
The isolation, identification and enumeration of the microorganisms were carried out by the methods described elsewhere (Karavaiko et al., 1988; Hallberg and Johnson, 2001).

RESULTS AND DISCUSSION

The fresh anaerobically digested sludge was rich in heavy metals but contained some pathogenic microorganisms and eggs of helminths which made this sludge not suitable for application in agriculture (Tables 1 and 2). Most of the heavy metals, including the copper and zinc, were present mainly in the relevant oxidizable mobility fractions, as the relevant sulphides or connected with organics. Smaller portions of these metals were present in the easily leachable mobility fractions, mainly as carbonates. The pH and the net neutralization potential of the sludge were closed to the neutral point. The indigenous microflora of this sludge consisted mainly of different anaerobic microorganisms and the number of the aerobic microorganisms was much lower (Table 2). Only few of them were able to oxidize sulphidic sulphur (present as hydrogen sulphide or as some water soluble sulphides) and S^0 under aerobic conditions. Most of these aerobic sulphur-oxidizers were related to the genera *Thiothrix* and *Beggiatoa* which are typical inhabitants of the aerobically digested sludge. *Thiobacillus thioparus* was also present but in very low concentrations (about 10 cells/ml). The facultative anaerobic denitrifying microorganism *Thiobacillus denitrificans* was the prevalent sulphur oxidizer in the anaerobically digested sludge. Iron-oxidizing bacteria, both acidophilic chemolithotrophs and heterotrophs able to grow at pH values around neutral point, were found only in some samples and were present in very low concentrations (less than 10 cells/ml).

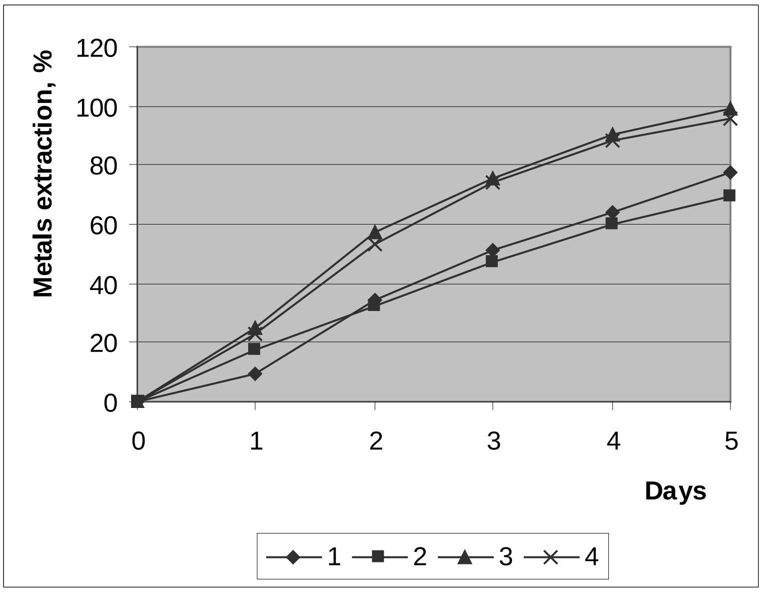
The preliminary experiments revealed that the removal of metals from the sludge proceeded at highest rates under aerobic conditions and acidic pH. It was found also that the treatment under such conditions eliminated most of the undesirable biological pollutants. However, some spores of the pathogenic microorganisms related to the genus *Clostridium* were still viable after the treatment. For that reason, the sludge was subjected to prior autoclaving (at 121 ^oC, 1.2 atm, for 15 min) to kill undesirable biological pollutants. The autoclaving was efficient in this respect and, at the same time, the distribution of the heavy metals into the different mobility fractions was changed only to a small extent. The oxidizable mobility fraction of the Cu and Zn consisting mainly of sulphides was by far the prevalent fraction of these metals in the pretreated sludge.

The bioleaching of the pretreated sludge by the mixed cultures of acidophilic chemolithotrophic bacteria at 37 and 50 ^oC was efficient, especially when Fe^{2+} ions were added from outside at the start of the leaching (Figures 1 and 2). Slightly higher extractions of metals were obtained by the moderately thermophilic chemolithtrophic bacteria but for a shorter period of leaching (Figure 2). Sulphuric acid was generated during the leaching as a result of the bacterial oxidation of sulphides. However, the initial acidification of the pH 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 towards the sulphides. It must be noted that ferrous ions were generated as a result of the solubilization of the iron present in the sludge (mainly as FeS but also as iron present in carbonate or connected with some organic components of the sludge). However, the addition of soluble ferric iron from outside at the start of the leaching accelerated this process.



1. Cu extracted without Fe added
2. Zn extracted without Fe added
3. Cu extracted in the presence of Fe^{2+} added
4. Zn extracted in the presence of Fe^{2+} added

Figure.1 Bioleaching of activated sludge by means of mesophilic chemolithotrophic bacteria at 37 °C



1. Cu extracted without Fe added
2. Zn extracted without Fe added
3. Cu extracted in the presence of Fe^{2+} added
4. Zn extracted in the presence of Fe^{2+} added

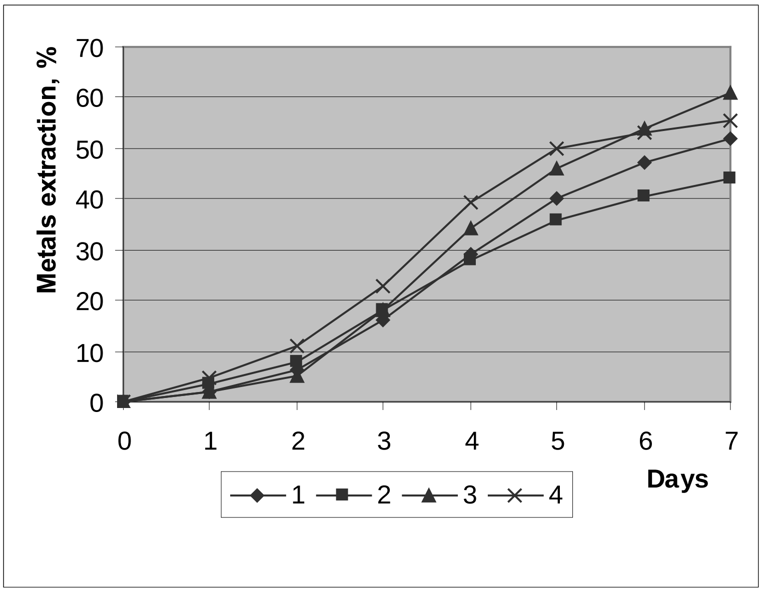
Figure 2. Bioleaching of activated sludge by means of moderaty thermophilic chemolithotrophic bacteria at 50 °C

The bioleaching by means of the acidophilic chemolithotrophic bacteria resulted in relatively good extractions of the other heavy metals present in the sludge, with the exception of the lead which was not soluble as $PbSO_4$. The residual concentrations of all these non-ferrous metals were decreased below the relevant permissible levels in the sludge intended for application in agriculture (Table 3). The addition of ferric ions instead the ferrous ions at the start of leaching (in the same concentrations, i.e. 5 g/l) also slightly decreased the leaching time.

Table 3. Data about the removal of heavy metals from the sludge by means of mesophilic chemolithotrophic bacteria

Metal	Content before treatment, mg/kg dry sludge	Content after treatment, mg/kg dry sludge	Extraction, %	Permissible level for agriculture, mg/kg
Cu	3250	748	73.0	1500
Zn	6840	1218	64.0	3000
Ni	510	208	59.2	400
Pb	770	640	16.9	1000
Cd	280	28	55.0	40
Cr	1310	590	90.0	1000

The bioleaching of the sludge by means of the heterotrophic mesophilic microbial cultures was less efficient (Figure 3). The addition of citric acid (in concentrations of 2 – 5 g/l) increased the extraction of lead (up to 38.5 %) and had not a negative effect on the acidophilic chemolithotrophs and on the extractions of the non-ferrous metals. However, the residual concentrations of the most heavy metals also were decreased below the relevant permissible levels.



1. Cu extracted by the culture producing peroxide and complexing agents
2. Zn extracted by the culture producing peroxide and complexing agents
3. Cu extracted by the culture producing citric acid
4. Zn extracted by the culture producing citric acid

Figure 3. Bioleaching of activated sludge by means of heterotrophic microbial cultures

These cultures were more efficient than the chemolithotrophic cultures only towards the lead which was solublized mainly as complexes with the organic acids produced by the heterotrophic cultures. The submerged culture of *Aspergillus niger* at pH about 2.0 solubilized 53.2 % of the lead within the 7-day leaching period.Chemical leaching by means of solutions contained citric acid (5 g/l) and sulphuric acid to maintain the pH in the range of about 1.9 – 2.0 at 50 ^oC and enhanced aeration was more efficient than the leaching by the heterotrophic microbial cultures but was less efficient than the bacterial leaching by the acidophilic chemolithotrophic bacteria, even at 37 ^oC (but in the presence of ferrous ions added from outside).

CONCLUSION

The data from this study revealed that different microorganisms were able to solubilized efficiently considerable portions of the heavy metals present in the pretreated by autoclaving anaerobically digested sludge. The best results were achieved by the mixed cultures of acidophilic chemolithotrophic bacteria in which the relevant microbial communities as a whole possessed both iron- and sulphur-oxidizing activities. The addition of iron ions (mainly in the ferric state) and sulphuric acid from outside at the start of the leaching facilitated the growth of bacteria and the solublization of metals. It must be noted, however, that the bacteria were able to produce the desired amounts of ferric ions and sulphuric acid in situ oxidizing the ferrous ions solubilized from the sludge, as well as the sulphide sulphur present in the sludge. The pregnant solutions after leaching contained copper and zinc in concentrations which were suitable to obtain these valuable non-ferrous metals as the relevant cathode forms with a very high purity by means of solvent extraction plus elecrowinning. At the same time, the residual concentrations of these metals in the sludge treated in this way, like the residual concentrations of the other heavy metals, present in the sludge, were decreased below the relevant permissible levels. The residual treated sludge contained no toxic chemical and biological pollutants and the bioassimilable forms of C, N, P, K and some essential microelements were still present in sufficient concentration making this sludge suitable for application in agriculture.

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