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The Influence of Sludge Concentration and Sulphuric Acid on Bioleaching Efficiency of *Thiobacillus thiooxidans* on Sewage Sludge

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ABSTRACT

Microbial leaching using *Thiobacillus thiooxidans* was investigated to solubilize heavy metals (HMs) from domestic sludge at different concentrations. Results obtained showed that 100% Cr was solubilized with 50 ml sludge when the bioleached sludge was digested with 50% (v/v) H_2SO_4, while 90% Cr was also solubilized with the same amount of sludge when the bioleached sludge could not be digested with H_2SO_4. However, 3.0% Cd was the metal least solubilized overall with 100 ml sludge when the bioleached sludge could not be digested with H_2SO_4. For high HM extraction efficiency, digestion using H_2SO_4 must be performed after bioleaching of sludge.

Keywords: acidophilic, bacteria, concentration, contamination, extraction, precipitation, substrate, wastewater

INTRODUCTION

The treatment and final disposal of sewage sludge (SS) is an expensive exercise in wastewater treatment. Ocean and landfill disposal methods are two procedures practiced in discharging sludge, though alternative methods such as incineration, solidification, composting and pyrolysis have been used (Donatello *et al.* 2010; Gautam *et al.* 2011). Composting, followed by land application is the most economical method for sludge disposal (Chang *et al.* 2006; Taiwo 2011). Nevertheless, when SS is applied to land, heavy metals (HMs) present in the sludge can enter the food chain via human consumption of contaminated animal and vegetation and produce harmful effects. The content of HMs in SS from domestic facilities often exceeds the minimum tolerable limits due to the activities performed in this system. The removal of HMs from SS reduces potential health risk during its application to land.

The commonly used procedures for removing HM ions from SS and aqueous streams include: chemical precipitation, lime coagulation, ion exchange, reverse osmosis, solvent extraction, etc. However, these methods are generally ineffective in practical application due to their high cost, operational difficulties, and low HM leaching efficiency (Sreekrishnan and Tyagi 1996). An alternative approach to replace chemical methods in removing HMs from SS is microbial leaching (bioleaching) involving numerous ferrous- and sulphur-oxidizing bacteria, including *Acidithiobacillus ferrooxidans* and *A. thiooxidans* (Sreekrishnan *et al.* 1996; Ryu *et al.* 1998; Cho *et al.* 1999). Bioleaching has several advantages over chemical leaching due to its simplicity, high yield of HM extraction, lower acid and alkali consumption, minimum reduction in sludge nutrients such as N and P; moreover, its has been shown to be less expensive and environmentally friendly (Chen *et al.* 2003).

A study on leaching characteristics of HMs from SS by *A. thiooxidans* revealed that this bacterium has sulphur-oxidizing ability at both acidic and neutral conditions, and allowed HM leaching at a high sludge solid concentration (Ryu *et al.* 2003). The study also observed that HM leaching was mainly influenced not by solid sludge concentration but by pH of the sludge solution. Chen and Lin (2000) also studied the effects of ferric ion on bioleaching of HMs from contaminated sediment and found that sediment pH apparently decreased in the bioleaching process after the addition of ferric ion, while HM solubilization increased after the addition of ferric ion, especially for Cr and Pb. Bioleaching of HMs from contaminated soil using metabolites, mainly weak organic acids, produced by a fungus, *Aspergillus niger* was investigated by Ren *et al*. (2009). *A. niger* exhibited good potential in generating a variety of organic acids necessary for HM solubilization. Results obtained showed that after a one-step process, maximum removal of 56, 100, 30, and 19% was achieved for Cu, Cd, Pb, and Zn, respectively. Optimization of the addition of an energy source required for bioleaching HMs from undigested SS after secondary treatment was also studied by Chen *et al.* (2008). Bioleaching was conducted in a batch system with both inoculation of iron-oxidizing bacteria and the addition of $FeSO_4{\cdot}7H_2O$ in the range of 0-17.5 g L^{-1}. The pH of the sludge decreased with an increase in the ferrous ion concentration and reached maximum acidity of pH 3.0 for treatment receiving both bacterial inoculation and substrate addition. This led to significant solubilization for different HM species. It has also been demonstrated that it is possible to develop an economical process for the removal of Pb from contaminated sediments. Different strategies to increase the removal of Pb were tested by Mercier *et al.* (1996) during the application of a biological remediation procedure to treat highly-contaminated aquatic sediments. The use of $FeCI_2$ instead of $FeSO_4{\cdot}7H_2O$ as substrate for *Thiobacilli* bacterium did not interfere with biological solubilization, which occurred in sediments acidified to pH 4.0 with H_2SO_4. With $FeCI_2$, twice as much Pb was solubilized than the same strain acclimated to $FeSO_4{\cdot}7H_2O$. Bioleaching using iron-oxidizing microorganisms and ferrous sulphate as an energy source is considered superior to other bioleaching processes as there is less risk of soil re-acidification (Chan *et al.* 2003). Besides substrate concentration, the solid content of the sludge also plays an important role in determining the efficiency of bioleaching. Bioleaching with higher sludge solids provides an attractive opportunity since a higher amount of the sludge can be treated in a given time. However, at a higher sludge solid content, the

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Table 1 Summary of research works using sulpur- and iron-oxidizing bacteria.

Reference	Microbe (substrate)	HMs removal (% efficiency)	HMs removal (% efficiency) of control	Bioleaching time (range)	pH
Zhou *et al.* 2000	*A. ferrooxidans* (Fe^{2+})	Cr (80%), Cu (100%), Zn (100%)	NA	4 – 10 days	2.0
Xiang *et al.* 2000	*A. ferrooxidans* (Fe^{2+})	Cr (55.5%), Cu (91.5%), Zn (83.3%)	Cr (2.6%), Cu (42.9%), Zn (72.1%)	16 days	2.0
Kim *et al.* 2005	*A. ferrooxidans* (Fe^{2+})	Cr (10%), Cu (39%), Zn (42%)	NA	40 days	2.0
Bayat and Sari 2010	*A. ferrooxidans* (Fe^{2+}, S^0)	Cr (34%), Cu (93%), Zn (96%)	NA	10 days	2.0
Shanableh and Ginige 2000	*A. thiooxidans* (S^0)	Cr (71%), Cu (90%), Zn (91%)	Cr (5%), Cu (4%), Zn (4%)	11 days	2.0

NA = not applicable

reduction in pH due to higher buffering capacity of the sludge ultimately requires more time for attaining the pH value necessary for solubilization of HMs (Pathak *et al.* 2009a). Hence, it is worth examining the optimum sludge solids concentration at which efficient bioleaching can take place. The effect of ferrous sulphate concentration and total solids on bioleaching of HMs from SS revealed that using an indigenous iron-oxidizing microorganism (*A. ferrooxidans*) to optimize the concentration of 10 g L^{-1} ferrous sulphate for maximum bioleaching, 69, 52, 46 and 45% for Zn Cu, Cr and Ni, respectively were leached. It was also observed that prolonged bioleaching using 10 g L^{-1} of ferrous sulphate decreased the pH of SS to a value needed for significant HM solubilization (Wong *et al.* 2004).

An investigation on the effect of sludge solids concentration on bioleaching of Cr (III) and other HMs from tannery sludge by indigenous sulphur-oxidizing bacteria was studied by Shen *et al.* (2003). The concentration of sludge solids ranged from 13-60 g L^{-1}. The study further demonstrated that the lowest pH reached after 25 days of bioleaching at all studied solids concentration was about 1.3. The optimum concentration of sludge solids for maximum HM leaching from tannery sludge was 40 g L^{-1} and 73% of Cr was leached. Zhou *et al.* (2005) also examined the effects of initial sulphuric acid addition and recycling of acidified bioleached sludge to recover Cr from tannery sludge. Their study noted that tannery sludge had a higher buffering capacity than SS and there was an increase in the rates of pH reduction and 100% Cr solubilization with an increase of initial sulphuric acid addition. Sequential selective extraction procedure has been shown as an effective way to reflect the variation in forms of HMs. Coupled with bioleaching, this procedure becomes an efficient approach to investigate the transformation of HM forms during SS bioleaching with elemental sulphur. Chena *et al.* (2005) suggested that the exchangeable form of Cu, Pb and Zn after bioleaching accounted for 81.6, 40.2 and 75.8%, respectively. Cu existed mainly as sulphide precipitate form while Pb and Zn mainly existed as carbonate precipitate and organically bound forms, respectively. The exchangeable Cu and Pb achieved an obvious increase at pH 2.0, while Zn showed higher percentage at pH 3.0. The study also revealed that the transformation of chemical forms for Cu had a good relationship with oxidation reduction potential (ORP) during bioleaching, but Zn was not influenced by the ORP of sludge (Chena *et al.* 2005). Removing metals from SS by bioleaching (mainly by sulphur and iron-oxidizing bacteria) have been studied extensively by other authors, including Zhou *et al*. (2008), Xiang *et al*. (2000), Kim *et al*. (2005), Bayat and Sari (2010), Pathak *et al.* (2008) and Shanableh and Ginige (2000). These authors besides Shanableh and Ginige (2000) used *A. ferrooxidans* in a comparative study to evaluate microbial and chemical leaching processes for HMs removal from de-watered SS. However, the latter authors examined the use of controlled bio-acidification using *A. thiooxidans* in decontamination process to recover HMs from SS. A summary of these studies is shown in **Table 1**.

The performance of the bioleaching process is affected by buffering capacity and, oxygen transfer coefficient of the sediment solid content. These parameters determine the size of the bioreactor and the operational time of the bioleaching process. Changes in the pH values of the bioleaching process are influenced by the buffering capacity of the sediment solids. Hence, with high buffering capacity, there is a reduction of pH in sediment with high solid contents. Metal leaching from the sludge occur directly with the microorganism or indirectly with H_2SO_4 formed in the presence of $FeSO_4$ and S^0. The reaction time required to leach HMs from sludge to a tolerable level is about 8-32 days at an initial pH of 2.5-4.0 (Cho *et al.* 1999). To solve the problem of time factor in bioleaching, mixed cultures of microbes have been developed by adding elemental sulphur during digestion. These mixed cultures allowed leaching of HMs without controlling the initial pH. Mixed cultures of a neutral sulphur-oxidizing bacterium (*A. thioparus*) and acidophilic bacterium (*A. thiooxidans*) have been tested successfully (Chen and Lin 2000; Ryu *et al*. 2003). However, Zhu *et al*. (2011) examined bioleaching of metal concentrates of wastes printed circuit boards (PCB) by mixed cultures of acidophilic bacterial (MCAB). Their findings demonstrated that metals could be efficiently leached from metal concentrates of PCBs by using MCAB, and the leaching period could also be reduced. Other combination protocols tested for bioleaching of contaminated sediment include the studies of Fang *et al*. (2011) and Liu *et al*. (2011). The former author investigated a linked microbial process comprising bioleaching with sulphate-oxidizing bacteria and bioprecipitation with sulphate-reducing bacteria operating sequentially to remove contaminating metals from dredged sediment. Results obtained showed that sediment bioleaching resulted in a sharp decrease in sediment pH from 7.6-2.5 within 10-20 days. This result demonstrates the ability of combined bioleaching process as particularly attractive for the treatment of different types of metal contaminants. Nevertheless, the latter author also studied the bioleaching potential of *A. thiooxidans* when the bacterium was introduced to a consortium of other bacteria, including, *A. caldus*, *Leptospirillum ferriphilum*, *A. ferrooxidans*, *Sulfobacillus thermosulfidooxidans*, *Acidiphilum* spp., and *Ferroplasma thermophilum*. Funtional gene arrays results obtained revealed that after addition of *A. thiooxidans*, most genes involved in iron, sulphur, carbon, and nitrogen metabolism were up-regulated. Mixed contamination by organic and inorganic compounds in soil is a serious problem for soil remediation. A recent study investigating the use of metal-reducing bacterial for bioremediation of soil contaminated with mixed organic and inorganic pollutants has shown that concurrent microbial reaction is a feasible approach for biodegradation and bioleaching of toluene and arsenic (As) simultaneously (Lee *et al.* 2011a). Similarly, to determine the effect of organics (yeast extract) on microbial community during chalcopyrite bioleaching at different temperature, real-time polymerase chain reaction was employed to analyze community dynamics of major bacteria applied in bioleaching. Results indicated that the yeast extract exerted great impact on microbial community and, hence, influenced the bioleaching rate (Li *et al*. 2011).

The objective of the present study was to investigate the effect of sludge concentration and sulphuric acid on bioleaching of sludge using *Thiobacillus thiooxidans* to solubilize HMs from domestic sewage sludge.

MATERIALS AND METHODS

The SS sample used in this research was obtained from the septic tank of the male hostel A of the Federal University of Technology, Owerri, Nigeria. The tank has a capacity of approximately 900 m^3/day. Stratification sampling method was used to select the sam-

Table 2 Concentrations of heavy metals in sewage sludge of bioleaching experiment without digesting with sulphuric acid.

Sludge solid concentration / Heavy metals	150 ml[(i)] Mean (mg/l) ± SD	100 ml[(ii)] Mean (mg/l) ± SD	50 ml[(iii)] Mean (mg/l) ± SD	10 ml[(iv)] Mean (mg/l) ± SD
Pb	0.142 ± 0.0	0.202 ± 0.0	0.282 ± 0.0	0.213 ± 0.0
Ni	0.227 ± 0.0	0.297 ± 0.0	0.271 ± 0.0	0.237 ± 0.0
Co	0.034 ± 0.0	0.081 ± 0.0	0.299 ± 0.0	0.147 ± 0.0
Cr	0.350 ± 0.1	0.378 ± 0.1	0.450 ± 0.1	0.400 ± 0.1
Cd	0.039 ± 0.0	0.016 ± 0.0	0.022 ± 0.0	0.020 ± 0.0

F-test i and ii = 1.00; ii and iii = 1.00; iii and iv = 4.00

Table 3 Concentrations of heavy metals in sewage sludge of bioleaching experiment digested with sulphuric acid

Sludge solid concentration / Heavy metals	150 ml[(a)] Mean (mg/l) ± SD	100 ml[(b)] Mean (mg/l) ± SD	50 ml[(c)] Mean (mg/l) ± SD	10 ml[(d)] Mean (mg/l) ± SD
Pb	0.430 ± 0.2	0.262 ± 0.0	0.308 ± 0.0	0.210 ± 0.0
Ni	0.366 ± 0.1	0.352 ± 0.2	0.411 ± 0.3	0.230 ± 0.0
Co	0.250 ± 0.0	0.258 ± 0.0	0.338 ± 0.2	0.147 ± 0.0
Cr	0.500 ± 0.2	0.498 ± 0.5	0.501 ± 0.3	0.480 ± 0.1
Cd	0.080 ± 0.0	0.085 ± 0.0	0.091 ± 0.0	0.024 ± 0.0

F-test a and b = 1.96; b and c = 1.30; c and d = 1.60

ple site. Random sampling was then applied within each stratification sub-groups to improve representativeness of the sample.

A 25 L container was used to collect the SS sample from the septic tank. The container was sealed and allowed to stand for 24 hrs for particles to sediment. The sedimented sludge was then collected and dehydrated (filter-press) using cheese-cloth. The sludge solid obtained was made up with deionized water to give a 50 g L^{-1} sludge solid concentration in a 2 L conical flask. This procedure was repeated and five 50 g L^{-1} conical flasks of the sludge solid was obtained. From this stock solution, samples for the bioleaching experiment were continually drawn.

An isolate of *Thiobacillus thiooxidans* sub-cultured on nutrient agar and stored at 4°C until used was obtained from the Department of Biotechnology, Federal University of Technology, Owerri, Nigeria. To 150 ml of the (stock) sludge in a 500 ml conical flask was added 10 ml of the sub-cultured microbe and stoppered. The flask was agitated slightly at 30 rpm using a microcentrifuge (MC 5415C; Akson Scientific) for 10 min. The pH of the mixture was 9.0, About 2 g of elemental sulphur powder was added and the flask agitated as above for another 10 min. The flask was placed in a water bath at 30°C and this temperature was maintained until the pH was reduced to 2.0 (about 8 days). After this period, about 40 ml of the supernatant liquid was decanted and filtered using Whatman No. 42 filter papers. The filtrate was divided into two parts of 20 ml each. One 20 ml part of the filtrate was used (without digesting the filtrate) to determine HMs concentration (mg L^{-1}) using Atomic Absorption Spectrophotometer (ALFA 4) for metals Pb, Ni, Co, Cr and Cd according to the method as described by Ukiwe and Oguzie (2008). Three treatments were made and the mean HMs concentration (mg L^{-1}) was obtained. The remaining 20 ml of the filtrate was poured into a 100 ml beaker and 10 ml of a 50% (v/v) H_2SO_4 was added. The mixture was stirred for 5 min and digested as described by Ukiwe and Oguzie (2008). About 20 ml of this solution was used to determine concentration (mg/l) of HMs; Pb, Ni, Co, Cr and Cd as described by Ukiwe and Oguzie (2008). Three treatments were also made and mean HMs concentration was also obtained. These procedures were repeated using 100, 50, and 10 ml stock sludge solution.

Statistical analysis

Data were statistically analyzed using SPSS software (Version 14). Data were also presented as arithmetic mean and standard deviation. The *F*-test was used to estimate significant difference between bioleaching protocols. Analysis of variance (ANOVA) was used to determine difference between sludge solid concentrations.

RESULTS AND DISCUSSION

The bio-chemical solubilization of metals is a complex phenomenon that depends on many indicators such as pH. *A. thiooxidans* under aerobic conditions can oxidized elemental sulphur to produce sulphuric acid which brings down the pH of the sludge to a value necessary for metal solubilization. **Table 2** show mean values of concentration (mg L^{-1}) of HMs of supernatant liquid of sludge solution at various concentration of the bioleaching process with and without digestion with sulphuric acid. When the supernatant sludge solution was not digested with 50% (v/v) sulphuric acid, it was observed that Cr (0.350, 0.378, 0.450 and 0.480 mg L^{-1}) was the highest metal leached at 150, 100, 50 and 10 ml sludge concentration respectively. However, Cd (0.039, 0.016, 0.022 and 0.020 mg L^{-1}) was the least metal solubilized at the above sludge concentrations. However, when the sludge solution was digested with 50% (v/v) sulphuric acid, **Table 3**, Cr (0.500, 0.498, 0.501, 0.480 mg L^{-1}) was the highest metal extracted, while Cd (0.080, 0.085, 0.091, and 0.024 mg L^{-1}) was the least extracted metal at the above mentioned sludge concentrations. There was no trend in metal solubilization as sludge concentration increased from 10 to 150 ml. Nevertheless, 50 ml sludge concentration was the most effective sludge concentration for metal solubilization, especially when the supernatant sludge was digested with sulphuric acid.

Tables 2 and **3** also give values of the standard deviation and *F*-test of extracted metals between sludge concentrations. For both protocols (bioleaching with digestion and bioleaching without digestion using 50% (v/v) sulphuric acid), at 50 ml sludge concentration, the *F*-test was 64 (4 and 4 df, $P < 0.01$).

A. thiooxidans and *A. ferrooxidans* have been successfully tried in both pure and mixed cultures in bioleaching experiments. In one study comparing inorganic arsenic resistance of several strains of *A. thiooxidans* and *A. ferrooxidans*, results obtained revealed that in the presence of up to 120 mM arsenate, different strains of these microbes showed different inorganic arsenic resistance (Leng *et al.* 2009). Another study on bioleaching of arsenic from medicinal realgar, a Chinese mineral drug, using pure cultures of *A. ferrooxidans* and *A. thiooxidans* and mixed cultures of both microbes revealed that the leaching rate of arsenic in realgar after 20 days was higher (43%) in *A. ferrooxidans* cultures but the leaching rate of *A. thiooxidans* cultures only increased from 21-23% in the presence of ferrous ion (Zhang *et al.* 2007). The leaching of arsenic in mixed cultures was greatly enhanced indicating that bioleaching was preferred in mixed cultures for the dissolution of realgar. Two studies on bioleaching of chalcopyrite by pure and mixed cultures of *A. ferrooxidans*, *A. thiooxidans* and *Leptospirillium* specie have demonstrated that these microbes have the ability to leach chalcopyrite thus preventing jarosites accumulation on the substrate and allow further Cu solubilization through the action of ferric ion (Qiu *et al.* 2005; Zhang *et al.* 2008). Some conditional parameters such as pH, ORP, nature and dosage of the SS, and agitation time influence the removal of HMs using the bioleaching process. Lower sludge content invariably leads to higher yields of metal extraction (Kim *et al.* 2005). As earlier noted, it is necessary that the bioleaching process is maintained at pH 2.0 for maximum metal recovery efficiency. However, it has been reported that the process generally requires a longer period of operational time compared to chemical leaching process (Bayat and Sari 2010). Substrate dosage (Sd) to solid content (Sc) also significantly influence bioleaching of HMs from SS (Zhang *et al*. 2009). With increase in Sd/Sc ratio, bioleaching is enhanced and efficient to solubilize HMs such as Zn, Cu and Pb. This is achieved when bioleaching is performed at a maximum time range between 10-30 days (Zhou *et al.* 2008). Other authors have studied several aspects of bioleaching using microbes of mixed cultures. Cabrera *et al.* (2005) studied the kinetics of *A. ferrooxidans* in the presence of HMs ion. Cheng and Hu (2007) studied bioleaching of anilite using pure and mixed cultures of *A. ferrooxidans* and *A. caldus*. Zhang *et al*. (2009) studied the ratio of the effect of substrate dosage to solid content on SS bioleaching by indigenous sulphur-oxidizing bacteria, while Wang *et al.* (2009) and Tsai *et al.* (2003a, 2003b) all studied different approaches on the use of bacteria oxidizing activity of solubilization of HMs from different substrates. Other bioleaching studies for the extraction of HM include the studies of Darezereshki *et al.* (2011) who used mesophilic and thermophilic bacteria to improve copper extraction from a low-grade ore and that of Lee *et al*. (2011b) who also examined a feasibility study on bioelectrokinetics for the removal of organic and inorganic contaminants from soil. However, Das *et al*. (2011) had reasoned that the study of microbial genomes, metabolites and regulatory pathways could provide novel insights to the metabolism of bioleaching microorganisms and their synergistic action during bioleaching operations. Hence, this could also promote understanding of the universal regulatory responses that the biomining microbial community uses to adapt to their changing environment leading to high metal recovery. Bioleaching may be an alternative approach to replace chemical leaching in removal of HMs from SS (Cho *et al.* 1999). Nevertheless, there are several technical problems associated with the bioleaching process which need to be addressed while developing the process on a larger scale. Pathak *et al.* (2009) have taken an overview into the various bioleaching studies carried out in different modes of operation with a view to develop the process as an environmentally friendly and cost-effective technology for the removal of HMs from SS.

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