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Research
Araştırma

BIOREMEDIATION OF SEWAGE SLUDGE FOR LAND APPLICATION AS A FERTILIZER USING BIOLEACHING

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Abstract: Huge amount of sewage sludge is generated worldwide that needs a proper discharge strategy, which retains both future sustainability and present needs. Land application of sewage sludge can be a good solution, whereas it is cost-effective disposal method for treatment plants and also can provide a favorable fertilizer for farm lands. It provides an economical alternative for the final disposal of the sewage sludge, but heavy metals in sewage sludge is always an issue restricting its general use. Therefore, removal of heavy metals prior to land application is likely to be a possible and practical means for reducing metal content in sewage sludge. Bioleaching appear to be a promising technology in removing heavy metals from contaminated sewage sludge. The effect of bioleaching on heavy metals solubilization and also their DTPA-extractable (Diethylene Triamine Pentaacetic Acid) form changes was investigated in this work. The samples of activated sludge were collected from three of most important sewage sludge treatment plant of Tehran. Total metal concentration and DTPA-extractable metal content of samples were determined. Bioleaching using Acidithiobacillus ferrooxidans was carried out in two experiments to study solubilization rate and changes in DTPA-extractable Fe, Cu, Ni and Pb. Results showed that bioleaching could affect metal DTPA-extractable form significantly but, there was no definite behavior for each metal and also each metal in different samples. However, bioleaching can efficiently remove mentioned metals from sewage sludge solid phase. Bioleaching removed approximately 24.73 % of Fe, 83.96 % of Cu, 81.46% of Ni and 38.96 % of Pb from sewage sludge samples. In fact, bioleaching is more efficient and economic than chemical leaching. Indeed Acidithiobacillus ferrooxidans is a powerful bacterium in metals removal and environmental remediation programs.
Key Words: Bioleaching, Heavy metal, Sewage sludge, Bioremediation, Acidithiobacillus ferrooxidans, DTPA-extractable

1. INTRODUCTION

Wastewater treatment plants generate millions of tons of sludge worldwide every year. The management of this sludge is a major part of waste treatment, involving substantial cost and effort (Babel and Dacera, 2005).The basic disposal methods for such large quantities of sludge are land application, land filling, incineration, ocean dumping (Metcalf and Eddy, 2003). Ocean dumping is being phased out, incineration is costly and may contribute to air pollution and landfill space is becoming more and more scarce. One possible long- term solution appears to be recycling of the sludge nutrients and using it for beneficial purposes. Land application of sludge on agricultural lands, forest and disturbed lands represents one of the better options for utilization of this material by recycling plant nutrients and organic matter to soil for crop production (Wong et al., 2004). Agricultural utilization also provides a cost- effective method for sludge disposal (Smith, 1996). In Iran because of terrestrial climate in most regions, soils often have not sufficient organic matter.Therefore, land application of sewage sludge can be a good solution, whereas it has large amount of organic matter. The application of biosolids such as sewage sludge in to land, improves the water- holding capacity and nutritive value of poor soils due to its high content of organic matter and nutrients (Epstein, 1976). Until a few years ago, sewage sludge could be re- used directly in agriculture as fertilizer. Recently, however, there has been an increased concern because of high concentration of heavy metals in sewage sludge (Lue-Hing et al., 1998).The heavy metals therefore must be removed before land application to prevent environmental contamination and health hazards due to the presence of heavy metals in the sludge (Tyagi et al., 1991). Heavy metals removal can be achieved either by chemical or biological methods. Biological leaching referred as bioleaching. It has been proven that bioleaching technique is 80% cheaper than chemical methods (Tyagi et al., 1998). Bioleaching is based on the oxidation of sulfur or iron by chemiolithotrophic bacteria. The most widely used microorganisms in metal leaching are Acidithiobacillus thiooxidans and Acidithiobacillus ferrooxidans (Tyagi et al., 1991; Cho et al., 1999). In this work, we have investigated bioleaching of Cd, Zn, Mn and Co in comparison with chemical solubilization by acidification. This work was carried out to evaluate the ability of Acidithiobacillus ferrooxidans in heavy metal removal from sewage sludge. The effect of pH decrease and microbial activity on metal release was also compared and changes in DTPA-extractable form of metals also were investigated.

2. MATERIAL AND METHODS

2.1. Sample Preparation

The samples of activated sludge were collected from three of most important sewage sludge treatment plant of Tehran, namely Ekbatan, Shahrak-e-Gharb and Shosh. Samples immediately were transferred to the laboratory. The pH value and EC (Electrical Conductivity) were determined immediately by pH meter (Orion 920) and EC meter (Jenway 4230). Then samples of three treatment plant were mixed and finally a uniform sample obtained. To avoid any effect of solid content of sewage sludge on bioleaching rate, samples were dried at room temperature and stored at 4^{o}C until experiments.

2.2. Analysis

Total amount of metals, DTPA- Extractable form and soluble form were determined. To determine total metal concentration in sludge samples, the samples were digested in HNO_3 and $HClO_4$ (Page et al., 1982) and heavy metals were determined by Atomic Absorption Spectroscopy (Shimadzu A-660). In addition, DTPA-extractable amount of metals were determined (Lindsay and Norwell, 1978). DTPA extraction provides a chemical evaluation of the amount of metals that are available for plant uptake (Pretuzzelli, 1989; Su and Wong, 2003). The concentration of heavy metals in terms of soluble form was determined (Table 2) with the proportion of 5:1distilled water and dry sludge (v/w). Some physiochemical properties of sludge samples such as nutrient concentration, solid percentage, and organic C (Walkley and Black, 1934) were also determined and results are shown in Table 1.

2.3. Inoculum Preparation

The type of Acidithiobacillus ferrooxidans was ATCC (American Type Culture Collection) no.16466 that maintained in mineral salt medium which proposed by Tuovinen and Kelley (1973). Appropriate amount of inoculums were obtained through several steps of enrichment. Inoculum was maintained fresh and biologically active until experiment.

2.4. Bioleaching experiment

Two bioleaching experiments were designated; the first was to investigate the effect of microbial leaching on solubilization of heavy metals from sewage sludge solid phase in comparison with artificial acidification. The second was to investigate the effect of microbial activity on DTPA- extractable amount. Each experiment consisted of three treatments, which were triplicated. The treatments are inoculated, pH control and control treatments. At first, sludge samples were mixed with distilled water by proportion of 10:1 distilled water and dry sludge. To evaluate effect of microbial activity and pH decrease separately, another treatment was designated as pH control treatment, which used to add sulfuric acid to correspond the pH value with Inoculated treatment every day during experiment. So, we have two control treatments. pH control treatment which was exposed to pH values exactly same as inoculated treatment. Control treatment which only elemental sulfur was added. Samples were autoclaved (20 min at 121 ^oC) and inoculated in septic condition by appropriate inoculum (20% v/v). The experiments were carried out in fifty four 250 ml Erlenmeyer flask with 100 ml mixed sewage sludge and agitated at 120 rpm and 28 ^oC for 15 days. The sludge in all flasks also, was mixed with 5% (w/v) elemental sulfur powder as substrate. Determination of pH value for inoculated samples was done in septic condition every day during experiment. The sludge samples were centrifuged at 10000 rpm for 15 min to separate the solid from liquid fraction. The liquid fraction was filtered and stored at 4 ^oC prior to determination of heavy metals by Atomic Absorption Spectrometry (AAS). For the second experiment, solid fraction derived from centrifugation mixed with DTPA by the proportion of 1:5 (w/v) of solid fraction of sewage sludge and DTPA (1M). Then the mixture was filtered out by paper filter (Watman no.40). The solution which passed through filter, stored at 4°C until determinations by Atomic Absorption Spectrometry (AAS). All data were analyzed using the SAS statical package. One way ANOVA (Analysis of Variance) was carried out to compare the means of different treatments. Where significant F values were observed, the differences between individual means were tested using Duncan's test (Little and Hills, 1978).

Table 1. Some selected physiochemical properties of sludge samples

selected characteristics			
sludge sample	Ekbatan	Shahrak-e-gharb	Shosh
Solid (%)	1.8	2.0	1.7
pH	6.8	6.8	7.0
EC(ds.m-1)	0.7	1.0	1.3
Kjeldahl N (%) (Kjeldahl, 1883)	5.53	5.87	6.02
Organic Carbon (%)	26.3	26.6	28.5
Olsen(1954) P(mg.kg-1 dry sludge)	5700	16634	6154

Table 2. Heavy metals concentrations (mg.kg-1 dry sludge)

Total heavy metal concentration (mg.kg-1 dry sludge)	
Fe	29352.94
Cu	3808.24
Ni	121.65
Pb	55.52
DTPA-extractable form (mg.kg-1 dry sludge)	
Fe	364.67
Cu	-
Ni	3.52
Pb	18.83

3. RESULTS AND DISCUSSION

3.1. First Experiment

pH changes

As shown in Figures 1 and 2, pH value was decreased gradually during experiments in any of samples. Because of bacterial activity, acid produced and as a result, pH decreases during bioleaching. A.ferrooxidans uses elemental sulfur to produce sulfuric acid. A.ferrooxidans can also use sulfide form of metals as energy source and finally yield sulfuric acid. The addition of elemental sulfur, solely, did not affected pH, as it is shown in control treatment. Figures 1 and 2 show that pH decrease approximately after 4 days, because bacterial activity is not sufficient in initial days after inoculation. When pH begins to decrease, it gradually decreases approximately to value 1-2 and remains constantly to the end of the experiment. Samples from different sewage treatment plants did not different significantly in terms of pH decrease ($p< 0.05$).

Metal solubilization

The amount of Fe in liquid phase increases over time. Then after 10 days, decrease, probably because of production of other chemical forms of Fe, which are less soluble (Figure 3). Cu solubilized during experiment, but the rate of solubilized is different. At first five days, the solubilized rate is slow in comparison to next five days (5-10). The last five days (10-15), the solubilized rate decrease as slow as first days. The amount of soluble Cu increase significantly in inoculated treatment in comparison to the pH control and control treatments (Figure 4). Ni also solubilized in a rate such as that of Cu. All three treatments were significantly different (Figure 5).

Pb solubilized up to 38.96 % of total Pb of solid phase. Soluble Pb rises during experiment in approximately constant rate.Inoculation increased solubilized rate significantly in comparison to control treatments (Both pH control and control treatment). Regarding to significant difference between inoculated treatment and pH control treatment, it can be concluded that, bacterial activity play an important role in solubilization.

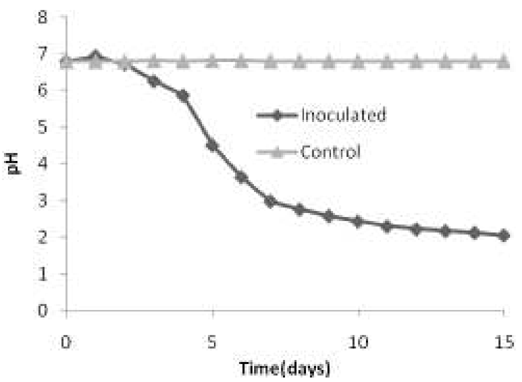

Figure 1. pH changes during first experiment

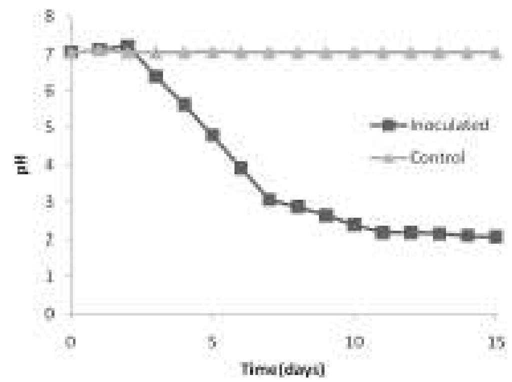

Figure 2. pH changes during second experiment

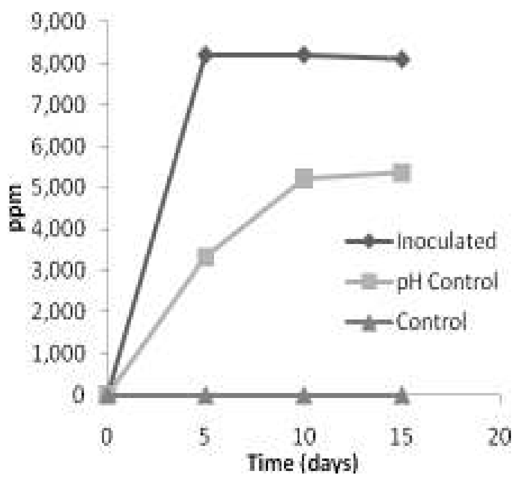

Figure 3. Fe changes in sewage sludge during bioleaching

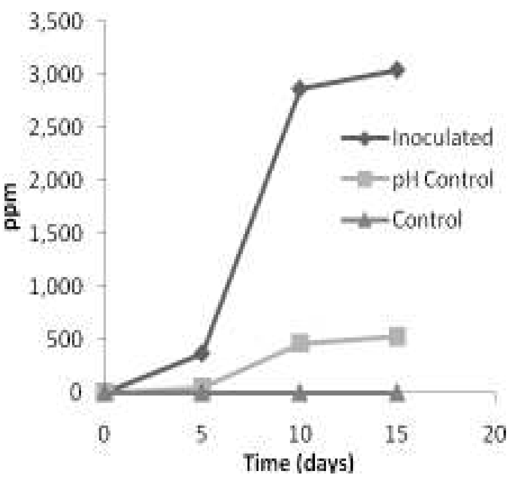

Figure 4. Cu changes in sewage sludge during bioleaching

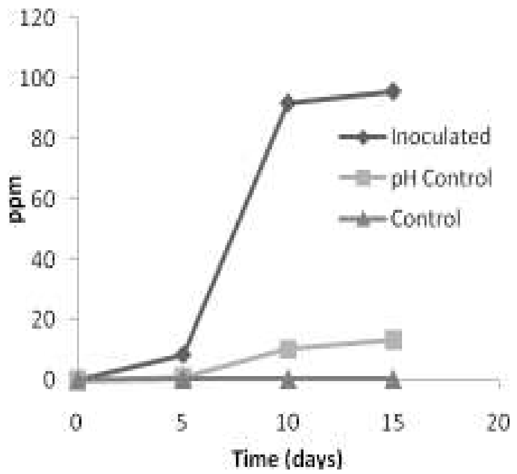

Figure 5. Ni changes in sewage sludge during bioleaching

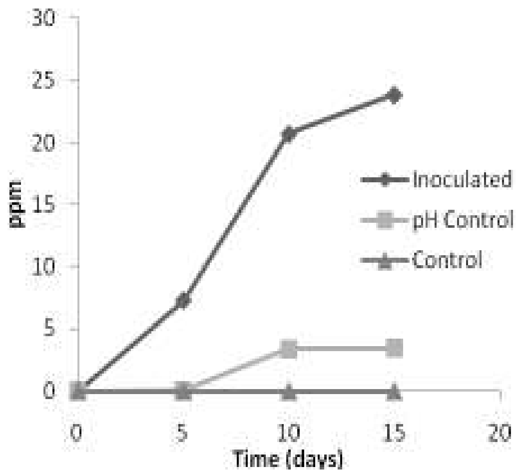

Figure 6. Ni changes in sewage sludge during bioleaching

3.2. Second Experiment

The effect of bioleaching on DTPA-extractable form of metal

In the case of all of four metals in second experiment, it is obvious that bioleaching did not affect DTPA-extractable forms of metals in a definite behavior. However DTPA-extractable forms of metals, change during experiments but it do not increase this form significantly. As we are to use the bioleached sludge as fertilizer, thus, this can be a good characteristic of bioleaching. As mentioned DTPA-extractable form of metals represents the amount of metals that can be uptake by plant roots. Whereas bioleaching decrease metals content of solid phase of sludge and also do not increase the absorbable form of metals, thus it can be recommended as a proper solution for high metal-polluted sludge. Cu can not be extracted with DTPA.

4. CONCLUSION

Results showed that bioleaching is an efficient and powerful tool for removal of heavy metals from sewage sludge. Sewage sludge samples were different in terms of response to bioleaching and consequent metal release likely because of organic matter nature or different forms of metals in sewage sludge. However, different metals are not same in the case of solubilization rate. Bioleaching removed approximately 24.73 % of Fe, 83.96 % of Cu, 81.46% of Ni and 38.96 % of Pb from sewage sludge samples. Generally, it can be concluded that bioleaching is an efficient approach in heavy metals removal form sludge and subsequent land application. In fact, bioleaching is more efficient and economic than chemical leaching. Indeed *Acidithiobacillus ferrooxidans* is a powerful bacterium in metals removal and environmental remediation programs.

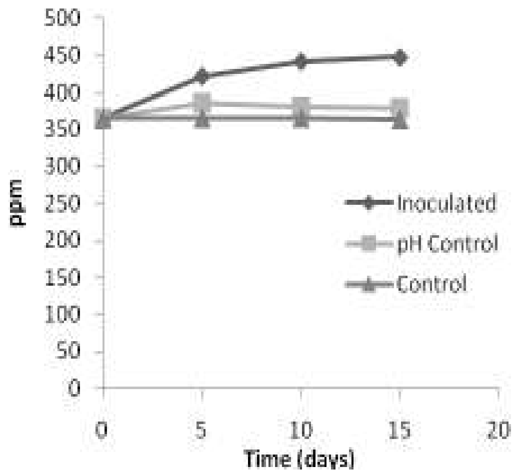

Figure 7. DTPA-extractable Fe changes in sewage sludge during bioleaching

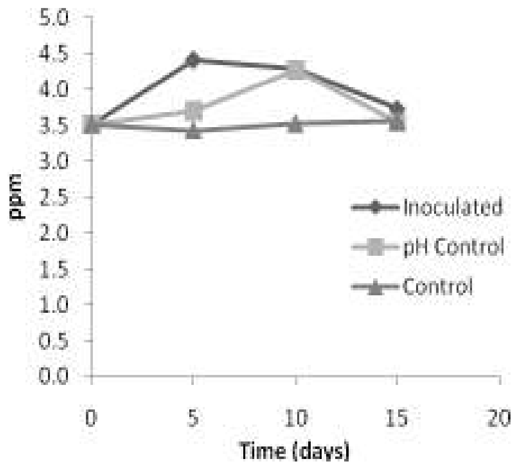

Figure 8. DTPA-extractable Ni changes in sewage sludge during bioleaching

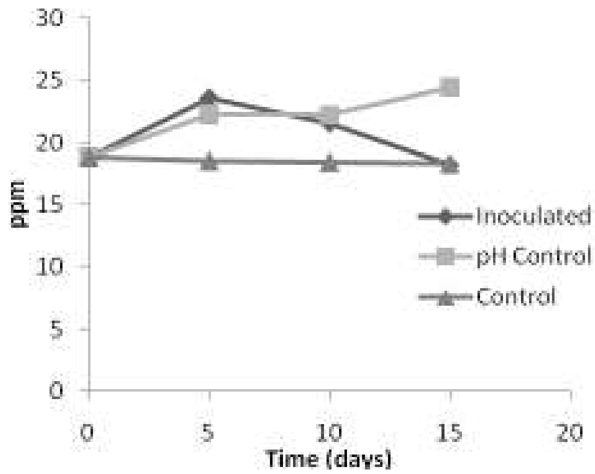

Figure 9. DTPA-extractable Ni changes in sewage sludge during bioleaching

5. ACKNOWLEDGMENT

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