



Bioleaching of heavy metals from sewage sludge by indigenous iron-oxidizing microorganisms using ammonium ferrous sulfate and ferrous sulfate as energy sources: A comparative study

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ABSTRACT

The potential of indigenous iron-oxidizing microorganisms enriched at initial neutral pH of the sewage sludge for bioleaching of heavy metals was investigated at initial neutral pH of the sludge using ammonium ferrous sulfate (FAS) and ferrous sulfate (FS) as an energy sources in two different sets of experiments. After 16 days of bioleaching, 56% Cu, 48% Ni, 68% Zn and 42% C were removed from the sludge using ammonium ferrous sulfate as an energy source. On the other hand, 64% Cu, 58% Ni, 76% Zn and 52% Cr were removed using ferrous sulfate. Further, 32% nitrogen and 24% phosphorus were leached from the sludge using ferrous sulfate, whereas only 22% nitrogen and 17% phosphorus were removed using ammonium ferrous sulfate. The BCR sequential extraction study on speciation of metals showed that using ammonium ferrous sulfate and ferrous sulfate, all the metals remained in bioleached sludge as stable form (F4 fraction). The results of the present study indicate that the bioleached sludge would be safer for land application. Also, the fertilizing property was largely conserved in the bioleached sludge using both the substrates.

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1. Introduction

Land application is being considered as one of the most economical methods for disposal of sewage sludge. This is because the sludge is an easily available nutrient source and hence can save substantial cost, if applied to soil as a fertilizer [1]. However, due to the various physicochemical processes involved in activated sludge treatment, the heavy metals present in the original wastewater get accumulated in the sludge and restrict its use as a fertilizer [2]. Therefore, removal of heavy metals from sewage sludge is an important step to ensure safe disposal of sludge on land.

Over the years, bioleaching process using iron-oxidizing microorganisms and sulfur-oxidizing microorganisms has been reported to be an efficient and economical method for removal of heavy metals from the sludge [3,4]. In iron-oxidizing process, ferrous sulfate is used as an energy source that has less risk of soil re-acidification. However, in the iron-oxidizing process pre-acidification of sludge (up to pH 3) is required to provide favorable conditions for the growth of *Acidithiobacillus ferrooxidans* [5]. The pre-acidification increases the process cost which hinders the application of bioleaching on a larger scale. On the other hand,

bioleaching process, if operated at neutral pH can be expected to be cost effective as the sludge generated in sewage treatment plants has near neutral pH. It has been reported that iron-oxidizing bacteria is naturally present in all types of sewage sludge (Primary, Secondary, aerobically or anaerobically digested) and can be adapted easily by amending sewage sludge with ferrous sulfate [6]. Bioleaching at neutral pH of the sludge using indigenous iron-oxidizing bacteria isolated from the sludge was reported [3,7]. In the above studies, the indigenous microorganisms used for inoculum were enriched at initial pH 2. This highly acidic pH condition was maintained by using acid and external nutrients were provided for the growth of *Acidithiobacilli*, ultimately making the process costly.

Further, it has been widely accepted that the toxic effect of heavy metals depends not only on the total concentration but also on the bioavailability of the metals [8]. The heavy metals in the sludge exist in different forms which eventually affect bioavailability of the metals as well as the efficiency of bioleaching process. The residual metals in the bioleached sludge may find their way on to the soil as a result of land application of the sludge. The previous study carried out with pure culture of *At. ferrooxidans* at sludge initial pH 3 has shown that bioleaching process affects the speciation of the metals remained in the sludge after bioleaching [9]. However, there is no information in the literature on the speciation of the metals in the bioleached sludge when the process is carried out at initial neutral pH of the sludge using indigenous iron-oxidizing

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Table 1
The characteristic properties of sewage sludge.

Parameters	Values
pH	7.1
Total solids (g L^{-1})	30.2
Organic matter (%)	43.0
Inorganic matter (%)	57.0
Nitrogen (%)	2.7
Phosphorus (%)	1.2
Cu (mg kg^{-1} dry sludge)	472
Ni (mg kg^{-1} dry sludge)	294
Zn (mg kg^{-1} dry sludge)	1310
Cr (mg kg^{-1} dry sludge)	332

microorganisms. Therefore, it is worth examining the speciation of those metals which remain in the bioleached sludge after the process has been operated at sludge initial neutral pH.

In the present study, bioleaching was attempted at initial neutral pH of the sludge using indigenous iron-oxidizing microorganisms enriched at initial neutral pH of the sludge and by using ferrous sulfate (FS) as an energy source without the addition of nutrients. The sewage sludge being rich in nutrients does not require additional nutrients for the growth of microorganisms. Further, a comparative study at initial neutral pH of the sludge was carried out using ammonium ferrous sulfate ($[\text{NH}_4]_2[\text{Fe}][\text{SO}_4]_2 \cdot 6\text{H}_2\text{O}$) as an alternative energy source. Ammonium ferrous sulfate (FAS) contains ferrous iron which can serve as an energy source and ammonium ion which can act as a source of nitrogen for the growth of indigenous *Acidithiobacilli*. The speciation of heavy metals in the sludge before and after bioleaching using both ferrous sulfate and ammonium ferrous sulfate has also been investigated using the sequential procedure developed by Bureau of Community Reference (BCR) [10].

2. Materials and methods

2.1. Characterization of the sludge

The anaerobically digested sewage sludge was procured from a sewage treatment plant (treatment capacity: 100 MGD) located in Delhi, the capital city of India. The pH, oxidation–reduction potential (ORP), total solids content, organic matter content, total kjeldahl nitrogen (TKN) and total phosphorus content of the sludge were determined according to the standard methods [11]. The concentration of ferrous and ferric iron in the supernatant of the sludge was determined by the o-phenolthroline method. For total heavy metal determination, the sludge samples were subjected to di-acid digestion ($\text{HNO}_3 + \text{HClO}_4$) and the heavy metals in the digested liquid were determined by using atomic absorption spectrophotometer (PerkinElmer AAnalyst 200). The characteristic properties of the anaerobically digested sludge before bioleaching are shown in Table 1.

2.2. Preparation of inoculum and metal bioleaching experiments

For inoculum preparation, the secondary activated sludge was fortified with 3% (w/v) of ferrous sulfate at 28 °C and 180 rpm. When pH of the sludge reduced from an initial value of 7 to 3, the culture was transferred to a fresh sample of the sludge. This procedure was repeated three times so as to get an active inoculum (enriched sludge) for using it in the subsequent bioleaching experiments.

The bioleaching experiments were conducted with 250 ml of sludge (30.2 g L^{-1} of the solid) using 10% (v/v) of the inoculum and 2% (w/v) of ferrous sulfate at 28 °C and 180 rpm. The addition of inoculum and instant auto-oxidation of added ferrous sulfate caused lowering of sludge pH, for which the initial pH of the

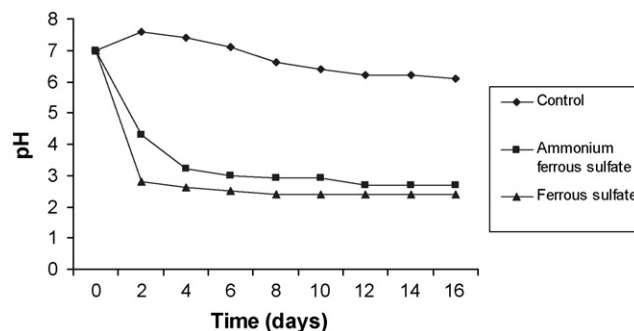


Fig. 1. Change in pH with time during bioleaching.

mixed slurry was readjusted to 7. Therefore, all the bioleaching experiments were performed at initial neutral pH of the sludge slurry. A control experiment was conducted similarly without using inoculum and ferrous sulfate. The experiments were performed in triplicates for 16 days. The change in pH, ORP and solubilization of heavy metals was monitored with time.

The above procedure was followed for inoculum preparation and bioleaching experiments using ammonium ferrous sulfate.

2.3. Sequential extraction study

For estimation of different forms of heavy metals in the dry sludge before and after bioleaching, the four-step BCR sequential extraction procedure was followed using 0.5 g of dry sludge [10]. In the BCR methodology, the first step involves extraction of exchangeable fraction (F1) representing the fraction of adsorbed metals, which is easily affected by the ionic composition of water. In the second step, the reducible fraction (F2) is extracted which represents the fraction that is bound to Fe–Mn oxide in the sludge and is susceptible to anoxic conditions. The oxidizable fraction (F3) is extracted in the third step, which represents the fraction bound to organic matter and to sulfide and may get extracted under oxidizing conditions. The residual fraction (F4) represents the most stable form of the metals remaining in the sludge after extraction of the fractions F1, F2 and F3.

2.4. Statistical analysis

The data determined in triplicate was analyzed by analysis of variance (ANOVA) using SPSS for Windows (version 16.0). The significance of difference was determined according to Duncan's multiple range test (DMRT). *P*-values < 0.05 are considered to be significant.

3. Results and discussion

3.1. Change in pH and ORP with time during bioleaching

The change in pH with time during bioleaching with ammonium ferrous sulfate and ferrous sulfate along with the control run is shown in Fig. 1. In the control, without the addition of substrates and inoculum, the pH decreased marginally from an initial value of 7 to 6.1 on the 16th day. This indicates lower acid production, as no inoculum of active microbes and no energy source were provided to support the in-situ iron-oxidizing microorganisms of the sludge. In the sludge containing ammonium ferrous sulfate and inoculum, the indigenous iron-oxidizing microorganisms were able to oxidize ammonium ferrous sulfate resulting in rapid decrease in pH from 7 to 4.3 in 2 days and then to less than 3 gradually in 8 days and finally to 2.7 on the 16th day. In the sludge containing ferrous sulfate and inoculum, the pH decreased at a faster rate rapidly from 7 to 2.8

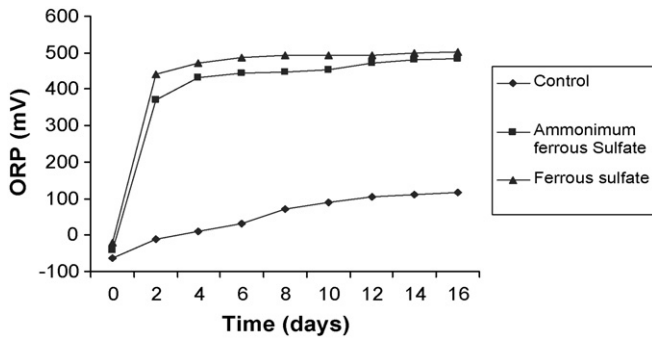
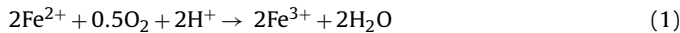


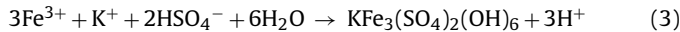
Fig. 2. Change in ORP with time during bioleaching.

in 2 days, then slowly to 2.4 on the 8th day and finally remained constant till 16th day.

The change in ORP is shown in Fig. 2. In the control, ORP reached a maximum of 116 mV from an initial value of -62 mV on the 16th day. In the sludge containing ammonium ferrous sulfate and inoculum, the oxidation of ammonium ferrous sulfate led to an increase in the ORP from -43 mV to 372 mV in 2 days and finally to 485 mV on the 16th day. The oxidation of ferrous sulfate in the sludge led to a rapid increase in the ORP from -20 mV to 442 mV in 2 days and finally to 502 mV on the 16th day. The increase in ORP coupled with a low pH value during bioleaching is an indicator of substantial growth of microorganisms [12]. The increase in ORP is due to the oxidation of Fe^{2+} to Fe^{3+} , which occurs naturally in the presence of air and also through biological oxidation:



The ferric iron is hydrolyzed into ferric hydroxide and jarosite resulting in decrease in pH:



The oxidation of ferrous iron during bioleaching is shown in Fig. 3. In the sludge containing ammonium ferrous sulfate and inoculum, 64.2% of the total Fe^{2+} (2.85 g L^{-1}) was oxidized to Fe^{3+} in 2 days and finally oxidized completely (100%) on the 12th day. In the sludge containing ferrous sulfate and inoculum, 94.5% of the total Fe^{2+} (4 g L^{-1}) was oxidized to Fe^{3+} in 2 days and almost complete oxidation (99.55%) of Fe^{2+} was observed in 4 days.

The results of the present study show that indigenous iron-oxidizing microorganisms were efficient at initial neutral pH of the sludge and were able to use both ammonium ferrous sulfate and ferrous sulfate as an energy source for bioleaching. The similar results indicating decrease in pH were also obtained in the previous

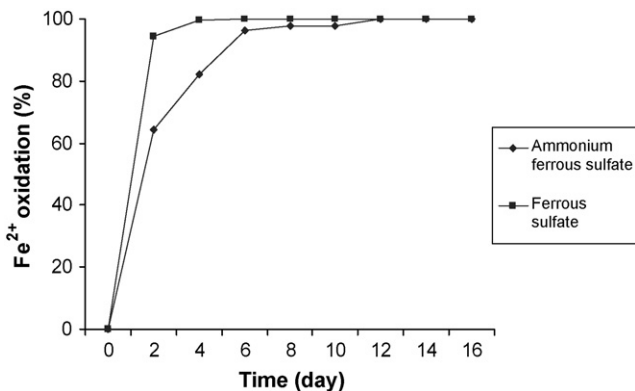


Fig. 3. Percentage oxidation of ferrous iron with time during bioleaching.

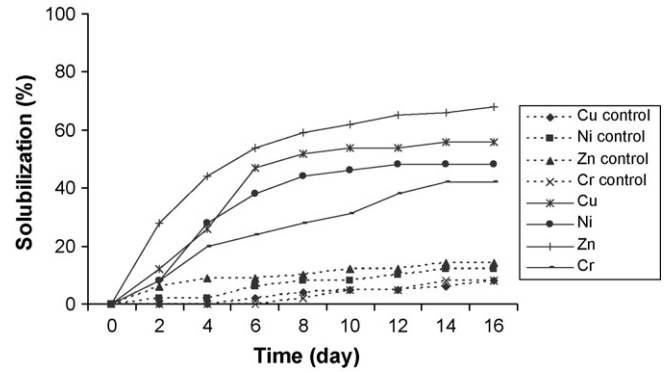


Fig. 4. Percentage solubilization of heavy metals with time using ammonium ferrous sulfate.

study carried out at initial neutral pH of the sludge using indigenous iron-oxidizing bacteria [3].

3.2. Change in solubilization of heavy metals with time during bioleaching

The percent solubilization of Cu, Ni, Zn and Cr with time in control and in the sludges containing ammonium ferrous sulfate and ferrous sulfate during bioleaching is shown in Figs. 4 and 5. In the control, only 8% Cu was solubilized in 16 days at sludge pH 6.1. In the sludge containing ammonium ferrous sulfate, 52% Cu was solubilized on the 8th day when pH of the sludge reached less than 3. After 8 days, there was a gradual increase in Cu solubilization and a maximum of 56% Cu was solubilized at sludge pH 2.7 on the 16th day. On the other hand, using ferrous sulfate, comparatively higher solubilization of Cu was achieved. About 51% Cu was solubilized on the 6th day, which increased to a maximum of 64% on the 16th day when pH of the sludge reached 2.4. The higher solubilization of Cu obtained using ferrous sulfate than what was obtained using ammonium ferrous sulfate can be attributed to the lower pH and higher ORP obtained in the sludge using the former. It is worth mentioning that besides pH, ORP also plays an important role in Cu solubilization. The ORP of the sludge should be more than 250 mV to initiate solubilization of Cu [12]. In the present study, the ORP of both the sludges containing ammonium ferrous sulfate and ferrous sulfate reached a maximum of 485 mV and 502 mV, respectively, on the 16th day.

While correlating the solubilization of Cu with ORP and pH, the coefficients ($r^2 = +0.805$ for ORP and $r^2 = -0.786$ for pH) calculated for the sludge containing ammonium ferrous sulfate and the coefficients ($r^2 = +0.856$ for ORP and $r^2 = -0.841$ for pH) calculated for the sludge containing ferrous sulfate strongly suggest that high ORP and low pH play an important role in solubilization of Cu.

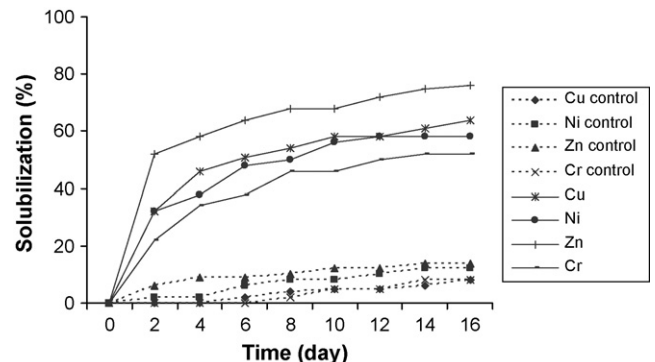


Fig. 5. Percentage solubilization of heavy metals with time using ferrous sulfate.

Table 2
Heavy metals speciation in sewage sludge before bioleaching and after bioleaching.

Sludge	Heavy metals (mg kg ⁻¹)	F1	F2	F3	F4	Sum
Before bioleaching	Cu	0a	37.76a	302.08a	132.16a	472
	Ni	52.92a	29.4a	94.08a	117.6a	294
	Zn	183.4a	314.4a	668.1a	144.1a	1310
	Cr	6.64a	0a	189.24a	136.12a	332
After bioleaching (FAS)	Cu	20.77b	18.69b	39.46b	128.78a	207.7
	Ni	3.06b	10.7b	19.88b	119.26a	152.9
	Zn	74.62b	79.65b	112.93b	152a	419.2
	Cr	3.85b	3.85b	55.9b	129a	192.6
After bioleaching (FS)	Cu	3.4c	10.2c	27.2b	129.2a	170
	Ni	2.47b	8.65c	8.65c	103.74b	123.51
	Zn	56.59c	40.87c	75.76c	141.18a	314.4
	Cr	6.38b	4.78c	22.31c	125.93a	159.4

Values given in the columns (means of three replicates) represented by different letters (a, b and c) to indicate significant differences ($P < 0.05$).

The solubilization of Ni was only 44% using ammonium ferrous sulfate within 8 days of bioleaching and 48% on the 16th day when pH was 2.7 as compared to 12% in the control at sludge final pH 6.1. In the sludge having ferrous sulfate, higher solubilization of Ni was achieved. About 50% Ni was leached from the sludge in first 8 days, with maximum of 58% solubilization on the 16th day at sludge final pH 2.4.

In comparison with all other metals, the highest solubilization was achieved for Zn. In the control, only 14% Zn was solubilized on the 16th day at sludge final pH 6.1. In presence of ammonium ferrous sulfate, 68% Zn and using ferrous sulfate, 76% Zn were solubilized on the 16th day, when sludge final pH reached 2.4.

The solubilization of Cr was less compared to the other heavy metals. Within first 8 days, only 28% Cr was solubilized using ammonium ferrous sulfate, which increased gradually and reached a maximum of 42% on the 16th day at sludge final pH 2.7. In the sludge having ferrous sulfate, higher Cr removal (52%) was observed on the 16th day at sludge final pH 2.4. In the control, only 8% Cr was removed at sludge final pH 6.1.

As can be seen from the above results, the enriched indigenous iron-oxidizing microorganisms were able to grow at sludge initial neutral pH and were using both ferrous sulfate and ammonium ferrous sulfate. The oxidation of ferrous sulfate and ammonium ferrous sulfate resulted in a significant decrease in pH and solubilization of metals from the sludge. However, ferrous sulfate proved better substrate compared to the ammonium ferrous sulfate as higher solubilization (8% Zn, 10% Ni, 10% Cr and 12% Cu) was obtained using the former. The solubilization efficiencies of heavy metals achieved in the present study appear to be very close to the values reported in the literature for bioleaching started at initial neutral pH of the sewage sludge using indigenous iron-oxidizing bacteria. In the present study, after 16 days of bioleaching 64% Cu, 58% Ni, 76% Zn and 52% Cr were removed using ferrous sulfate compared to the 63.7% Cu, 15.5% Ni, 74.9% Zn and 50.2% Cr solubilization as reported earlier [3].

3.3. Speciation of heavy metals in sludge before bioleaching

Table 2 and Fig. 6 clearly show the different fractions of Cu, Ni, Zn and Cr in the anaerobically digested sludge before bioleaching. The fractions correspond to different chemical forms of the metals. The Cu was primarily found in F3 fraction accounting about 64% of the total Cu content. According to the BCR scheme, the F3 fraction represents the portion of metals bound to the organic matter and sulfides. It has been reported that Cu has a strong tendency to remain in sulfide minerals or associated with organic matter of the sludge [8]. The second most important fraction (F4) of Cu was 28% indicating the presence of residual fraction of Cu in the sludge. The sum of these two forms (F3 + F4) accounted 92% of the total

Cu suggesting that highly oxidizing and acidic conditions would be required for solubilization of Cu from the sludge.

Compared to Cu, about 40% Ni remained in F4 fraction which was followed by F3 (32%), F1 (18%) and F2 (10%) fractions. A similar trend for different fractions of Ni has also been reported in previous study [8].

Zn was majorly distributed in F3 (51%), F2 (24%) and F1 (14%) fractions suggesting that the potential mobility of Zn is very high. This is also supported by other authors who mentioned that most of the Zn remained in more mobile fractions [13,14].

Cr was predominantly distributed in F3 (57%) and F4 (41%) fractions constituting 98% of the total Cr content in the sludge. The remaining two fractions (F1 and F2) constitute only 2% of the total Cr content, which suggest that the potential mobility of Cr is low. This fact is supported by the results obtained in earlier studies [8,14].

3.4. Speciation of heavy metals in sludge after bioleaching

Although, during bioleaching there was a substantial solubilization of different fractions of heavy metals (Cu, Ni, Zn and Cr) using ammonium ferrous sulfate (Fig. 7) and ferrous sulfate (Fig. 8) with respect to initial metal content of the sludge, a significant quantity of the metals was still present in the bioleached sludge. The different fractions of the metals present in the bioleached sludge will govern the ultimate fate of the sludge for safe disposal on land. Figs. 9 and 10 show distribution of all the fractions of Cu, Ni, Zn and Cr remained in the bioleached sludge using ammonium ferrous sulfate and ferrous sulphate, respectively. On the basis of the results obtained, it was observed that the bioleaching process using ammonium ferrous sulfate and ferrous sulfate significantly affects the percentage distribution of various fractions of the metals present in the bioleached sludge.

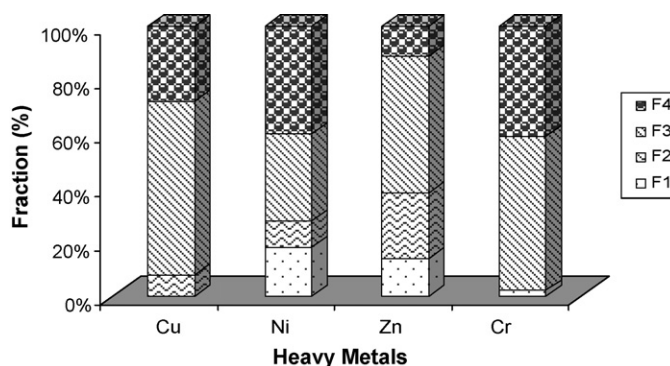


Fig. 6. Percentage distribution of fractions of Cu, Ni, Zn and Cr in anaerobically digested sludge before bioleaching.

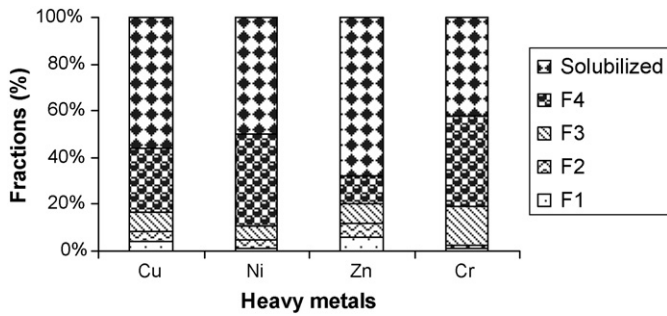


Fig. 7. Percentage distribution of Cu, Ni, Zn and Cr in bioleached sludge with respect to initial metal content using ammonium ferrous sulfate.

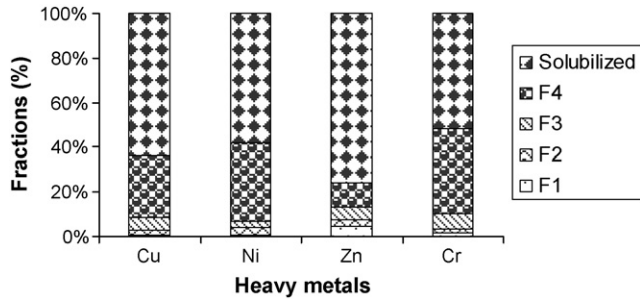


Fig. 8. Percentage distribution of Cu, Ni, Zn and Cr in bioleached sludge with respect to initial metal content using ferrous sulfate.

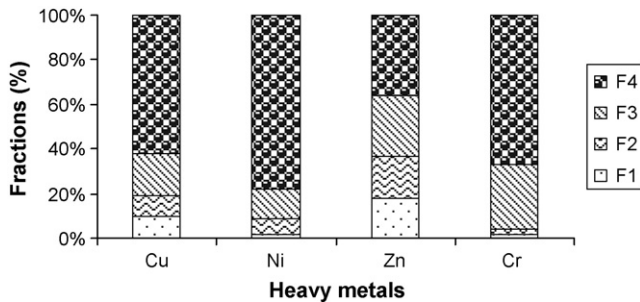


Fig. 9. Percentage distribution of remaining Cu, Ni, Zn and Cr in bioleached sludge using ammonium ferrous sulfate.

After bioleaching using ammonium ferrous sulfate, the F4, F3 and F2 fractions of Cu reduced from initial values 28%, 64% and 8% to 27.28%, 8.36% and 3.96%, respectively (Fig. 7). During bioleaching, the sulfide portion was well oxidized by in-situ iron-oxidizing microorganisms. The highly oxidizing conditions (ORP 451 mV) coupled with low pH led to the liberation of metal associated with organic matter of the sludge resulting in significant solubi-

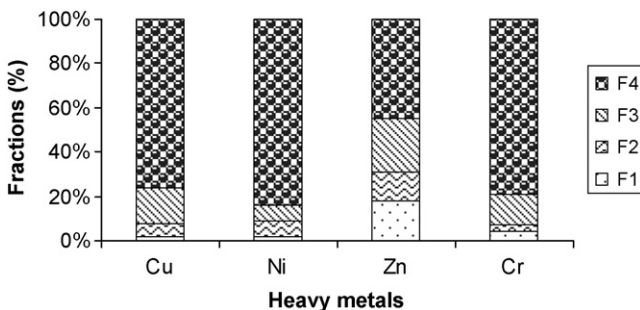


Fig. 10. Percentage distribution of remaining Cu, Ni, Zn and Cr in bioleached sludge using ferrous sulfate.

lization of Cu. Another interesting observation was an increase of 4.4% Cu in the F1 fraction which was not present in the sludge before bioleaching. In the sludge containing ferrous sulfate, the F4, F3, F2 and F1 reduced to 27.37%, 5.76%, 2.1% and 0.7%, respectively, with respect to the total initial Cu content of the sludge (Fig. 8).

In the bioleached sludge using ammonium ferrous sulfate, the remaining Cu was predominantly present in F4 fraction (62%) followed by F3 (19%), F1 (10%) and F2 (9%) fractions (Fig. 9). In the sludge bioleached with ferrous sulfate, similarly most of the remaining Cu was found in F4 fraction (76%) followed by F3 (16%), F2 (6%) and F1 (2%) fractions (Fig. 10).

A similar trend was observed in case of Zn, where F3, F2 and F1 fractions reduced from initial values 51%, 24% and 14% to 8.62%, 6.08% and 5.7%, respectively, in the bioleached sludge using ammonium ferrous sulfate (Fig. 7). The F4 fraction remained nearly unchanged (from 11 to 11.6%). The F4, F3, F2 and F1 fractions reduced to 10.78%, 5.78%, 3.12% and 4.32% in the sludge containing ferrous sulfate (Fig. 8).

In bioleached sludge using ammonium ferrous sulfate, the remaining Zn was found in the stable fraction F4 (36.3%) followed by F3 (26.94%), F1 (19%) and F2 (17.8%) fractions (Fig. 9). In the sludge containing ferrous sulfate, the majority of Zn (44.9%) existed in the residual fraction (F4), followed by F3 (24.08%), F1 (18%) and F2 (13%) fractions (Fig. 10).

In the sludge bioleached using ammonium ferrous sulfate, the F3, F2 and F1 fractions of Ni reduced from the initial values 32%, 10% and 18% to 6.76%, 3.64% and 1.04%, respectively, whereas F4 fraction remained largely unaffected (Fig. 7). In the bioleached sludge using ferrous sulfate, the F4, F3, F2 and F1 reduced to 35.29%, 2.94%, 2.94% and 0.8%, respectively (Fig. 8).

In the bioleached sludge using ammonium ferrous sulfate, remaining Ni was mostly found in F4 (78%) fraction, followed by F3 (13%), F2 (7%) and F1 (2%) fractions (Fig. 9). In the sludge containing ferrous sulfate, relatively higher Ni content was in F4 fraction (84%), followed by F2 (7%), F3 (7%) and F1 (2%) fractions (Fig. 10).

In the case of Cr, the F4, F3 and F1 fractions reduced to 38.85%, 16.83% and 1.16% from the initial values 41%, 57% and 2%, whereas F2 fraction increased slightly to 1.16% with respect to the total initial Cr in the sludge in bioleached sludge using ammonium ferrous sulfate (Fig. 7). In the sludge bioleached using ferrous sulfate, the F4 and F3 fractions reduced to 37.93% and 6.72%, respectively, whereas F2 fraction increased to 1.44%. The percentage Cr in F1 fraction remained nearly the same (1.92%) with respect to the total initial Cr content (Fig. 8).

In the bioleached sludge using ammonium ferrous sulfate, the remaining Cr was present in the F4 fraction (67%), followed by F3 (29%) and 2% each in F1 and F2 fractions (Fig. 9), whereas using ferrous sulfate comparatively higher proportion of Cr content (79%) was found in F4 fraction suggesting that most of the Cr was in stable form (Fig. 10).

From the above findings, it was observed that most of all the metals studied in the present work, after bioleaching remained in the bioleached sludge as F4 fraction. The following trend in F4 fraction of Ni (78%) > Cr (67%) > Cu (62%) > Zn (36.3%) was observed in the bioleached sludge containing ammonium ferrous sulfate, whereas in the sludge containing ferrous sulfate the following trend was observed: Ni (84%) > Cr (79%) > Cu (76%) > Zn (45%). This suggests that except for Zn, more than 60% of other metals are in stable forms in the bioleached sludge, ensuring the safe disposal of bioleached sludge on land. This was also reported in the previous study carried out on mine tailing showing that after bioleaching most of the metals remained in stable fraction [15]. Other interesting observation was that a drastic reduction in F3 fraction took place in all the cases. This supports significant solubilization of the metals during bioleaching.

4. Nutrient property

One of the major concerns with bioleaching is the potential loss of nutrients from sludge during bioleaching, which reduces the fertilizer value of the sludge. The loss of sludge nutrients during bioleaching can be attributed to the low pH conditions coupled with highly oxidizing environment which leads to the oxidation of organic matter causing dissolution of the sludge bound nutrients. The loss of nitrogen from sludge could also be due to the destruction of microbial proteins in the sludge. In the present study, after bioleaching 32% nitrogen and 24% phosphorus were leached from the sludge using ferrous sulfate as a substrate, whereas only 22% nitrogen and 17% phosphorus were lost using ammonium ferrous sulfate. The higher dissolution of sludge bound nutrients using ferrous sulfate as an energy source could be due to the lower final pH achieved in the sludge. The results show that during bioleaching although higher solubilization of metals can be achieved using ferrous sulfate, the fertilizer property can be better conserved using ammonium ferrous sulfate.

5. Conclusions

The results of the present study show that indigenous iron-oxidizing microorganisms enriched at sludge initial neutral pH can successfully carry out bioleaching of sewage sludge using both ammonium ferrous sulfate and ferrous sulfate as an energy source. After 16 days of bioleaching, 56% Cu, 48% Ni, 68% Zn and 42% Cr were removed using ammonium ferrous sulfate, whereas 64% Cu, 58% Ni, 76% Zn and 52% Cr were leached from the sludge using ferrous sulfate as an energy source. The metals speciation study suggests that all the heavy metals in the bioleached sludge were in stable form (F4 fraction) using both the substrates. The ammonium ferrous sulfate proved better substrate in conserving the fertilizing properties of the bioleached sludge, although higher solubilization of heavy metals were achieved using ferrous sulfate.

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