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ORIGINAL ARTICLE

Bioleaching of heavy metals from sewage sludge, direct action of *Acidithiobacillus ferrooxidans* or only the impact of pH?

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Abstract The bioleaching process comprises two mechanisms: direct action of the bacteria and indirect effect of low pH. In this work, the effect of bacteria and the effect of low pH on dissolution of the metals were compared. To study these two mechanisms, bioleaching and the chemical treatment were operated simultaneously at the same pH. Results showed that the effect of bacteria played the main role in dissolution of metals, and regarding metal dissolution, there was a significant difference between these two effects. Although the chemical leaching by means of low pH could dissolve metals, the metals are dissolved mainly by the function of the bacteria rather than dissolution because of low pH. Bioleaching could dissolve Cd (71.90%), Mn (92.5%) and Zn (89.14%), whereas the chemical leaching could dissolve Cd (22.03%), Mn (25.06%) and Zn (14.23%). These results indicate that the main cause of metal dissolution during the bioleaching treatment is the unique impact of bacterial activity, which changes the redox state of the metal rendering them to a more soluble form.

Keywords *Acidithiobacillus ferrooxidans* · Heavy metals · Iron-oxidizing bacteria · Sewage sludge · Sulphur-oxidizing bacteria

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Introduction

Environmental issues are gaining more and more concerns. Along with all other problems, huge amounts of sewage sludge are generated worldwide every day. Its production is also expected to increase as a consequence of population growth and industrial development [1]. Disposal of such a large quantity of sewage sludge is a serious environmental concern. Several methods have been proposed for the disposal of sewage sludge, including land application, landfilling, incineration, ocean dumping and lagooning [2]. The land application is the best option for the sludge disposal, since it recycles nutrients and adds organic matter to soil [3, 4]. Sewage sludge has also been shown that acts as a soil conditioner and improves physical, chemical and biological properties of the soil [5].

However, high concentration of heavy metals in the sewage sludge restricts its usage as a conditioner in agricultural soils [6, 7]. Due to decomposition of sludge in the soil, its heavy metal content may release and lead to soil contamination [1]. Therefore, the removal of metals from sewage sludge is an essential practice prior to land application.

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In order to decrease heavy metal content of sewage sludge, leaching of metals using chemical and biological approaches has been studied. As a chemical treatment, leaching of the heavy metals by inorganic [8, 9] and organic acids [10, 11] has been well documented. The results of these studies indicated that although both inorganic and organic acids could dissolve some of the heavy metals, the efficiency of dissolution varies by the type of metal which should be dissolved and the nature of sludge itself [12].

Bioleaching is being used commonly because it is efficient and economical [12–14]. It has been widely studied for removal of heavy metals from sewage sludge and has demonstrated to be a cheap and efficient over chemical leaching [12, 13, 15]. It has been proved to be 80% cheaper than chemical leaching [16]. *Acidithiobacillus ferrooxidans* is able to affect metal sulphide dissolution by "contact" and "non-contact" mechanisms. By non-contact mechanism, the bacteria oxidize only dissolved iron(II) ions to iron(III) ions. The latter can be reduced to iron(II) ions again, through oxidizing metal sulphides [17].

The bacteria that are used in bioleaching have been shown that perform metal dissolution by both direct and indirect mechanisms. By the direct mechanism, the bacteria change the chemical state of metals working on them directly [18]. Sometimes bacteria use metals as a final electron acceptor. As a consequence, bacteria reduce them altering their oxidation state that eventually leads to increase in their solubility [19]. The indirect effect of bacteria on dissolution of metals occurs as a result of decrease in pH. Some bacterial species such as those so-called sulphur-oxidizing bacteria (SOB) [20] and iron-oxidizing bacteria (IOB) decrease the pH value as they grow [21]. Consequently, low pH leads to increased solubility of heavy metals. Therefore, bacterial activity increases metal solubility indirectly. Most of the related studies which have compared chemical and biological leaching such as [6] have not differentiate the action of bacteria and the effect of low pH happened during the chemical leaching practices. Here we aimed to separate these two factors that affect metal leaching from sewage sludge.

Another unique aspect of current study was the study of the effect of sludge type on the efficiency of the leaching treatments. In this regard, Solisio et al. [22] have studied bioleaching of Zn and Al from two different sludge samples. Their results showed that the efficiency of bioleaching was different in the sludge samples. They pointed out that it might be due to difference in chemical composition of sludge samples. Sewage sludge treatment process was also supposed to affect the efficiency of metal removal from the sludge via either bioleaching or chemical acidification [14]. Fuentes et al. [23] have also showed that different sludge types had different chemical composition and different phytotoxicity effects. They showed that the mobility of metals in sewage sludge and their chemical bound are different in various sludge types. In this case, most of the related papers have not investigated the effect of chemical composition on bioleaching efficiency and its fate in the agricultural lands. However, we supposed that it could be an influencing factor on metal removal efficiency.

To our knowledge, there were no studies addressing the different aspects of bioleaching with SOB and IOB bacteria. Hence, we aimed to differentiate the action of bacteria and the effect of low pH. We also aimed to investigate the effect of sludge composition which derived from different origins (sludge treatment plants), on efficiency of bioleaching treatment. We chose three most important heavy metals: Cd, Zn and Mn (according to the initial analysis of sludge samples).

Materials and methods

Collection and properties of sewage sludge

Samples of fresh activated sewage sludge were collected from three large municipal wastewater treatment plants of Tehran, Iran. As the rate and performance of metal dissolution in the sludge are possibly related to its quality and composition, in order to study the effect of sludge quality on the bioleaching process, three wastewater treatment plants were selected. These plants were located in different part of the city and were far enough to show differences in quality and composition. The treatment plants had a facultative lagoon system with the depth of 5–6 m for the lagoons and the retention time of 25–30 days. The samples transferred to the laboratory immediately and stored in refrigerator at 4 °C until the experiment. These three types of sludge samples were named as E (Ekbatan treatment plant), G (Shahrak-e-Gharb) and S (Shush). The initial pH (Method 2310 A) and electrical conductivity (EC, Method 2520 B) were measured using pH meter (Orion 920, Thermo Fisher Scientific, USA) and EC meter (Jenway 4230, UK) as explained in Standard Methods [24].

Some nutrient concentrations in sludge samples including organic carbon (Method 5310), nitrogen (Method 4500-N B) and phosphorous (Method 4500-P C) were determined according the Standard Methods [24]. The total solids were also measured by drying at 105 °C, and the results were presented as per cent of initial weight (Standard Methods Part 2540 B). The concentration of heavy metals also were determined [24] using atomic adsorption spectrophotometry (Shimadzu, AA-6200, Japan). The concentration of heavy meals was measured as the total amount (Method 3030 F), diethylene triamine pentaacetic acid (DTPA)-extractable form [25] and the soluble form (Method 3030 B).

Inoculum preparation

The bacterium used for bioleaching was *A. ferrooxidans*-type strain PTCC 1646 (Persian Type Culture Collection) which corresponds to DSM 586. The bacterium was grown in liquid salt media for 3 days, before the experiments [13]. The composition of the medium was as follows (g/l): KH_2PO_4 0.4, $MgSO_4·7H_2O$ 0.4, $(NH_4)_2SO_4$ 0.4, $FeSO_4·7H_2O$ 33.3. The pH should be set to 1.4 by addition of H_2SO_4 (2 N). Inoculum was prepared in 250-ml Erlenmeyer flask, and 10 ml of each culture was used for inoculation. Fresh and biologically active culture of bacteria was used as inoculum for bioleaching experiments.

Bioleaching experiment

To study the effect of sludge type on the rate of dissolution of the metal, three different types of sludge were used for the experiments. The experiments were carried out separately on each of the three types of sludge. The experiment was carried out in 250-ml Erlenmeyer flasks containing 100 ml of sludge. Experimental design was completely randomized design for each type of sludge samples (E, G and S). There were three treatments including (1) bioleaching, (2) chemical treatment and (3) control. There were three replications for each of the treatments. Elemental sulphur and $FeSO_4·7H_2O$ were added to the flasks as a substrate in all experimental units of all treatments at a concentration of 4 g/l [26]. Neither the chemical treatment nor control were inoculated. Furthermore, the pH value in the chemical treatment was being equalized every day during the experiments to the pH value of the bioleaching treatment. Therefore, there was only one difference between the bioleaching treatment and the chemical treatment, as the bioleaching treatment was inoculated. For control treatment, there was no adjustment of pH. The control was to evaluate the metal dissolution without any changes in pH value. The control was only to let the other effects such as chemical changes to be separated from the effect of pH. Since the optimum pH range for *A. ferrooxidans* growth is between 1.5 and 4 and the best growth occurs at pH values about 2 [27, 28], at the beginning of the experiments the pH had been adjusted for all of the treatments at four (pH 4) by addition of sulphuric acid (2 N) to make the environment more favourable to bacteria. Flasks were incubated in gyratory shaker at 28 °C and 150 rpm for 15 days.

For bioleaching treatment, the flasks were inoculated with 10% (v/v) of the cultures. The water loss due to evaporation was replenished with sterilized distilled water. During the experiments, pH was measured in each of the flasks every day. To determine the concentration of heavy metals during the experiment, 5 ml of samples was taken from each flask every 3 days. Then the samples were centrifuged at 10,000 (rpm) and the supernatants were used for the analysis of the soluble metal content.

Calculations

To calculate the dissolution percentage in the figures, the amount of each metal in a given day (1–15) of experiment was divided by the total amount of that metal in the sludge sample which was measured before the experiment.

Results and discussion

Characteristics of the sludge samples

Some of the physicochemical properties of sludge samples are presented in Table 1. The results of nutrient analysis in the sludge samples revealed that the samples had sufficient amount of nutrient to be used as a fertilizer. However, there is a concern about the metal content of samples. The heavy metal content of sludge samples is presented in Table 2. The concentration of iron was also included in the table because it was an important factor for *A. ferrooxidans*. This bacterium uses ferrous ions as final electron acceptors and produces ferric ions. As shown in the table, we have measured soluble form, total amount and DTPA-extractable form of metals as well. Soluble form comprises less than 1% of metal content of sludge samples. One of the most important factors in metal solubility is the pH of surrounding environment. Most of the metals reveal their best solubility rate at lower pH values. As the pH in

Table 1 Some selected physicochemical properties of sludge samples

Selected characteristics			
Sludge sample types	Ekbatan (E)	Shahrak-e-Gharb (G)	Shush (S)
Total solids (%)	1.8 ± 0.3	2.0 ± 0.3	1.7 ± 0.2
pH	6.8 ± 0.3	6.8 ± 0.3	7.0 ± 0.2
EC (dS/m)	0.7 ± 0.1	1.0 ± 0.1	1.3 ± 0.2
Total organic N (dry wt%)	5.53 ± 0.6	5.87 ± 0.5	6.02 ± 0.6
Organic carbon (dry wt%)	26.3 ± 3	26.6 ± 5	28.5 ± 6
P (mg/kg dry sludge)	5700 ± 450	16,634 ± 820	6154 ± 320

Table 2 Concentration and speciation of the heavy metals in the samples

Heavy metals	Type of sludge	Concentration (mg/kg dry sludge)		
		Soluble	DTPA-extractable	Total
Fe	E	1.67 ± 0.2	364.67 ± 28	$2.4 \times 10^4 \pm 1800$
	G	0.84 ± 0.1	120.73 ± 19	$1.8 \times 10^4 \pm 890$
	S	1.23 ± 0.3	245 ± 32	$2.9\ 10^4 \pm 1320$
Cd	E	0	0.24 ± 0.2	4.32 ± 1
	G	0.01 ± 0.001	1.01 ± 0.2	9.15 ± 2
	S	0	0	9.27 ± 2
Zn	E	2.25 ± 0.2	230 ± 22	$2.8 \times 10^3 \pm 318$
	G	2.25 ± 0.3	256 ± 16	$2.9 \times 10^3 \pm 297$
	S	0.25 ± 0.1	85.83 ± 7	$5.6 \times 10^3 \pm 721$
Mn	E	0.42 ± 0.1	24.19 ± 3	$4 \times 10^2 \pm 48$
	G	0.46 ± 0.2	49.83 ± 4	$1.09 \times 10^3 \pm 111$
	S	0	19.63 ± 2	$8.14 \times 10^2 \pm 67$

these samples was close to neutral, low solubility of metals was expected. Soluble heavy metals are more toxic than other forms, since the bioavailability and toxicity of heavy metals in the environment occur mostly in soluble form [23]. Thus, to apply a remediated sludge to the agricultural lands as a fertilizer, soluble form should be removed prior to use. As the bioleaching process cause the metals to be dissolved and released to the liquid phase, proper remediation plan should consider a separation step, to separate the dissolved metals from the sludge matrix to prevent metal release to the farm lands.

Variation of pH during the experiment

The variation of pH during the experiment is shown in Fig. 1. The chemical treatment was not plotted in these figures, since the pH value for this treatment was the same as the inoculated treatment. As mentioned previously, the pH value in this treatment was to be equalized exactly with the same pH as the inoculated treatment. Because the pH is an important factor for *A. ferrooxidans*, the pH had been adjusted to 4 (pH 4) before starting bioleaching. Whilst this bacterium can grow in pH ranges from 3 to 7 [29], the low pH values are more preferred and its growth is limited in neutral pH. Furthermore, it takes time for bacteria to decline pH to a favourable range extending the experimental time. In addition, due to the buffering capacity of sludge, it takes 2 or 3 days for pH to start decreasing. Therefore, we have shortened the delay phase by manual reduction of the pH prior to experiment. The bacterium oxidizes elemental sulphur into sulphate ions, thereby decreasing the pH. Then, the variation in pH in the inoculated treatment was merely a consequence of bacterial growth.

Bioleaching of heavy metals

Metal dissolution during the experiment is shown in Figs. 2, 3 and 4. It has been well demonstrated that bioleaching could successfully dissolve metals from contaminated sludge, sediments and soil [22, 30]. We also aimed to show that whether the dissolution of the metals happens because of bacterial specific effect directly or reduction in pH as an indirect res ult.

Kinetics of the dissolution

The kinetics of the dissolution in the inoculated treatment was probably according to the growth of the bacteria. At first 2 or 3 days, the dissolution curve was too slow and after 4 days (for most of the experiments), the dissolution rate increased rapidly. This point for some of the experiments was in the fifth day of the experiment. Then after a rapid dissolution step, the dissolution rate slowed down again reaching to almost a constant rate. In contrast, in the chemical treatment the kinetics of dissolution ran in a different way. Once the decreasing of pH started, the dissolution process began and continued approximately in a constant rate until the end of the experiment. The reason is that the chemical treatment dissolves metals according to the chemical reaction kinetics of the dissolution.

Cadmium

Changes in dissolved cadmium in the liquid phase are shown in Fig. 2. The results indicated that bioleaching could dissolve 71.90, 75.84 and 67.96% of Cd from E-, G- and S-type sludge samples, respectively. However, the artificial acidification could dissolve only 32.40, 20.32 and 13.37% of Cd from E-, G- and S-type sludge samples, respectively. There

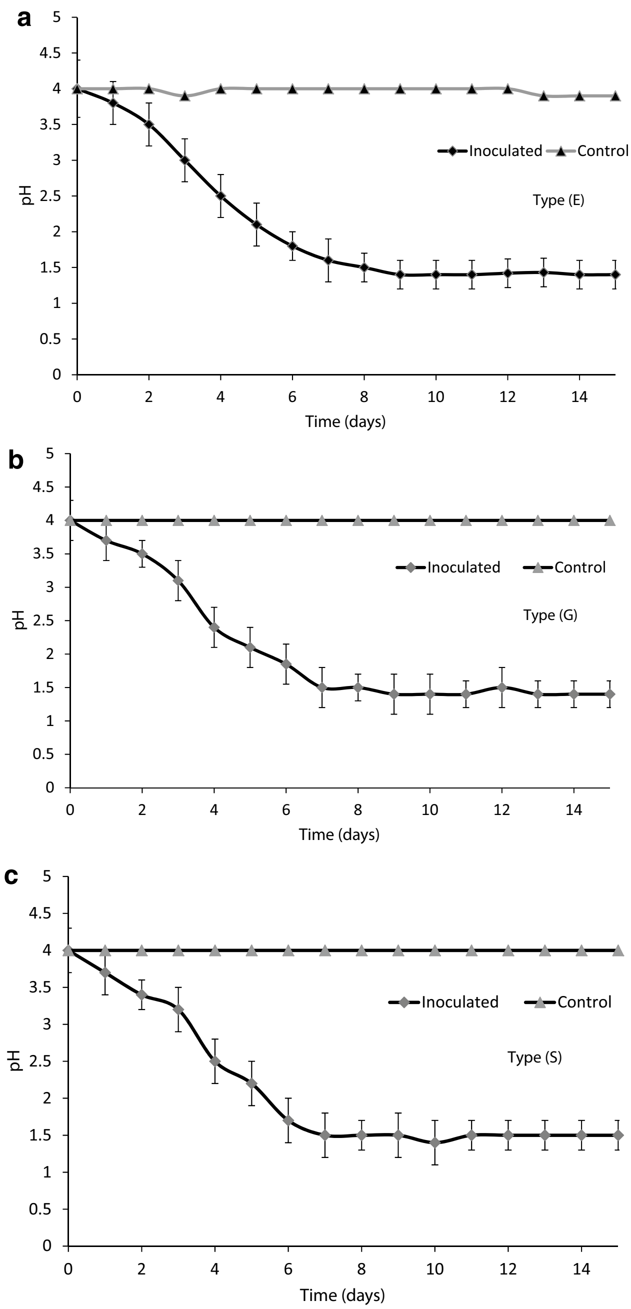

Fig. 1 Variation of pH in sewage sludge type (E), type (G) and type (S) during the experiments

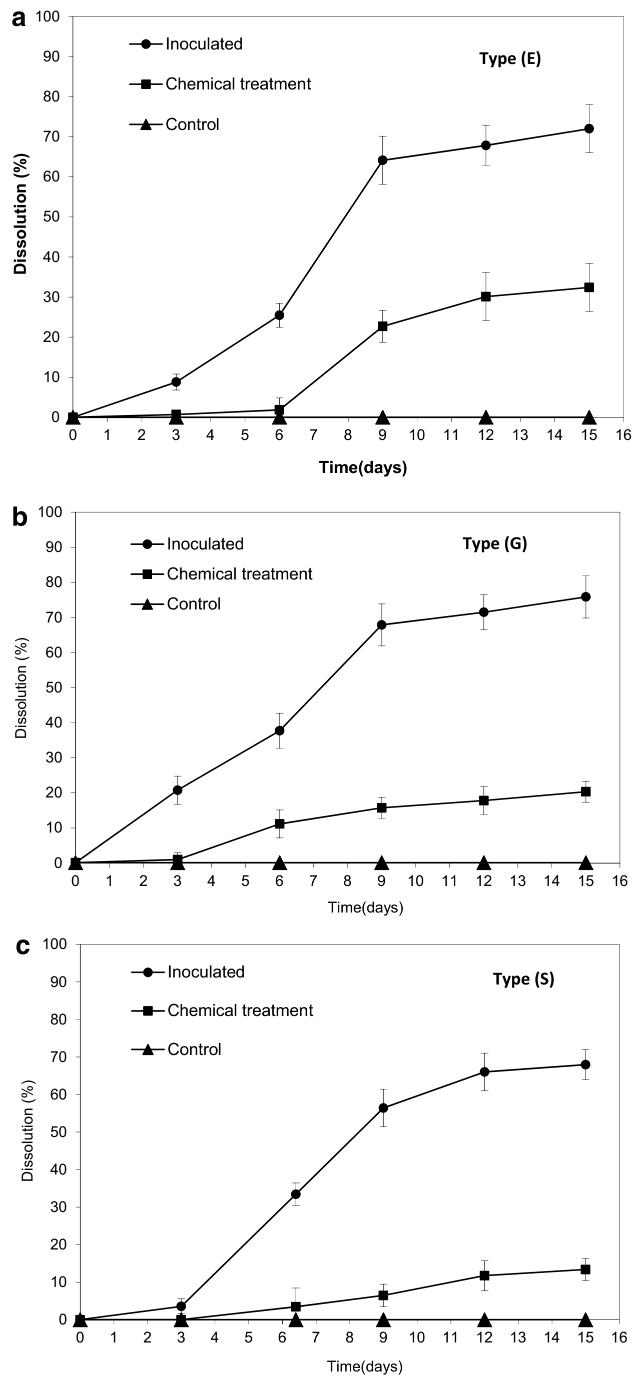

Fig. 2 Dissolution of Cd in sludge type (E), type (G) and type (S) during the experiments

was a significant difference between inoculated treatment and the chemical treatment in this regard. It means that regarding Cd dissolution, the action of bacteria is significantly important that the effect of low pH.

Manganese

As it is shown in Fig. 3, the bioleaching could dissolve Mn from solid phase of sludge successfully. The rate of dissolution was 91.80, 91.08 and 94.67% for E-, G- and S-type sludge samples, respectively. However, this rate for chemical treatment was 49.80, 10.04 and 16.06% for E-, G- and S-type sludge samples, respectively. There was a significant difference between chemical and biological treatment of sludge to dissolve its Mn content. Like Cd

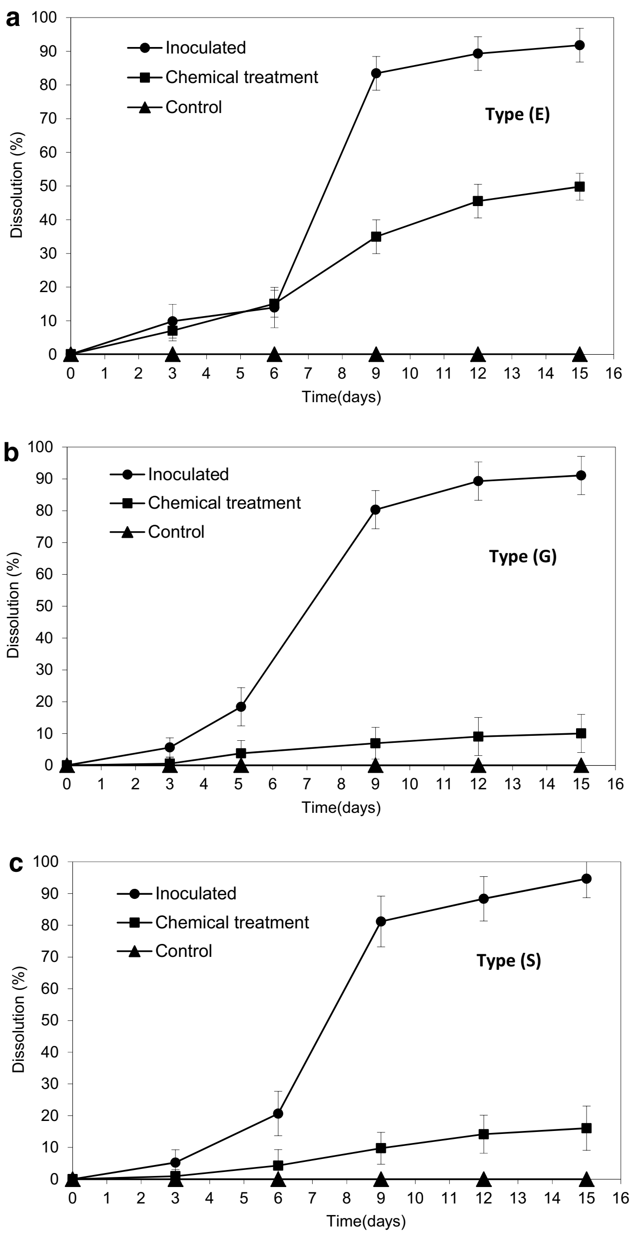

Fig. 3 Dissolution of Mn in sludge type (E), type (G) and type (S) during the experiments

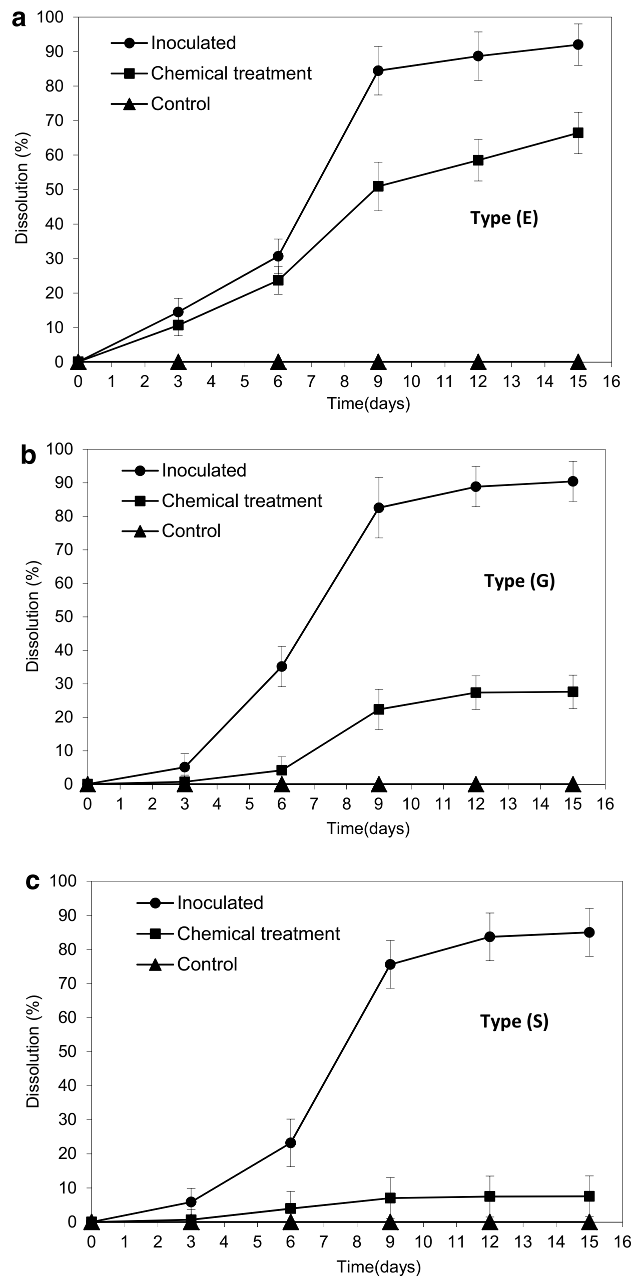

Fig. 4 Dissolution of Zn in sludge type (E), type (G) and type (S) during the experiments

dissolution, the results showed that the bacteria play the main role in Mn dissolution rather than low pH.

Zinc

The dissolution rate of Zn is shown in Fig. 4. The dissolution rate for bioleaching of Zn was 92.01, 90.44 and 84.99% for E-, G- and S-type sludge samples, respectively. Despite bioleaching, the rate of the dissolution by the chemical treatment was 7.54, 27.63 and 7.54% for E-, G- and S-type sludge samples, respectively. The bioleaching and the artificial acidification (the chemical treatment) were significantly different in dissolving Zn from the sludge. The same results were achieved regarding Cd and Mn, indicating that the bacteria activity rather than low pH contributes to Zn dissolution.

Comparison of two different strategies for leaching of the metals

The effect of the bacteria in dissolution of metals from the sludge samples has been attributed to two aspects. First aspect is the action of bacteria on metallic ions, which leads them to be more dissolved, which is known as the direct effect. Second aspect deals with the oxidizing ability of the SOB and IOB, which enables them to produce sulphate anions and subsequently decrease pH in the environment. Our results showed that even at the same pH values the bioleaching was much more efficient than the chemical treatment. The results showed a significant difference between these two aspects of metal leaching. Although we compered two different strategies for removal of heavy metals from sewage sludge, it was not the direct aim of this work. Here we were to separate the effect of bacteria and the effect of low pH on metal dissolution in the sludge samples. In this regard, other studies [6] have compared the chemical and biological leaching strategies; however, they did not conduct the leaching process in the same pH. Hence, they could not differentiate the action of bacteria and the effect of low pH on metal dissolution.

Leaching of heavy metals using different biological and chemical approaches has been practiced in many studies. To investigate the chemical leaching approach, mineral acids [31] and also organic acids such as citric, malic, acetic and lactic acids were used to leach heavy metals from municipal waste [32]. However, in the bioleaching approach using *A. ferrooxidans* in other studies [33], the rate of dissolution was lower in the case of chemical leaching. Our results confirmed that the action of bacteria was the main factor affecting metal dissolution rather than the dissolution caused by low pH. According to the results, bioleaching seems to be a promising technique in dissolution of Mn, Zn and Cd from sewage sludge. In particular, it could successfully dissolve mentioned metals in a short period. Similar results have been reported by other researchers [22]. There are also some studies that have compared artificial acidification and bioleaching in metal dissolution [6]. Separation of two aspect of bacterial action on metal dissolution is a different look to this issue [6]. This work showed that action of bacteria leads to metal dissolution rather than the reduction of pH alone. Although pH plays an important role in the dissolution of the metals, it is not the main factor in the bioleaching. There is another explanation that the bacterium can only function in a favourable range of pH. In other words, bacteria need an appropriate environment to grow and function properly. If the conditions are not in a preferred state, the growth will be limited; accordingly, dissolution rate will be lower than the optimal conditions. Since sludge is rich in nutrients, this factor is not the limiting factor for the growth of *A. ferrooxidans*. Hence, pH is probably the most important factor for the growth, in environments such as sewage sludge. Moreover, the effect of pH on the dissolution of the metals is known already.

Chemical leaching has been also proved that could dissolve metals from sludge. However, the rate of dissolution was significantly less than that of bioleaching. Despite bioleaching, the rate of dissolution for the chemical approach was not acceptable. At the best, it could dissolve only 49.5% (average of all studied metals) in sludge type E (Fig. 5), keeping in mind that the chemical treatment requires more cost and time to achieve a satisfied point [34]. The chemical approach seems to be an alternative only when a bioleaching approach is limited.

There was a significant difference among the types of sludge in terms of chemical leaching rates (Fig. 5). It showed that the type of sludge was an affecting factor. According to Tables 1 and 2, we know that there are some chemical differences among the sludge samples. Therefore, it can be concluded that the chemical composition of sludge may be an influencing factor in either chemical or biological leaching of heavy metals. For example, it seems that there is an obvious difference among the samples regarding their EC; since the EC represents the amount of soluble ions, then this difference might have affected the leaching process. Other study [22] has shown that the type of sludge showed different leaching behaviours due to the various chemical compositions. It has also been shown that the rate of bioleaching (or may be chemical leaching) depends on the sewage sludge treatment process [23]. For instance, aerobic and anaerobic sludge samples showed different dissolution rates possibly because of difference in their chemical composition, metal content, the nature of organic matrix, etc.

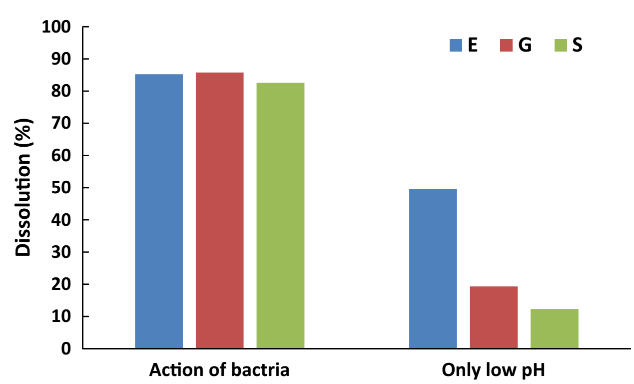

Fig. 5 Comparison of dissolution rate of three sludge samples by bioleaching (action of bacteria) and chemical leaching (only low pH)

Conclusion

Bioleaching (the effect of bacteria) could dissolve Cd (71.90%), Mn (92.50%) and Zn (89.14%), whilst the chemical leaching (low pH) by only lowering the sludge pH could dissolve Cd (22.03%), Mn (25.06%) and Zn (14.23%). Therefore, bacterial direct action on the metallic ions plays the main role in dissolution of Cd, Mn and Zn. As the results show, the lowering of pH can also lead to dissolution of metals from the solid phase; however, it is not as efficient as bioleaching by action of bacteria. In fact, bioleaching exploits not only bacterial distinctive function but also the effect of low pH. The leaching rate of metals was also depending on the sludge type and chemical composition. The sludge samples reveal different rates of dissolution, indicating that the nature of chemical compounds in the sludge is an influencing factor on metal dissolution using either bioleaching or chemical treatments. For further study on this field, different bacteria and different metals should be considered to prove the effect of different bacteria on metal dissolution.

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