Optimization of Fe2+/solids content ratio for a novel sludge heavy metal bioleaching process

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Abstract: A novel bioleaching process, preincubation bioleaching (PB) has been recently developed in our laboratory for decontamination of heavy metal-laden sewage sludge by removing the toxic low molecular weight organic acids using an isolated degradative yeast species. The objective of the present study was to optimize Fe2+/solids content ratio required for removing heavy metals from sewage sludge by using this novel process at high solids content. Results from the present study showed that regardless of the Fe2+/solids content ratio iron oxidation and sludge acidification could be achieved within 2 days confirming the effectiveness of PB in reducing the inhibitory organic acids. When PB was conducted at an elevated solids content of 3%, Fe2+/solids content ratio could be reduced to 0.75:1, 1:1 and 1.25:1 for solubilization of Zn, Cu and Cr respectively. Under this optimized bioleaching conditions, the following solubilization efficiencies were obtained after 8 days of bioleaching: 100% Zn, 96% Cu, 60% Cr with no reduction in nutrient contents. Hence, bioleaching at elevated solids contents represents a more economical way for decontamination of heavy metal-laden sewage sludge while preserving its fertility values.

Keywords: Bioleaching; Acidithiobacillus ferrooxidans; Sewage sludge; Heavy metals

INTRODUCTION
Iron-based bioleaching has been proven to be an effective means for decontamination of heavy metal-laden sewage sludge prior to land application. During this process metal solubilization can be achieved by acidification of sludge through ferrous iron oxidation by Acidithiobacillus ferrooxidans (Wong and Henry, 1988; Tyagi and Couillard, 1991; Lombardi and Garcia, 1999). This process is usually initiated at pH 4.0 due to acidophilic feature of the bioleaching bacterium and subsequent acidification is generally attributed to precipitation of ferric iron in the form of jarosite (Eq. 1) although formation of ferric hydroxide (Eq. 2) also contributes to the reduction of sludge pH (Wong and Henry, 1988). Wong et al. (2002) reported that heavy metal bioleaching efficiency was not significantly affected by initial sludge pH and sludge preacidification could be avoided if the amount of ferrous iron was raised to 4 g l⁻¹.

\[
3\text{Fe}^{3+} + X^- + 2\text{HSO}_4^- + 6\text{H}_2\text{O} \rightarrow \text{XFe}_3(\text{SO}_4)_6(\text{OH})_6 + 8\text{H}^+ \quad (1)
\]

\[
\text{Fe}^{3+} + 3\text{H}_2\text{O} \rightarrow \text{Fe(OH)}_3 + 3\text{H}^+ \quad (2)
\]

Where X represents monovalent metal ion.

However, the survival of this bacterium in sewage sludge will be adversely affected by the presence of various kinds of organic compounds (Tuttle and Dugan, 1976; Martin, 1978). Growth of A. ferrooxidans has been found significantly inhibited by the presence of acetic acid and propionic acids during bioleaching of anaerobically digested sewage sludge (Gu and Wong, 2004a).

Significant inhibitory effects of sewage sludge on iron oxidation and metal solubilization have also been observed by other researchers (Fournier et al., 1998; Cho et al., 2002). Hence, the initiation of this bioleaching is highly dependent on degradation of organic acids by heterotrophic microbes. Our previous study showed that coinoculation of A. ferrooxidans and acetate and propionate degrading yeast Blastoschizomyces capitatus Y5 accelerated iron oxidation and metal solubilization during the bioleaching of sludge. One drawback of this
coinoculation system is that these inhibitory effects persisted in the course of biodegradation of acetate and propionic acids (Wong and Gu, 2004). Fortunately this inhibition can be successfully removed by preincubating the sludge with inoculation of *B. capitatus* Y5 for 24 hours before inoculation of iron-oxidizing bacteria, and this newly developed bioleaching process was named as preincubation bioleaching (PB) (Gu, 2004).

Usually bioleaching is performed at a fixed concentration of energy source. Theoretically, such a system would be inapplicable for practical operation since solids content changes continuously even in the same sewage treatment plant. Actually reduced metal solubilization efficiency has been reported for bioleaching process at high solids contents (Blais et al., 1993; Cho et al., 2002). Recently iron/solids content ratio has been proposed as a controlling parameter to solve this problem and results show that metal solubilization rates remain unchanged at solids content ranging from 1% to 3% when bioleaching is initiated at an iron/solids content ratio of 2:1. However, concentration of ferric iron in the liquid phase at the end of PB process could be as high as 6600 mg l⁻¹ at a solids content of 3% (Gu, 2004). This indicates that energy source could be further reduced when PB process is initiated at the elevated solids content. Therefore, the objective of the present study is to optimize the iron/solids content ratio required for removing heavy metals from anaerobically digested sewage sludge by using PB process at elevated solids content.

**METHODS**

*Physicochemical properties of the anaerobically digested sewage sludge*

The anaerobically digested sludge was collected from the anaerobic digester at the Yuen Long Sewage Treatment Plant, Hong Kong SAR, P. R. China and stored at 4°C in a cold storage room to prevent any changes in the physicochemical properties. Selected physicochemical properties of the sludge are shown in Table 1. This sludge was considered toxic to plants due to high levels of Zn, Cu and Cr and would be unsuitable for land application according to the limits of metals set out in the Netherlands (National Research Council, 2002). It was also considered highly toxic to *A. ferrooxidans* due to the presence of high concentrations of acetic and propionic acids (Gu and Wong, 2004a).

| Table 1 Selected properties of Yuen Long anaerobically digested sludge |
|-----------------|-----------------|-----------------|
| Parameter       | Parameter       |                |
| pH              | 7.11            | 42.7           |
| ORP (mV)        | -222            | 25.5           |
| Solids content (%) | 0.85        | 1.00           |
| Organic matter (%) | 74.6          | 21,685         |
| Acetic acid (mM) | 5.58           | 204            |
| Propionic acid (mM) | 2.53         | 108            |

*Microorganisms and culture media*

*A. ferrooxidans* ANYL-1 was an indigenous strain of iron-oxidizing bacterium isolated from local Yuen Long sludge (Gu and Wong, 2004b). This bacterium was grown and maintained in liquid FeP medium (Johnson, 1995) at pH 2.0 with 20 g l⁻¹ FeSO₄·7H₂O as the energy source. The inoculum was prepared by growing the bacterium in 500 ml conical flasks containing 150 ml FeP medium. The stock culture was first activated in fresh medium for three consecutive times before being used in the bioleaching experiments. *Blastoschizomyces capitatus* Y5 was a yeast strain capable of degrading either acetate or propionate, and was isolated from local anaerobically digested sludge in our previous study (Gu and Wong, 2004b). The inoculum was prepared by activating the stock culture three times in liquid MSAP medium at pH 3.5, which contained: (NH₄)₂SO₄ 2g, MgSO₄·7H₂O 0.25g, K₂HPO₄ 0.25g, KCl 0.1g, Ca(NO₃)₂·2H₂O 0.01g, CH₃COOH 0.5 ml, CH₃CH₂COOH 0.5 ml, H₂O 1,000 ml.
Bioleaching experiments

Two sets of experiments using the newly developed preincubation bioleaching process (PB) were designed in the present study. The first bioleaching experiment was performed for optimization of Fe\(^{2+}\)/solids content ratio for bioleaching heavy metals from anaerobically digested sludge at elevated solids content. The solids content in all treatments were adjusted to 3%. The bioleaching experiment was carried out in 500 ml flasks, each containing 165 ml sludge slurry, into which 2.5, 3, 3.75 and 4.5 g of FeSO\(_4\)·7H\(_2\)O, was added and mixed thoroughly to obtain Fe\(^{2+}\)/solids content ratios of 0.75:1, 1:1, 1.25:1, 1.5:1 and 2:1, respectively. In order to remove organic acids present in the sludge, 5 ml of activated culture of *B. capitatus* Y5 was inoculated and the mixture was incubated at 30°C and 180 rpm for 24 hours in a gyratory shaker to remove inhibitory organic acids present in this sludge. The bioleaching process was initiated by inoculating 30 ml of actively growing culture of *A. ferrooxidans* into the sludge mixture at the end of preincubation. All treatments were conducted in triplicates. During the bioleaching experiments, 10 ml of sludge sample was removed periodically from each of the flasks and added to a 30 ml centrifuge tube. After measurement of pH and ORP using an Orion 720A pH meter, the sludge was centrifuged at 10,000 rpm for 10 min to separate the solid phase. The liquid fraction was filtered, acidified with concentrated HNO\(_3\) to about pH 1.0 and then stored at 4 °C prior to determination of soluble Zn, Cr and Cu by atomic absorption spectrometry (AAS). Ferrous and ferric iron was determined by 1, 10-phenanthroline methods immediately before acidification of the filtrate (APHA, 1985).

The second experiment was performed at the optimized Fe\(^{2+}\)/solids content ratio to assess nutrient leachability as affected by the PB process. Other bioleaching conditions were the same as that of the first bioleaching experiment. During the bioleaching period, 100 ml of sludge samples were removed daily and added to a dried and pre-weighed 250 ml centrifuge tube. The sludge was centrifuged at 10,000 rpm for 10 min to separate the solid fraction. After measurement of solids content by oven-drying at 105 °C, the sludge was pulverized for chemical analysis. Total organic matter was determined by loss on ignition of oven-dried sludge samples in a muffle furnace at 550 °C for 16 hours. Total N and total P were extracted by semi-micro Kjeldahl digestion and the N and P content in the digest were determined as NH\(_4\)-N and PO\(_4\)-P by indophenol blue method and molybdenum blue method, respectively (Page et al., 1982). Total metals were extracted by di-acid digestion (conc. HNO\(_3\) + conc. HClO\(_4\)), and determined by atomic absorption spectrometry (Page et al., 1982).

RESULTS AND DISCUSSION

Effects of Fe\(^{2+}\)/solids content ratio on sludge acidification during the bioleaching process

Trends of pH and ORP changes during the bioleaching period were very similar in all treatments with different Fe\(^{2+}\)/solids content ratios (Fig. 1). After a 24-hour lag period sludge pH decreased quickly to its lowest level at day 2 and then remained unchanged till the end of the bioleaching period. The final pH of the bioleaching matrix decreased slightly to 2.07 to 2.57 with increasing Fe\(^{2+}\)/solids content ratio in the treatments with Fe\(^{2+}\)/solids content ratio ranging from 0.75:1 to 2:1. Unlike the effects on pH, Fe\(^{2+}\)/solid content ratio did not show any influence on sludge ORP (Fig. 1). After a 24-hour lag time, sludge ORP of all the different treatments increased to the maximum level after 2 days of bioleaching with no further change in ORP in the remaining bioleaching period.
Changes of pH and ORP during the bioleaching of anaerobically digested sewage sludge. Symbols: □, 0.75:1; ■, 1:1; △, 1.25:1; ▲, 1.5:1; ◊, 2:1.

Figure 1. Changes of pH and ORP during the bioleaching of anaerobically digested sewage sludge.

**Effects of Fe<sup>2+</sup>/solids content ratio on iron oxidation during the bioleaching process**

Changes of ferrous and ferric iron during the bioleaching process are shown in Figure 2. Acetic and propionic acids were identified as the major inhibitory compounds involved in the bioleaching of anaerobically digested sewage sludge (Gu and Wong, 2004a). However, in the present study there was only one-day lag time before iron oxidation occurred and complete iron oxidation could be achieved on day 2 in all treatments, confirming the effectiveness of the PB process in consuming inhibitory organic acids (Gu, 2004). As a result the residual ferrous iron in the liquid phase decreased to below 50 mg l<sup>-1</sup> while ferric iron increased to its maximum level, which remained, almost unchanged during the remaining bioleaching period. In iron-based bioleaching process, biooxidation of ferrous iron is a key reaction controlling pH and ORP of sludge. The complete iron oxidation at day 2 explains the reason why the sludge ORPs of all treatments were the same after day 2. The concentration of ferric iron in the liquid phase increased with an increase in Fe<sup>2+</sup>/solids content ratio. This can explain the slightly lower pH in those treatments with higher Fe<sup>2+</sup>/solids content ratios (Fig. 1). At the end of the bioleaching period, the concentration of ferric iron in the liquid phase from the bioleaching systems ranged from 453 to 5538 mg l<sup>-1</sup>.

Changes of pH and ORP during the bioleaching of anaerobically digested sewage sludge. Symbols: □, 0.75:1; ■, 1:1; △, 1.25:1; ▲, 1.5:1; ◊, 2:1.

Figure 2. Effects of Fe<sup>2+</sup>/solids content ratio on iron oxidation during the bioleaching process.
Effects of Fe\textsuperscript{2+}/solids content ratio on metal solubilization during the bioleaching process

Solubilization of Zn, Cu and Cr during the bioleaching process is given in Figure 3. Zinc was solubilized immediately after inoculation with iron-oxidizing bacteria. Although a decrease in Fe\textsuperscript{2+}/solids content ratio caused a reduction in Zn solubilization at day 1, the maximum Zn solubilization was reached at day 2 and no significant difference was noted among all treatments except the treatment with the highest Fe\textsuperscript{2+}/solids content ratio of 2:1. Only one day was required to reach maximum solubilization of Zn due to its lower pH in this treatment (Fig. 1).

The different Fe\textsuperscript{2+}/solids content ratios did not show a significant effect on the pattern of Cu solubilization during the bioleaching process. Following a lag period of one-day, Cu solubilization in all treatments increased sharply from day 2 to the maximum level at day 3 and then maintained more or less the same level till the end of bioleaching period. The different Fe\textsuperscript{2+}/solids content ratios did not affect the final Cu solubilization efficiency of about 90% except that the sludge with Fe\textsuperscript{2+}/solids content ratio of 0.75:1 had a significantly lower final Cu solubilization rate of about 80% (Fig. 3). This might be due to a slightly higher pH observed in this treatment (Fig. 1).

Irrespective of the Fe\textsuperscript{2+}/solids content ratio, Cr started to solubilize after a lag period of one day but the solubilization rate started to level off after 4 days of bioleaching. An increase in Fe\textsuperscript{2+}/solids ratio significantly increased the final Cr solubilization rate. The maximum solubilization rate obtained at the end of bioleaching was 18.6%, 35.5%, 51.6%, 56.5% and 60.0% for the sludge with a Fe\textsuperscript{2+}/solids content ratio of 0.75:1, 1:1, 1.25:1, 1.5:1 and 2:1, respectively. The treatment with a Fe\textsuperscript{2+}/solids content ratio of 0.75:1 had the lowest solubilization rate of Cr, which could partly explained by the higher pH in this treatment. However, it is difficult to explain the difference in the amount of Cr solubilized in other treatments since only a little difference in sludge pH and ORP was observed in the sludge with Fe\textsuperscript{2+}/solids content ratio ranging from 1:1 to 2:1 (Fig. 1).

The optimum Fe\textsuperscript{2+}/solids content ratio for metal solubilization differed among the metal species studied. Satisfactory solubilization of Cr required a Fe\textsuperscript{2+}/solids content ratio ≥1.25:1. However a low Fe\textsuperscript{2+}/solids content ratio of 0.75:1 and 1.00:1 was sufficient to achieve significant removal of Zn and Cu respectively. Sewage sludge was usually contaminated by multiple metal species, therefore Fe\textsuperscript{2+}/solids content ratio 1.25:1 was recommended for bioleaching of sewage sludge at an elevated solids content of 3%. Compared with a Fe\textsuperscript{2+}/solids content ratio 2.1 required for bioleaching process at low solids content of 1%, energy source consumption could be reduced by 37.5% under these conditions.

Nutrient leachability as affected by PB process

One major concern about bioleaching of sewage sludge is the potential loss of nutrient such as N, P, and K, which will reduce the fertilizer values of the decontaminated sewage sludge. In the present study there was a significant reduction in the concentration of total N and total P content in sludge solids after 8 days of bioleaching (Table 2). However this reduction was caused mainly by the dilution effect due to an increase in solids content from 3.00% to 4.65% rather than acidic hydrolysis of organic matter during the preincubation period. Increasing solids content could be attributed to the precipitation of Fe\textsuperscript{3+} in the form of Fe(OH)\textsubscript{3} or jarosite during the bioleaching period (Blais et al., 1993a). If the increase in solids content is also considered, only 6.25% of N and 7.87% of P was lost through acidic dissolution at the end of bioleaching. The changes of total K in the solid phase were totally different from those of N and P (Table 2). In spite of dilution effect caused by increased solids content, total K content still showed a significant increase of 16% at the end of bioleaching. Chemical analysis demonstrated that the decrease in soluble K\textsuperscript{+} was exactly accompanied by an increase in total K of solid phase during the bioleaching period (data not shown), indicating immobilization of soluble K due to the formation of jarosite (Eq. 1). This can explain the presence of a slightly K-enriched decontaminated sludge after 8 days of bioleaching.
Table 2 Effects of bioleaching on fertilizer values of the decontaminated sludge

<table>
<thead>
<tr>
<th></th>
<th>Total N</th>
<th>Total P</th>
<th>Total K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before bioleaching</td>
<td>42.7</td>
<td>25.5</td>
<td>1.00</td>
</tr>
<tr>
<td>After bioleaching</td>
<td>25.5</td>
<td>15.5</td>
<td>1.16</td>
</tr>
<tr>
<td>Nutrient loss (%)</td>
<td>6.25</td>
<td>7.87</td>
<td>-80</td>
</tr>
</tbody>
</table>

CONCLUSION

Effects of Fe²⁺/solids content ratio on bioleaching of heavy metals from anaerobically digested sewage sludge was studied at elevated solids content of 3% using the preincubation process. The results showed that optimum Fe²⁺/solids content ratio for metal solubilization differed among the metal species studied. The optimum Fe²⁺/solids content ratio for solubilization of Zn, Cu and Cr during the bioleaching process was 0.75:1, 1:1 and 1.25:1, respectively. Energy source consumption could be reduced by 37.5% by performing PB with the recommended Fe²⁺/solids content ratio of 1.25:1 and at an elevated solids content of 3%. Furthermore this process led to a slightly K-enriched decontaminated sludge while preserving more than 90% of total N and total P.

ACKNOWLEDGEMENT

The work described in this paper was fully supported by a Faculty Research Grant from Hong Kong Baptist University (project No. FRG 04 05/II 28).

REFERENCES


