DOCTORAL THESIS

Improving heavy metal bioleaching efficiency through microbiological control of inhibitory substances in anaerobically digested sludge

Gu, Xiangyang

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Improving Heavy Metal Bioleaching Efficiency Through Microbiological Control of Inhibitory Substances in Anaerobically Digested Sludge

GU Xiangyang

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Principal Supervisor: Dr Jonathan W. C. WONG

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Abstract

Iron-based bioleaching process has been proven to be a promising technique for remediation of heavy metal-laden sewage sludge before land application. However sewage sludge is composed mostly of organic matter, which was generally considered to be toxic to *Acidithiobacillus ferrooxidans*. The aim of the present study was to improve heavy metal bioleaching efficiency through identifying and controlling the potential inhibitory substance(s) present in anaerobically digested sewage sludge.

A simple and reliable plating method was essential for understanding the roles of iron-oxidizing bacteria in the bioleaching process. However isolation and enumeration of acidophilic iron-oxidizing bacteria from sludge samples is always challenged with contamination of fungal growth on solid medium. The present study showed that filtering the sludge sample through sterile No. 5 Whatman filter paper before serial dilution was an effective way to overcome this problem. FeTSB was a reliable medium for both sludge samples and pure cultures, while medium TSM1 were suitable only for pure cultures. The optimum pH for FeTSB medium ranged from 2.5 to 2.75, while pHs out of this range resulted in lower counts and a long lag period of 12-14 days for colony formation. Double-lain technique could be simplified as single-layer plating technique since introduction of heterotrophic bacteria or yeast did not led to any improvement in plating efficiency. Using this simplified plating method, an indigenous mesophilic and acidophilic chemoautotrophic iron-oxidizing bacterium *Acidithiobacillus ferrooxidans* ANYL-1 was successfully isolated from local anaerobically digested sewage sludge in Yuen Long Sewage Treatment Plant.

When this bacterium was used in sludge bioleaching, a longer bioleaching period of 4 and 6 days was required to leach Cu and Cr from fresh anaerobically digested sludge (AD) as compared to 3 and 4 days for sludge sample collected after sludge holding tank (SHT). These inhibitory effects were reconfirmed in the sludge filtrate media of two sludges which were collected from the same sewage treatment plant but at two different time periods i.e., sludge A and N. The contents of potentially inhibitory substances including selected heavy metals, anions, reducing sugars, and organic acids were determined for these two filtrates. The inhibition of iron oxidation in filtrate of Sludge A was caused mainly by acetic acid while that in Sludge N was caused by both acetic and propionic acid. The bioleaching period for maximum solubilization of Cu and Cr was extended from only 3 and 4 days for Sludge J with low levels of organic acids to10 days for Sludge N which contained 10.8 mM acetic acid and 9.88 mM propionic acid required and 4 and 8 days for Sludge A containing 2.94 mM acetic acid.

During the bioleaching of Sludge N, there were sharp increases in total counts of both acetate- and propionate-degrader before vigorous growth of iron-oxidizing bacteria. Two yeasts (Y4 and Y5) capable of degrading acetate and/or propionate were isolated

and identified as *Pichia sp.* and *Blastoschizomycetes capitatus* respectively. These two yeasts were inoculated simultaneously with the iron-oxidizing bacteria to Sludge N at the beginning of bioleaching to remove the inhibitory effects caused by acetic and propionic acid. Metal solubilization was significantly accelerated by inoculation of *B. capitatus* Y5 and the bioleaching time was reduced from 10 day in the control to 3 and 4 days for Cu and Cr, respectively. However, *Pichia sp.* Y4 did not lead to any improvement in metal solubilization. *B. capitatus* Y5 alone was enough to remove both acetic and propionic acids.

However, the lag time for iron oxidation in Sludge N with inoculation of *B. capitatus* Y5 (2 days) was still one day longer than that of the sludge with low levels of organic acids (1 day), indicating that the organic acids still exerted an initial inhibition on the growth of iron-oxidizing bacteria. To overcome this problem, the organic acid-laden sludge slurry with *B. capitatus* Y5 inoculum and iron sulfate addition was preincubated at 30℃ for 24 hours before the inoculation of iron-oxidizing bacteria for bioleaching. Under this new bioleaching process (BPYb), the optimal bioleaching conditions could be achieved within 24 hours which was similar to that of the sludge with a low level of organic acids. As a result, optimum bioleaching efficiency could be reached 3 days after bioleaching while 5 days were required to reach similar metal solubilization efficiency with iron-oxidizing bacteria introduced simultaneously with *B. capitatus* Y5 after a 24-hour preincubation period.

The effect of solids content on metal bioleaching efficiency was investigated to optimize the BPYb process. The bioleaching was carried out at a Fe^{2+}/solids content ratio of 2:1. At a low solids content of 1%, no lag period existed before solubilization of Zn, Cu and Cr occurred, but a one-day lag period was observed when solids content was raised to 2-4%. Nevertheless there was no difference in the amount of Zn, Cu and Cr solubilized after 3 days of bioleaching with solids content ranged from 1-3% while treatment with 4% of solids content showed a lower solubilization of Cu and Cr. The present study demonstrated that the bioleaching could be performed at higher solids content of up to 3% without affecting the metal solublization efficiency. Furthermore, a lower Fe^{2+}/solids content ratio of 0.75:1 and 1.00:1 was enough for significant solubilization of Zn and Cu respectively, but a Fe^{2+}/solids content ratio ≥1.25:1 was required for Cr solubilization. Sewage sludge was usually contaminated by multiple metal species, therefore a Fe^{2+}/solids content ratio of 1.25:1 was recommended for bioleaching of sewage sludge at a solids content of no more than 3%.

Under the present optimized bioleaching conditions, a reduction of 40.3% and 40.6% in the concentration of total N and total P in the sludge solids was noted respectively. However this reduction in nutrient contents was caused partly by the "dilution effect" due to the increase in solids content. With the consideration of the increase in solids content, only 6.25% of N and 7.87% of P was lost through acidic dissolution at the end of bioleaching. Although there was a slight reduction in the content of organic matter in the solids, it should not affect much the soil conditioning properties of decontaminated sludge.

In can be concluded that the newly developed bioleaching process with a simultaneous inoculation of *B. capitatus* Y5 and iron sulfate addition at the beginning of a 24-hour preinoculation period could effectively remove the inhibitory effects caused by organic acids and remove the lag period.

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