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Degradation of inhibitory substances by heterotrophic microorganisms during bioleaching of heavy metals from anaerobically digested sewage sludge

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Abstract

The presence of organic acids was found to be inhibitory to the bioleaching of sewage sludge and the objective of the present study was to elucidate the roles of heterotrophic microorganisms in removing organic acids during the bioleaching of heavy metals from anaerobically digested sewage sludge. Microbiological analysis showed that acetic and propionic acids posed a severe inhibitory effect on iron-oxidizing bacteria as reflected by a sharp decrease in their viable counts in the first 4 d and it only started to increase 2 d after the depletion of both acids. Biodegradation of these inhibitory organic acids was revealed by sharp increases in total fungi and acidophiles between day 3 and day 5 which coincided with degradation of organic acids. This was further confirmed by the increases in total counts of both acetate and propionate degraders in the same period. Two yeast strains Y4 and Y5 with strong ability to degrade acetate and/or propionate were isolated and identified as *Pichia* sp. and *Blastoschizomycetes capitatus*, respectively. *B. capitatus* Y5 was an more important player in removing the inhibitory organic acids during the bioleaching process since it could utilize both acetate and propionate as sole carbon source while *Pichia* sp. Y4 was an strict acetate degrader. Results from the present study not only provided the evidence for biodegradation of organic acids by heterotrophs, but also disclosed a biological mechanism for the initiation of bioleaching of organic acid-laden sewage sludge.

Keywords: Bioleaching; Heavy metal; Sewage sludge; *Acidithiobacillus ferrooxidans*; Organic acid

1. Introduction

Bacterial leaching (or bioleaching) has been proven to be a promising means for decontamination of heavy metal-laden sewage sludge for facilitating its land utilization as fertilizer or soil conditioner. In principle, heavy metals in sewage sludge can be leached out through sludge acidification brought about either by oxidation of sulfur by *Acidithiobacillus thiooxidans* (formerly *Thiobacillus thiooxidans*) (Blais et al., 1992; Jain and Tyagi, 1992) or by ferrous iron oxidation by *Acidithiobacillus ferrooxidans* (formerly *Thiobacillus ferrooxidans*) (Tyagi et al., 1988; Wong and Henry, 1988; Blais et al., 1993; Tyagi et al., 1993; Xiang et al., 2000; Wong et al., 2002; Gu and Wong, 2004a). Iron-based bioleaching process is considered superior to sulfur-based bioleaching process because no residual sulfur formed in the decontaminated sludge, excluding the risk of secondary pollution caused by the acidification of soil due to sulfur oxidation (Wong et al., 2002). This process is usually initiated by preacidification of the sludge to pH 4.0 and the addition of ferrous iron followed by inoculation of iron-oxidizing bacteria (Tyagi et al., 1988; Wong and Henry, 1988). However, Wong et al. (2002)

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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 $4\ g\ l^{-1}$.

During the iron-based bioleaching process both direct and indirect mechanism are involved in the solubilization of metals in the sludge (Tyagi et al., 1988; Couillard and Zhu, 1992). Metal sulfide present in sewage sludge could be directly oxidized by *At. ferrooxidans* (Eq. (1)) or indirectly oxidized by ferric ion to metal sulfate (Eq. (2)). The newly formed ferrous iron will be reoxidized by the bacteria (Eq. (3)) and when the iron redox reactions repeat, increasing the overall metal solubilization efficiencies. The elemental sulfur in Eq. (4) will be oxidized to sulfuric acid by the bacteria. The production of sulfuric acid decreases the sludge pH which will further increase the solubilization of metals.

$$MS + 2O_2 \xrightarrow{At.\ ferrooxidans} M^{2+} + SO_4^{2-} \quad (1)$$

$$MS + 2Fe^{3+} \rightarrow M^{2+} + 2Fe^{2+} + S^0 \quad (2)$$

$$4Fe^{2+} + O_2 + 4H^+ \xrightarrow{At.\ ferrooxidans} 4Fe^{3+} + 2H_2O \quad (3)$$

$$2S^0 + 3O_2 + 2H_2O \xrightarrow{At.\ ferrooxidans} 2H_2SO_4 \quad (4)$$

($MS =$ Metal sulfide, $M^{2+} =$ soluble metal ions).

The optimal conditions for the culture of *At. ferrooxidans* have been extensively studied. It is well established that this bacterium does not tolerate various organic compounds, especially low molecular weight organic acids (Tuttle and Dugan, 1976; Martin, 1978; Leduc and Ferroni, 1994; Frattini et al., 2000; Gu and Wong, 2004b). Sewage sludge is composed mostly of organic matter, which may contain inhibitory substance(s) and thus affect the performance of this bioleaching process. In fact significant inhibitory effects of anaerobically digested sludge or sludge extract on iron oxidation and metal solubilization have been reported (Fournier et al., 1998; Cho et al., 2002). Unfortunately, no specific inhibitory compounds have been identified in these bioleaching systems. Our recent study showed that acetic and propionic acids constituted the major inhibitory substances and a prolonged bioleaching time of 10 d has been observed for maximum solubilization of Cu and Cr due to the presence of 10.8 mM acetic acid and 9.88 mM propionic acid in an anaerobically digested sewage sludge (Gu and Wong, 2004b).

Obviously organic acids, which are normal metabolic products of anaerobic digestion of organic matter, will pose an unfavorable condition for growth of *At. ferrooxidans* and subsequent metal solubilization from the sludge solid phase (Gu and Wong, 2004b). Detailed mechanism for bioleaching of heavy metals from anaerobically digested sewage sludge under this unfavorable condition has not yet been fully understood. Although sewage sludge contains a large variety of microorganisms, the potential of naturally-occurring heterotrophic microorganisms that can consume the inhibitory organic acids has not been explored. Therefore, the objective of the present study was to elucidate the roles of heterotrophic microorganisms through microbial population analysis during the bioleaching of anaerobically digested sewage sludge.

2. Materials and methods

2.1. Sludge sampling and characterization

Two anaerobically digested sludge samples were collected in September (sludge S), and November (sludge N) 2002, respectively, from the same anaerobic digester of the Sewage Treatment Plant at Yuen Long district, Hong Kong SAR, PR China. To avoid any changes in physicochemical properties, the sludge was stored at 4 °C and used throughout the study. Physicochemical properties of these two sludges have been reported in our previous study (Gu and Wong, 2004b). Sludge N used in the present study has been found to be highly toxic to *At. ferrooxidans* ANYL-1 due to the presence of 10.8 mM acetic and 9.88 mM propionic acid (Gu and Wong, 2004b). Filtrate from sludge S, which contained only 0.09 mM acetic acid and 0.08 mM propionic acid, was used for the preparation of the control treatment during the bioleaching experiments with sludge N.

2.2. Microorganisms and culturing conditions

Chemoautotrophic Fe/S-oxidizing bacterium *At. ferrooxidans* ANYL-1 was isolated from Yuen Long anaerobically digested sludge in our previous study. Its optimum growth conditions were 30–35 °C and pH 2–2.5 in mineral salt iron medium (Gu and Wong, 2004a). The inoculum was prepared by growing the bacterium in 500 ml conical flasks containing 150 ml of FeP medium (Johnson, 1995). The stock culture was initially activated in fresh medium three consecutive times, after which the active growing culture was used in the bioleaching tests.

2.3. Microbial population dynamics during the bioleaching process

A bioleaching experiment was performed to investigate microbial population dynamics in association with metal solubilization during the bioleaching of anaerobically digested sewage sludge in the presence of organic acids. Four grams of $FeSO_4 \cdot 7H_2O$ was mixed with 170 ml sludge slurry in a 500 ml flask with a final solids content of 1% and to the mixture 30 ml of active growing culture of *At. ferrooxidans* ANYL-1 was introduced as the inoculum. The initial cell concentration of iron-oxidizing bacteria was about 1.2×10^7 cfu ml^{-1}. The control treatment was prepared using the same sludge but with the liquid fraction replaced by sludge filtrate from sludge S, which contained only trace amount of organic acids (Gu and Wong, 2004b). The whole setup was incubated on a gyratory shaker at 30 °C and 180 rpm. During the incubation period,