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Enhanced dewaterability of anaerobically digested sewage sludge using *Acidithiobacillus ferrooxidans* culture as sludge conditioner

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HIGHLIGHTS

- Iron-oxidizing bacterial mediated acidification enhanced the sludge dewaterability.
- *Acidithiobacillus ferrooxidans* produced composite sludge flocculant.
- Different fractions of *A. ferrooxidans* culture were tested as sludge conditioner.
- *A. ferrooxidans* mediated conditioning resulted in no reversible sludge flocculation.

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ABSTRACT

Role of *Acidithiobacillus ferrooxidans* culture in bioacidification and dewaterability of anaerobically digested sewage sludge (ADS) was investigated. *A. ferrooxidans* culture grown in 9K medium along with Fe^{2+} produced iron flocculant containing, secondary iron minerals and biopolymeric substances as confirmed by FT-IR, XRD, and SEM-EDX. Bioacidification of ADS was performed using 10% (v/v) *A. ferrooxidans* culture, isolated cells and cell-free culture filtrate; and dewaterability was assessed using the capillary suction time (CST) and specific resistance to filtration (SRF). Isolated bacterial cells significantly ($P < 0.05$) reduced the sludge dewaterability when supplemented with Fe^{2+} while the whole culture and cell-free filtrate rapidly acidified the sludge without Fe^{2+} and showed significant reduction of CST (71.3–73.5%) and SRF (84–88%). Results clearly indicated that the culture and filtrate of the *A. ferrooxidans* facilitated rapid sludge dewaterability while the cells supplemented with Fe^{2+} also enhanced dewaterability but required 2–4 days.

1. Introduction

Conventional activated sludge treatment of municipal sewage generates a large quantity of waste activated sludge as the end product. Sludge management is one of the major problems in the sewage treatment. The waste activated sludge needs to be stabilized and dewatered before disposing or recycling. Anaerobic digestion is a biological process generally adopted to stabilize sewage sludge as it can generate the useful biogas. Sewage sludge generally contains about >95% water, thus the dewatering is the most challenging and expensive part of the wastewater treatment plants. Sludge management costs cover over 50% of the total cost of the wastewater plant operation (Neyens et al., 2004). Sludge dewatering generally is achieved through preconditioning using inorganic coagulant or synthetic polyelectrolytes such as polyacrylamide (PAM) followed by centrifugation or filter pressing (Bolto and Gregory, 2007). This process generally yields sludge cake with moisture of around 80% or even higher (Liu et al., 2012a). In addition to high cost, the synthetic polymers can cause secondary pollutions (Chang et al., 2005; Ho et al., 2010). Inorganic polymeric flocculants have more advantages over conventional inorganic salts due to high charge density, stable hydrolysis, higher coagulation efficiency (Wu et al., 2008) and ability to make the sludge compact (Niu et al., 2013) during dewatering. However, instead of chemically synthesized inorganic flocculants, environmentally benign, and *in situ* bioprocess to produce such flocculant would be of great interest for sludge conditioning to improve the dewaterability.

Bioleaching, driven by acidophilic iron-oxidizing and sulfur-oxidizing bacteria, has been recognized as a potential method for

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the removal of heavy metal from sewage sludge (Wong and Gu, 2008; Wong et al., 2004; Zhou et al., 2013). The two common acidophilic chemolithoautotrophic bacteria involved in bioleaching process are *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. Bioleaching with iron-oxidizing bacteria requires the addition of iron sulfate as an energy substrate and the process can acidify the sludge to a pH level lower than 2.0, which is quite efficient in removing metals from sewage sludge. Tyagi et al. (1997) reported that in addition to metal leaching, a substantial amount of solids reduction also occurred during sludge bioleaching. Fang et al. (2007) observed that bioleaching was better than chemical leaching in terms of the settled sludge quantity and the suspended solids content of the effluent. Furthermore, recent studies reported that bioleaching by the co-inoculation of *A. ferrooxidans* and *A. thiooxidans* showed improvement in dewaterability of activated sludge compared to other physical treatments (Liu et al., 2012a,b). However, co-presence of sulfur-oxidizing bacteria extremely acidified the sludge pH (pH <2) and deteriorated the sludge dewaterability during bioleaching. This might be due to disintegration of flocs and deflocculation under extreme acidification, suggesting that *A. thiooxidans* mediated bioacidification of sludge may be suitable for metal leaching rather than sludge dewatering.

Unlike metal bioleaching, which requires strong acidic pH, the bioleaching mediated sludge dewatering requires an optimum pH of 2.4 to achieve better dewaterability (Liu et al., 2012b). Compared to the co-inoculation of *A. ferrooxidans and A. thiooxidans* that negatively affects the dewaterability during bioleaching (Liu et al., 2012b), *A. ferrooxidans* mediated bioleaching could be appropriate for sludge dewatering as it acidifies the sludge to the optimum pH range suitable for dewaterability. Moreover, inclusion of sulfur to the sludge system leaves residual elemental sulfur, and sulfate in sludge/wastewater would create an acidic environment when the biosolids are disposed. During the sludge bioleaching, biooxidation of ferrous iron by *A. ferrooxidans* generates ferric iron flocculant that can effectively coagulate and flocculate the sludge particles that improved the dewaterability. Hydrolysis of the ferric iron produces secondary iron minerals and H^+ ions, which lower the sludge pH (Wong and Gu, 2004; Xiang et al., 2000). In addition, *A. ferrooxidans* produces extracellular polymeric substances (EPS) that also facilitate the bioleaching process (Fazzini et al., 2011; Gehrke et al., 1998; Zeng et al., 2010).

Considering the large volume of sludge and rapid dewatering in practical scale, the use of effective flocculant that simultaneously improving the sludge settleability, dewaterability, effluent quality, and reduction in pathogen and dissolved organics would be of great interest. In this view, the use of *A. ferrooxidans* culture could be used as the sludge conditioning agent as it contains soluble Fe^{3+} ions, polymeric ferric compounds, and secondary iron-minerals from the biooxidation of Fe^{2+} (Wang et al., 2011) and biological compounds released by *A. ferrooxidans* that might enhance the dewaterability. Thus, the properties and activities of biologically derived iron flocculant may vary from commercial ferric iron flocculant employed in the sewage treatment. However, the roles of different components of *A. ferrooxidans* culture in sludge acidification and dewaterability remain to be elucidated. Bioacidification process will be an interesting area of research to achieve efficient sludge dewaterability. Therefore, the objective of this study was to characterize the different fractions of the *A. ferrooxidans* culture and their efficiency on dewaterability of anaerobically digested sludge (ADS) to understand the process. Here, we characterized the fractions of *A. ferrooxidans* culture using Fourier transform infrared spectrophotometry (FT-IR), X-ray diffraction (XRD) and Scanning electron microscopy-Energy-dispersive X-ray spectroscopy (SEM-EDX) and elucidated the dewaterability using standard laboratory filtration methods.

2. Methods

2.1. Sludge sampling

Anaerobically digested sludge (ADS) was collected from Shatin Sewage Treatment Works, Hong Kong, in clean polypropylene cans; quickly transferred to laboratory and stored at 4 °C for further use. Selected sludge properties were characterized according to the Standard Methods (APHA, 2005). The ADS used in this investigation had the following properties: pH, 7.45; oxidation reduction potential (ORP), −178 mV; total solids (TS), 2.05%; total organic matter (TOM), 43.5%; CST, 30.4 s; and SRF 1.61×10^{13} m kg^{-1}.

2.2. Microorganisms and inoculum

A. ferrooxidans ANYL-1, an indigenous strain of iron-oxidizing bacteria isolated from ADS (Gu and Wong, 2004) was used in this study. The active bacterial culture was maintained in modified 9K medium at pH 2.5, with 44.2 g/L $FeSO_4{\cdot}7H_2O$ as the energy source. Erlenmeyer flasks (500 ml) containing 225 ml of modified 9K medium was inoculated with 10% (v/v) of *A. ferrooxidans* inoculum. The culture flasks were incubated at 30 °C on an orbital shaker at 180 rpm for 3 days, and then the culture was used for sludge treatment.

2.3. Preparation of culture fractions

Well grown culture of *A. ferrooxidans* ($\sim 10^8$ cells/ml) had slight precipitation of brownish red colored iron minerals. The culture was filtered through filter paper (Advantech No. 1) to remove the coarse precipitates. Filtered bacterial culture was used for sludge conditioning. Then, the bacterial culture was fractionated to separate the cells and cells-free filtrate by passing through 0.22 μm membrane filter. The bacterial cells were recovered from the membrane by washing the membrane twice in sterile 9K medium (pH 2.5). Whole *A. ferrooxidans* culture, isolated cells and cell-free culture filtrate were evaluated for sludge bioacidification and dewaterability. To isolate the extracellular polymeric substances (EPS) from the *A. ferrooxidans* culture filtrate, the filtrate was extensively dialyzed against distilled water (pH 2.5) using 3 kDa cut off membrane to remove the Fe^{3+}, residual Fe^{2+} and other inorganic salts. The resulted dialysate was freeze dried and analyzed.

2.4. Characterization of culture fractions

To elucidate the properties of *A. ferrooxidans* culture fractions, the Fe^{2+} grown culture was collected after 3 days of growth and fractionated as stated above and characterized using Fourier transform infrared spectrophotometry (FT-IR) (Nicolet Magna 550 Series II, Mid to Far-IR), X-ray diffraction (XRD) (Bruker AXS D8) and Scanning electron microscopy-Energy-dispersive X-ray spectroscopy (SEM-EDX) (LEO 1530 Field Emission SEM with EDX from OXFORD).

2.5. Sludge treatment

Sludge treatment was carried out in 500 ml Erlenmeyer flasks containing 270 ml of ADS and 30 ml of different fractions of *A. ferrooxidans* culture: whole culture, cell-free culture filtrate and cells isolated from the culture. Then, the flasks were incubated at 30 °C in a shaking incubator at 180 rpm. To study the effect of energy substrate on dewaterability, $FeSO_4{\cdot}7H_2O$ was amended to the sludge at 0.05% to the total solid content of the sludge (0.05:1 ratio of Fe^{2+} and sludge solid). All treatments were performed in duplicate. Samples were collected from each flask