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Bioleaching of incineration fly ash by *Aspergillus niger* – precipitation of metallic salt crystals and morphological alteration of the fungus

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ABSTRACT

This study examines the bioleaching of municipal solid waste incineration fly ash by *Aspergillus niger*, and its effect on the fungal morphology, the fate of the ash particles, and the precipitation of metallic salt crystals during bioleaching. The fungal morphology was significantly affected during one-step and two-step bioleaching; scanning electron microscopy revealed that bioleaching caused distortion of the fungal hyphae (with up to 10 μm hyphae diameter) and a swollen pellet structure. In the absence of the fly ash, the fungi showed a linear structure (with 2–4 μm hyphae diameter). Energy-dispersive X-ray spectroscopy and X-ray diffraction confirmed the precipitation of calcium oxalate hydrate crystals at the surface of hyphae in both one-step and two-step bioleaching. Calcium oxalate precipitation affects bioleaching via the weakening of the fly ash, thus facilitating the release of other tightly bound metals in the matrix.

1. Introduction

Incineration offers a management option for treating incinerable municipal solid waste (MSW). In general, the volume of waste is reduced by about 90%, and energy is recovered in the process. Although all organic matter is oxidized during incineration, the less volatile inorganic waste remains in the bottom ash while the more volatile inorganic wastes are captured as residues (termed fly ash) in air pollution control devices (for instance, electrostatic precipitator [9]). MSW incineration fly ash is a granular material that contains many hazardous constituents, amongst which are heavy metals (e.g. Cd, Cu, Ni, Pb, Zn). When in contact with water, these hazardous constituents may potentially be leached [25]. Due to its toxicity, most of the fly ash is landfilled after detoxification, or recycled as a secondary material [26]. Since some of the elements (e.g. Cu and Zn) are present in high concentration and may permit an economic recovery, fly ash may be considered as an artificial ore [5]. The leached and recovered metals may be recycled for re-use as raw materials [17].

Conventional pyro- or hydro-metallurgical techniques in fly ash detoxification and heavy metal recovery include thermal treatment, chloride evaporation process and chemical leaching. Although these techniques provide a rapid treatment and complete destruction of toxic compounds in fly ash, they are very energy intensive and result in the release of hazardous emissions during treatment. The high cost and the negative environmental impact of conventional methods have led to the investigation of bioleaching (considered a clean technology) as an alternative in the extraction of heavy metals from fly ash [24,26].

The main focus in bioleaching was initially the recovery of metals from insoluble metal sulfide minerals in mining ores, based on the ability of microorganisms to oxidize reduced iron and sulfur compounds, via the production of organic or inorganic acids. There are patents on pilot- or commercial-scale bioleaching plants, with most focused on low-grade ore [8]. Recently, however, there have been interests in the application of bioleaching in industrial wastes as increasingly vast quantities of hazardous industrial wastes (such as spent catalyst, electronic waste, MSW incineration fly ash etc.), are generated [4,30]. Although much has been reported on bioleaching by the chemolithoautotrophic acidophilic microorganisms of the genus *Acidithiobacillus,* fly ash is not a suitable substrate for bioleaching due to its high pH [26]. *Acidithiobacillus* sp. grow well under pH 2–3, while fungi are generally able to grow over a wide pH range, from 1.5 to 9.8 [7,26].

Fungal bioleaching of heavy metals have been reported for solid wastes including electronic scrap material [6], spent refinery processing catalyst [2,27] and incineration fly ash [5,31,33]. In general, bioleaching may be conducted using either one-step or two-step. In the former, the microorganism is incubated together with the metal-bearing waste. In two-step bioleaching, the

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microorganism is first cultured in the growth media and incubated for a period of time before the metal-bearing waste is added to the culture and the incubation continued.

In order to better exploit this intrinsic capability of selected microorganisms for metal leaching and recycling, more efforts are needed to understand the behavior of both the microorganisms and the metal substrate during bioleaching. The objective of this study is to examine the fungal growth behaviour and its morphology in the presence of the ash, the fate of fly ash particles, and the precipitation of nano-sized metallic salt crystals during bioleaching.

2. Materials and methods

2.1. Fly ash

Municipal solid waste (MSW) incineration fly ash used in this study was obtained from Tuas South Incineration Plant in Singapore. The fly ash was autoclaved at 121 °C for 15 min prior to use.

2.2. Fungi inoculum preparation

A. niger was obtained from Dr H. Brandl (University of Zürich, Switzerland) and was cultured as previously described [32]. 7-day old conidia were harvested from the surface of potato dextrose agar (Becton Dickinson Co.) using sterile deionized (DI) water. The number of spores was counted under a microscope (Olympus CX40) at 400× magnification using a Superior Marienfeld 0.1 mm depth haemocytometer. The spore suspension was diluted with DI water to the desired spore suspension concentration (10^7 spores/ml).

2.3. Shake flask pure culture

1 ml of spore suspension was added to 100 ml of standard sucrose medium with composition (g/l): sucrose (100), $NaNO_3$ (1.5), KH_2PO_4 (0.5), $MgSO_4{\cdot}7H_2O$ (0.025), KCl (0.025), yeast extract (1.6), and incubated at 30 °C with rotary shaking at 120 rpm [32]. All reagents were of analytical grade. The liquid medium was autoclaved at 121 °C for 15 min prior to inoculation.

2.4. Shake flask one-step bioleaching

One-step bioleaching was conducted following reported protocol [32]. In one-step bioleaching, the fungus was incubated with ash at 1% pulp density. Sterile medium was added to autoclaved flasks containing the fly ash, followed by inoculation of fungal spore suspension. Samples of fungi pellet were withdrawn after Day 7, 8, 17, and 27 for SEM, EDX and XRD analyses.

2.5. Shake flask two-step bioleaching

In two-step bioleaching, the fungus was first cultured in an autoclaved sucrose medium (as in pure culture) and incubated at 30 °C with rotary shaking at 120 rpm without fly ash. After 2 days, when a large pH drop occurred, sterile fly ash at 1% pulp density was added to the culture and the incubation was continued. Samples of fungi pellet were withdrawn after Day 2, 3, 7, 8, 17 and 27 for SEM, EDX and XRD analyses.

2.6. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX)

Fungi pellet taken from pure culture, one-step bioleaching, and two-step bioleaching were washed with deionized water for three changes. The pellets were fixed with 3% (v/v) glutaraldehyde in deionized water at 4 °C overnight before being washed with deionized water and dehydrated over an ethanol gradient. Samples were dried using a critical point dryer, mounted on copper stub and sputter-coated for 120 s using a JEOL JFC-1300 Auto Fine Coater fitted with a Pt target. A JEOL JSM-5600LV scanning electron microscope (SEM) was used to examine the morphology of the fungi and fly ash. For high magnifications, field emission scanning electron microscope (FESEM), JEOL JSM-6700F was used. The images obtained were analyzed using Image-Pro Premier software to obtain the size of particles and fungal hyphae. Energy-dispersive X-ray spectroscopy (EDX) (OXFORD Instruments 6647) was coupled to the SEM for surface elemental analysis of the fungal samples. The EDX data were analyzed using INCA Suite Version 4.01.

2.7. X-ray diffraction (XRD)

Fungal pellet samples taken from pure culture, one-step bioleaching, and two-step bioleaching were washed with DI water (three changes). After lyophilization, the pellet was mixed with liquid nitrogen, ground in a mortar and pestle, and placed in the sample holder for X-ray diffraction (XRD) analysis using a SHIMADZU X-ray diffractometer (XRD-6000). The diffraction data from the fungal samples were compared with that obtained from JCPDS-International Center for Diffraction Data.

2.8. Analysis of organic acid

Citrate, oxalate and gluconate were analyzed using HP 1100 series high performance liquid chromatography with variable wavelengths detector at 210 nm, and carried out at 30 °C. The mobile phase used was 5 mM sulphuric acid (Merck, analytical grade), at a flow rate of 0.5 ml/min. Standards of the compounds mixture were prepared using analytical grade reagents of citric acid (Aldrich Chemical Co.), disodium oxalate (Merck) and D-gluconic potassium salt (Sigma Chemical Co.) at concentrations of 0, 5, 50, 100, 200 mM for citrate and gluconate; and 0, 5, 10, 20, 50 mM for oxalate.

3. Results and discussion

3.1. Characteristics of fly ash

Fly ash obtained from the Tuas incineration plant in Singapore was of very small particle size (averaging 26 μm) and was rich in metals. Ca was the most dominant followed by K, Mg and Zn. Pb, Al and Fe were also found in significantly amounts. A more detailed description of the physical and chemical characteristics of fly ash has been given in the supplementary material (Tables S1 and S2).

3.2. Acid formation in the presence and absence of ash

The quantity of acids produced by the fungi in the presence and absence of ash is given in Table 1. The growth of fungi in sugar-containing media results in the production of organic acids such as oxalic acid, citric acid and gluconic acid. *A. niger* produces citric acid at a higher concentration in the absence of fly ash, while gluconic acid is produced at a higher concentration in its presence. When the fungus is grown in the absence of fly ash and in a manganese-deficient medium, the enzyme isocitrate dehydrogenase is unable to catalyse the oxidative decarboxylation of isocitrate to alpha-ketoglutarate (in the Krebs cycle) and citric acid is accumulated in the medium. In the presence of fly ash however, manganese (from the fly ash) which functions as a cofactor for isocitrate dehydrogenase is released into the medium,