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Alkaline bioleaching of municipal solid waste incineration fly ash by autochthonous extremophiles

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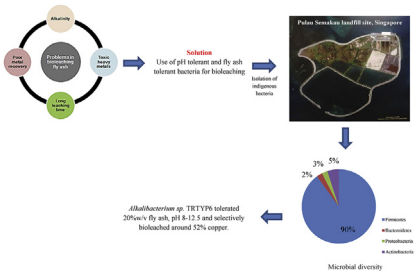
HIGHLIGHTS

- Indigenous bacteria were isolated from Pulau Semakau landfill site.
- 18 strains showed bioleaching potential, with alkaline pH or fly ash tolerance.
- Genetic characterization of the strains revealed a dominance of Firmicutes.
- *Alkalibacterium* sp. TRTYP6 tolerated 20%w/v fly ash and pH 8–12.5.
- TRTYP6 selectively bioleached around 52% copper.

GRAPHICAL ABSTRACT



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ABSTRACT

The increasing demand for energy and the generation of solid waste have caused an alarming rise in fly ash production globally. Since heavy metals continue to be in demand for the production of materials, resource recovery from the recycling of these wastes has the potential to delay the depletion of natural ores. The use of microorganisms for the leaching of metals, in a process called bioleaching, is an eco-friendly and economical way to treat the metal-laden wastes. Bioleaching of fly ash is challenging due largely to the alkaline nature and toxic levels of heavy metals which are detrimental to microbial growth and bioleaching activity. The present work reports the isolation of indigenous bacteria from a local fly ash landfill site and their bioleaching performance. 38 autochthonous strains of bacteria were isolated from eight samples collected and plated on five different media. 18 of the isolates showed bioleaching potential, with significant alkaline pH or fly ash tolerance. Genetic characterization of the strains revealed a dominance of Firmicutes, with *Alkalibacterium* sp. TRTYP6 showing highest fly ash tolerance of up to 20% w/v fly ash, and growth over a pH range 8–12.5. The organism selectively recovered about 52% Cu from the waste. To the best of our knowledge, this is the first time a study on bioleaching with extreme alkaliphiles is reported.

1. Introduction

Disposal of municipal solid waste (MSW) is of global concern due to the diminishing availability of landfill space, and the generation of toxic leachate with rainwater infiltration. Incineration is widely used to reduce the volume of MSW. The process of incineration results in the generation of a finely divided residue called fly ash which rises with flue gases and is collected using electrostatic precipitators. Several million tonnes of municipal solid waste incineration fly ash (MSWIFA) is generated globally annually (Ramanathan and Ting, 2014) and is usually landfilled.

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The increasing need for landfill space for fly ash disposal may be reduced by using or recycling the ash. Indeed, with increasing demand for metals, resource recovery from the ash has the potential to delay the depletion of natural ores. While conventional techniques such as thermal treatment, chloride evaporation and hydrometallurgical methods may be used for metal removal or recovery, bioleaching is an eco-friendly and economical alternative to achieve the same objective.

Metal leaching bacteria have been extensively used for the extraction of metals in the mining industry. The most common strains used for metal leaching include the autotrophic *Acidithiobacillus* species (Ishigaki et al., 2005). These bacteria are acidophilic and require sulphur or Fe(II) for energy production. Another class of organisms used for bioleaching is the heterotrophic fungi, such as *Aspergillus niger* (Bosshard et al., 1996) and *Penicillium simplicissum* (Amiri et al., 2011). Despite their bioleaching potential, the treatment of fly ash with commonly used microorganisms is extremely difficult because of the alkaline nature as well as the toxic heavy metal content of the ash, both of which are detrimental to microbial growth and leaching activity. Since organisms currently used in bioleaching are mainly acidophiles which thrive at pH close to 1–2, bioleaching of fly ash necessitates the acidification of the alkaline fly ash (Ramanathan and Ting, 2014).

The use of autochthonous microbes to address different environmental issues is a long-established approach and is commonly applied in biodegradation and bioremediation (Tiwari et al., 2008). Autochthonous bacteria (mainly acidophiles) have been exploited in the bioleaching of various Cu and Fe ores and secondary wastes (Pathak et al., 2009; Shen et al., 2013). The aim of this study is to identify indigenous bacteria capable of bioleaching MSWIFA in its characteristic alkaline environment. The study discusses the isolation and screening of autochthonous microbes with inherent alkaline pH and fly ash tolerance and their potential for the bioleaching of MSWIFA. To the best of our knowledge, there are no previous reports on the isolation of organisms from fly ash with inherent alkaline pH and metal tolerance.

2. Materials and methods

2.1. Fly ash

MSWIFA was obtained from Tuas Incineration Plant, Singapore. The incineration fly ash was collected after it was dosed with lime, and was ground and homogenized using a blender to particle size <500 μm. The elemental composition of fly ash was determined using ASTM D6357-11 (ASTM, 2011). The fly ash was autoclaved (Autoclave CL-40L, ALP Co. Ltd., Japan) at 121 °C for 15 min prior to use. TCLP analysis was performed using US EPA SW 846 Method 1311 (USEPA, 1992).

2.2. Bacteria

Bacteria used in this study were isolated from fly ash samples collected from Pulau Semakau Landfill site (1.20°N 103.77°E), located south of the main island of Singapore.

2.3. Sample collection from fly ash landfill site and isolation of autochthonous bacteria

Eight fly ash samples were collected, with two each from four different locations (called 'cells') at the landfill site (Fig. 1). The samples (1 g) were suspended in autoclaved distilled water and the supernatant from homogenized samples were diluted serially (10^4 to 10^{10} times) and plated on five types of agar media, using spread plate technique. Individual colonies obtained from the primary culture were streaked on respective agar plates using quadrant streak method. This step was repeated until pure cultures were obtained.

Different media were chosen based on the microbial properties of interest (pH and fly ash tolerance). The media compositions are specified in Table 1.

The reasons for the use of the five media are as follows. M1 (Nutrient agar +1% Fly ash) and M2 (Nutrient agar +1% Fly ash +3% NaCl) were designed based on the nature of their habitat with M2 promoting the growth of microbes which require NaCl for optimal growth (pH of media M1 and M2 were not adjusted). M3, M4 and M5 (Horikoshi medium (Horikoshi, 1999) with varying pH by adjusting the concentration of Na_2CO_3 in the medium) were used to screen for bacteria growing under extreme alkaline conditions. All chemicals used were of analytical grade. Nutrient broth and agar were obtained from Becton, Dickinson and Company, U.S.A; NaCl from Merck & Co., U.S.A; Na_2CO_3 from Alfa Aesar, U.S.A and glucose from Sigma-Aldrich, U.S.A.

2.4. Screening for autochthonous bacterial strains with bioleaching ability, pH and metal tolerance

2.4.1. Analysis of the metal leaching kinetics

Bioleaching was conducted in 250 mL Erlenmeyer flasks. 1% v/v of bacteria culture and 1% w/v of autoclaved MSWIFA sample (collected from Tuas Incineration plant) were introduced into 100 mL of fresh sterile medium (with same composition as the respective isolation medium). All experiments were conducted in triplicates. Samples were collected over a period of 30 days and centrifuged (16000*g*, 10 min). The supernatant was filtered (cellulose acetate syringe filters, 0.45 μm, VWR International, U.S.A) and stored at 4 °C prior to analysis using an Inductively Coupled Plasma-Mass Spectrometer, ICP-MS (Agilent 7500). The fly ash residue remaining after bioleaching was subjected to TCLP analysis as described in Section 2.1.

2.4.2. Fly ash tolerance limit

Five different types of agar media (M1–M5) were prepared and autoclaved (121 °C, 20 min), and autoclaved fly ash (collected from Tuas Incineration plant) at 1%, 5%, 10% and 20% w/v were added aseptically to the media. The suspension was well mixed before it was poured into plates. Bacterial pre-cultures in logarithmic growth phase were used as inocula to streak these plates using quadrant streaking. Depending on the isolate, growth of bacteria was observed between 24 h and 14 days. The isolates were considered tolerant to a specific fly ash concentration when a minimum of ten colonies appeared on the plates (Graham et al., 1994).

2.4.3. pH tolerance limit

pH of the agar media (M1–M5) were adjusted to four ranges, namely pH 7–8, 9–10, 11–12, 12–12.5. As before, bacterial pre-cultures in logarithmic growth phase were quadrant streaked on the agar plates, and colonies appeared between 24 h and 14 days. The isolates were considered tolerant to a specific pH when a minimum of ten colonies appeared on the plates (Graham et al., 1994).

2.5. Biochemical characterization of the isolates

Colony morphology was examined by visually observing the colonies on agar plates. Cell morphology was observed under immersion oil microscopy (Olympus CX40, magnification ×100). Gram characterization was determined using a rapid non-staining