GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Isolation and Characterization of Bacteria from Fly Ash Samples as Bioremediation Agents

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GSC Biological and Pharmaceutical Sciences, 2025, 33(01), 223-229

Publication history: Received on 11 September 2025; revised on 19 October 2025; accepted on 22 October 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.33.1.0403

Abstract

Fly ash, a residue generated from coal combustion at coal-fired power plants (CFPPs), presents both economic potential due to its rare earth element (REE) content and environmental risks as a hazardous pollutant. Therefore, an appropriate remediation strategy is required to mitigate its negative impacts while utilizing its valuable components. This study aimed to isolate and characterize bacteria from fly ash samples collected around the Tarahan CFPP, South Lampung, with potential as bioremediation agents for rare earth elements, particularly Yttrium (Y). The research employed an exploratory experimental approach, including sampling, bacterial isolation and cultivation, macroscopic and microscopic characterization, and optimization testing of bacterial tolerance to Yttrium. The results revealed two bacterial isolates (FA1.2 and FA2.2), identified as Gram-negative cocci that do not form spores but are capable of adapting to polluted environments. Optical Density (OD) analysis showed optimal growth at 50 ppm and resistance up to 400 ppm. These findings indicate that both isolates possess strong potential as bioremediation and bio-extraction agents for rare earth elements in fly ash from CFPPs.

Keywords: Coal Fly Ash; Bacteria; Bioremediation; Rare Earth Elements (REE); Coal-Fired Power Plants (CFPP)

1. Introduction

Composting Coal-fired power plants (CFPPs) that use coal as their primary fuel produce solid residues in the form of fly ash and bottom ash. Fly ash is a pollutant composed of very fine particles that can easily disperse in the air and settle in surrounding environments, leading to air, soil, and water pollution if not properly managed [1]. In addition to its small particle size (0.5–200 μm) and lightweight nature that allows it to remain suspended and spread through wind [2], fly ash also contains hazardous compounds such as heavy metals and silicates, which may negatively affect both the environment and human health when not properly mitigated [3].

Nevertheless, fly ash is known to contain rare earth elements (REEs) such as yttrium, neodymium, and europium, which have high industrial value, especially in modern technology. Indonesia, however, still relies heavily on imported REEs [4]. The limitations of extraction technology and the environmental risks of conventional mining, which ultimately require remediation, have restricted the utilization and management of fly ash residues [5]. Therefore, exploring alternative, eco-friendly approaches for utilizing fly ash is highly necessary.

One potential approach is bioremediation, which utilizes microorganisms such as bacteria to reduce toxicity or convert heavy metals into less harmful compounds [6]. Certain bacteria are even capable of extracting metals through a bioleaching process, which not only reduces contamination but also enables the recovery of REEs from solid waste such

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as fly ash [7]. Bacteria that can survive in contaminated environments often exhibit high resistance to toxic substances, making them promising candidates for this process [8].

Hence, the isolation and characterization of bacteria from the surrounding environment of the Tarahan CFPP in South Lampung, particularly from fly ash dump areas, were conducted to identify microorganisms that are not only resistant to extreme conditions but also capable of biologically extracting REEs, particularly yttrium (Y). This study is expected to contribute to the development of sustainable solutions for fly ash waste management and the utilization of rare earth elements (REEs) contained within these residues.

2. Material and methods

2.1. Time and Location

This research was conducted from January to June 2025. Sample collection of fly ash was carried out in the Tarahan Coal-Fired Power Plant (CFPP) area, South Lampung Regency, Lampung Province, Indonesia. Laboratory analyses were performed at the Integrated Mineral Laboratory, National Research and Innovation Agency (BRIN), KS Iskandar Zulkarnain, Tanjung Bintang, Lampung.

2.2. Equipment and Materials

The equipment used included sterile sampling spoons, droppers, sterile sample tubes, analytical balance, 50–500 mL Erlenmeyer flasks, Petri dishes, test tubes, test tube racks, shaker, spatula, inoculating loop, Bunsen burner, oven, bulb, incubator, laminar air flow, volumetric pipette, 250 mL measuring cylinder, micropipette, vortex, Drigalski spatula, microscope slides, wooden clamps, microscope, and UV-Vis spectrophotometer.

The materials used were fly ash samples, distilled water, standard yttrium (Y) stock solution, Nutrient Agar (NA), Nutrient Broth (NB), physiological saline (0.8% NaCl), 70% ethanol, cotton and gauze stoppers, spirit burner fuel, labels, plastic wrap, aluminum foil, crystal violet, iodine solution, 95% ethanol, safranin, and malachite green.

2.3. Sample Collection

Samples were collected from two different locations around the fly ash dump site of the Tarahan CFPP, South Lampung. Sampling points were determined based on field observations and visible fly ash accumulation. At each location, samples were taken from three points with two sub-samples per point (replicates) within a radius of 1 m and a depth of 20–30 cm using sterilized tools. Sub-samples were composited in sterile containers to obtain a representative sample from each point. Each sample was labeled according to location and sampling time, then stored in a cool box to maintain microbial stability during transport to the laboratory.

2.4. Bacterial Isolation

Fly ash samples were cultured by adding 5 g of fly ash into 100 mL of Nutrient Broth (NB) and incubating in a shaker for 24 hours to stimulate microbial growth. The culture was then serially diluted with sterile 0.8% physiological saline to obtain dilutions of 10^{-3} and 10^{-4}. Each dilution was inoculated onto Nutrient Agar (NA) medium supplemented with fly ash and 1 ppm yttrium using the spread plate method with a sterile Drigalski spatula. The plates were incubated at 37°C for 24 hours. Grown colonies were observed and sub-cultured to obtain pure isolates.

2.5. Bacterial Characterization

Pure bacterial colonies were characterized based on macroscopic and microscopic features.

Macroscopic characterization included observations of colony shape, color, edge, and elevation on solid media. Gram staining was performed to identify cell wall types (Gram-positive or Gram-negative) and cell shapes (coccus or bacillus). The Gram staining process consisted of primary staining (crystal violet), mordant (iodine), decolorization (ethanol), and counterstaining (safranin), followed by microscopic observation.

Spore staining was also carried out to detect sporulation ability using malachite green followed by safranin as a counterstain.

2.6. Bacterial Performance Optimization Test

The purified bacterial isolates were tested for tolerance limits by observing their growth in media containing yttrium (Y) at varying concentrations. Nutrient Broth media were prepared with yttrium concentrations of 50, 100, 200, 300, and 400 ppm, each inoculated with one loop of bacterial isolate. Positive and negative controls were also prepared. Incubation was carried out at 37°C for 72 hours. Every 24-hour interval, absorbance was measured using a UV-Vis spectrophotometer at 600 nm to determine growth rates. The absorbance data (Optical Density, OD) were then used to evaluate bacterial tolerance levels and efficiency in handling increasing concentrations of REEs.

3. Results and discussion

3.1. Macroscopic Characterization of Bacterial Isolates

Isolation of bacterial samples from fly ash and air was carried out using the spread method on media containing NA + fly ash (1 ppm) and NA + Yttrium (1 ppm). According to [9], isolation is the process of obtaining bacteria from their natural environment into a suitable artificial medium. In the initial stage of isolation, bacteria appear in the culture mixture, so a purification process using the streak method is necessary to obtain pure colonies.

Table 1 Macroscopic Characteristics of Bacterial Isolates from Fly Ash Samples

Isolate Codes	Colony Morphology Characteristic			
	Form	**Color**	**Margin**	**Elevation**
FA1.2	Irregular	Translucent White	Undulate	Raised
FA2.2	Irregular	Translucent White	Undulate	Flat

Two bacterial isolates were successfully obtained from fly ash samples collected around the fly ash dump area of the Tarahan Coal-Fired Power Plant, South Lampung. According to [10], the toxicity of environmental contaminants strongly influences microbial diversity, while [11] reported that fly ash contains various heavy metals that inhibit the growth of certain microorganisms. Factors such as pH, chemical composition, and medium texture also affect bacterial growth and activity [12].

Macroscopic morphological identification was carried out for both isolates. The colony of isolate FA1.2 appeared irregular in shape, with raised elevation, undulate margins, and a translucent white color. Similarly, isolate FA2.2 exhibited an irregular form, flat elevation, undulate edges, and a transparent white color. According to [13], variations in bacterial colony morphology are closely associated with physiological responses to environmental stress, nutrient availability, and secondary metabolite activity, including extracellular polymeric substance (EPS) secretion. The production of EPS enhances bacterial adhesion, provides protection from desiccation, and acts as a physical barrier against toxic compounds, allowing bacteria to survive in harsh environments [14].

3.2. Microscopic Characterization of Bacterial Isolates

Microscopic characterization was carried out using gram staining and then observed under a microscope at 200X and 500X magnification.

Table 2 Microscopic Characteristics of Bacterial Isolates from Fly Ash Samples.

Isolate Codes	Test Type			
	Gram Staining			**Spore Staining**
	Gram Type	**Form**	**Arrangement**	
FA1.2	-	Coccus	Monococcus	-
FA2.2	-	Coccus	Monococcus	-

Notes: Gram staining: – (Gram-negative), + (Gram-positive) Spore staining: – (non-spore-forming), + (spore-forming)

Microscopic observation was conducted using Gram staining and spore staining to further identify the physiological characteristics of the isolates. Based on the Gram staining results, both isolates showed similar characteristics, identified

as Gram-negative cocci with a monococcal arrangement. Morphologically, the coccus form with a monococcal pattern helps minimize direct exposure to toxic substances and reduces water loss, particularly in dry environments or under high osmotic pressure [15].

Meanwhile, the results of spore staining indicated that neither isolate produced spores. Although these isolates did not form spores like certain Gram-positive bacteria, Gram-negative isolates possess distinct physiological and morphological mechanisms that enable them to adapt to extreme environments. Physiologically, the cell wall of Gram-negative bacteria consists of a thin peptidoglycan layer and a complex outer membrane rich in lipopolysaccharides (LPS), which provide protection against environmental stressors such as heavy metals, chemical agents, and antibiotics [16].

Furthermore, according to [14], bacteria are capable of producing extracellular polymeric substances (EPS), a polysaccharide matrix secreted by cells to form biofilms. EPS acts as a diffusion barrier against heavy metals and enhances water retention around the cell, thereby strengthening bacterial resistance to oxidative stress and environmental fluctuations [17]. Thus, even without forming spores, Gram-negative bacteria maintain efficient physiological and morphological adaptation systems, allowing them to survive in toxic environments such as fly ash.

3.3. Optimization Test of Bacterial Growth (Optical Density)

The two isolates that have been characterized as potential rare earth metal bioextraction agents were then tested for their resistance by observing their growth on selective media NA + Rare Earth Metal Yttrium (Y) with graded concentrations of 50 ppm, 100 ppm, 200 ppm, 300 ppm, and 400 ppm.

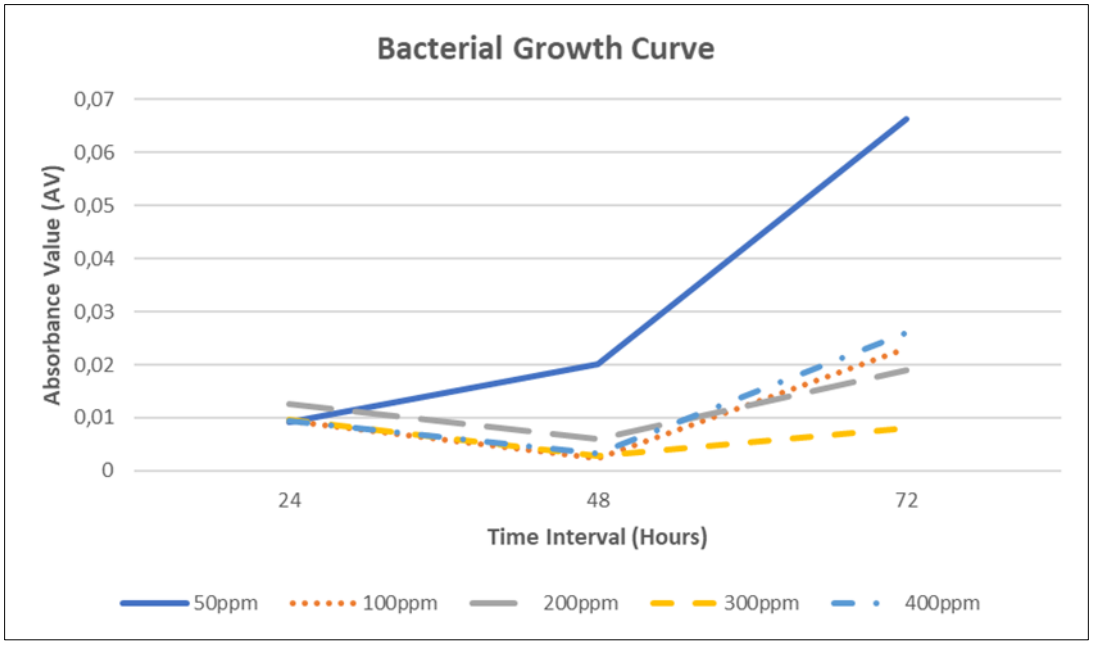

Figure 1 FA1.2 Isolate Optimization Test Curve

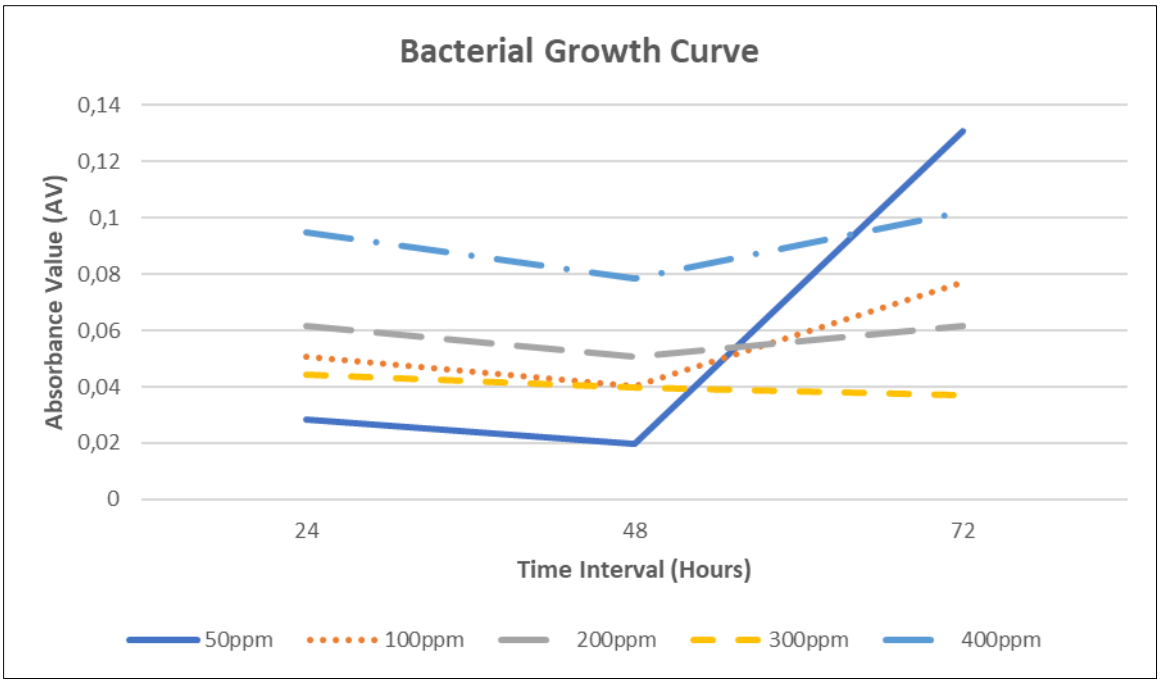

Figure 2 FA1.2 Isolate Optimization Test Curve

Bacterial resistance testing against rare earth elements (REE), specifically Yttrium (Y), was conducted on both isolates using media turbidity observation and Optical Density (OD) measurement with a spectrophotometer at a wavelength of 600 nm. Based on the turbidity test results, media turbidity began to appear across all tested concentrations after 72 hours of incubation, indicating a bacterial adaptation phase during the first and second days, particularly at concentrations of 300–400 ppm. According to [10], high turbidity indicates optimal microbial growth, suggesting that the isolates were able to withstand environmental stress caused by heavy metal contamination.

OD measurements of isolates FA1.2 and FA2.2 showed that significant bacterial growth occurred at 50 ppm, with absorbance values continuously increasing up to 72 hours. Growth at concentrations of 100–400 ppm showed a temporary decline during the 24–48-hour incubation period, but absorbance curves rose again after 48 hours and continued to increase until the third day, though less prominently at 400 ppm. This indicates that the bacterial isolates-maintained resistance even at high Yttrium concentrations, demonstrating strong potential as bioextraction agents. These results are consistent with [18], who noted that OD values reflect cell density and quantitatively represent microbial growth.

Therefore, both isolates exhibit a considerable ability to tolerate Yttrium contamination up to 400 ppm, highlighting their promising potential for bioremediation and bioleaching applications on a broader scale. As stated by [19], most microorganisms begin to experience growth inhibition at heavy metal concentrations between 100–200 ppm; thus, the ability to survive up to 400 ppm serves as an indicator of strong physiological resilience. Furthermore, [20] demonstrated that successful bioleaching of rare earth elements such as neodymium and praseodymium depends greatly on isolates with high resistance levels. Hence, the two isolates obtained in this study should be further explored and tested for potential application in pollutant bioremediation.

4. Conclusion

This study successfully isolated and characterized two bacterial isolates from fly ash, a residue from coal combustion at the Tarahan Steam Power Plant (PLTU), South Lampung. Based on macroscopic and microscopic characterization, the isolates exhibited diverse colony morphologies, indicating high potential for adaptation to extreme environments. The two Gram-negative, coccus-shaped bacterial isolates, although non-spore-forming, demonstrated good physiological adaptation through an outer membrane structure and possible EPS production, which supports resistance to toxic conditions.

Resistance testing to the rare earth metal yttrium (Y), showed that both isolates continued to grow up to a concentration of 400 ppm, with optimal growth at 50 ppm and a significant increase up to 72 hours, which is considered high in the

context of heavy metal contamination. The Optical Density (OD) values, which initially decreased but then increased again after 48 to 72 hours, indicated that the bacteria were able to undergo the adaptation phase and resume active growth, and exhibited considerable physiological resistance. Therefore, these two isolates are worthy of further development in advanced research and bioremediation and biometallurgy applications, particularly in the bioleaching process of rare earth metals.

Compliance with ethical standards

Acknowledgments

The authors would like to express sincere gratitude to the National Research and Innovation Agency, South of Lampung, Indonesia, for funding and providing the facilities to conduct this research.

Disclosure of conflict of interest

There is no conflict of interest.

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