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Exploration and Implementation of Indigenous Microbes from Coal Waste as Bioaccumulator Agents for Heavy Metals Cd and Pb

Nunung Eni Elawati [1*], Catur Retno Lestari [1], Fibra Resputri [1]

[1] Biomedical Science Study Program, Faculty of Health, Ivet University, Jl. Pawiyatan Luhur IV No.16, Bendan Duwur, Kec. Gajahmungkur, Semarang City, Central Java, Indonesia, 50235

* Correspondence: nunungenie@gmail.com

Abstract

Background: Heavy metals in the environment are a critical environmental pollution problem. Degrading and removing heavy metals does not degrade organic waste because heavy metals are non-biodegradable. Bioremediation is one technique that can be chosen to degrade metal waste. The purpose of this study was to obtain and identify native bacteria from coal waste that have the potential to reduce heavy metals or bioaccumulation. **Method:** Samples were taken from coal waste, then isolated using dilution, and incubated at 37^{0}C for 24 hours. Test for heavy metal content by applying a microbial consortium suspension to coal waste with a mixture of planting media, then analyze it with an atomic absorption spectrophotometer. **Results:** 10 isolates were obtained from microbial isolation with different color characteristics. The analysis of heavy metal content showed a decrease in the heavy metal Pb in the L1 and L2 treatments but did not affect the heavy metal Cd. **Conclusion:** The microbial consortium isolated from PLTU coal waste shows a decrease in the heavy metal Pb in treatments L1 and L2.

Keywords: Bioaccumulators; Indigenous Microbes; Heavy metal.

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Introduction

Steam Power Plants (PLTU) produce combustion residue in the form of coal ash. The type of PLTU combustion and the amount of coal significantly used influence coal ash (Firman, 2020). This combustion produces fly ash and bottom ash. Fly ash accounts for about 80–90% of the total, while bottom ash accounts for 10–20%. According to Mayfield & Lewis (2013), coal ash is landfill material for mine openings. The amount of coal waste produced is positively correlated with the amount of electrical energy produced by constructing new PLTUs. Environmental pollution problems arise when coal waste is not utilized effectively (Utami, 2018).

According to Presidential Decree Number 101 of 2014, coal ash is categorized as hazardous and toxic waste (B3), placed in category 2, requiring a unique approach (Febriana et al., 2021). In environmental management, heavy metals are one of the most concerning pollutants. This is because coal ash contains heavy metals, which are used in embankment materials, especially during the operation of steam power plants (PLTU) (Firman, 2020).

Heavy metals can cause significant ecological instability. Therefore, the presence of heavy metals in the environment is an essential environmental pollution problem (Zulaika et al., 2012). Mercury (Hg), cadmium (Cd), copper (Cu), silver (Ag), nickel (Ni), lead (Pb), arsenic (As), chromium (Cr), zinc (Zn), tin (Sn), and manganese (Mn) are some examples of heavy metals that are considered to have high levels of toxicity (Tchounwou et al., 2012). Heavy metals, such as lead (Pb), can harm humans directly or indirectly because they can inhibit the process of forming hemoglobin (Hb). This is because lead (Pb) compounds can have harmful effects on many organs in the human body (Rosita & Mustika, 2019). Heavy metals are also carcinogens, allergens, mutagens, teratogens, or teratogens for humans. Access is through the skin, breathing, and digestion (Rahadi et al., 2019).

Heavy metals cannot be biodegraded, so the decomposition and removal of heavy metals are not as easy as decomposing organic waste. However, physical and chemical methods can decompose and reduce heavy metals through ion exchange, precipitation, coagulation, inverse osmosis, and adsorption (Sarie, 2020). Even though all the techniques mentioned above are pretty effective, processing industrial waste using these techniques will be detrimental because of the high costs, high energy consumption, and the need for significant chemicals (Zulaika et al., 2012).

According to Wijayanti (2017), a biotechnological approach that utilizes bacteria is an option that can be used in the future. This approach is very profitable both from a technical and economic point of view. The bioremediation process is one method that might be used to decompose heavy metal waste. Bacteria use in bioremediation is more practical because bacteria can degrade and undergo heavy metal transformation. One of these mechanisms is biosorption and bioagglutination, which will not affect the survival of bacteria (Zulaika et al., 2012). Bioremediation refers to removing pollutants from specific soil using microorganisms grown on the pollutants (Parhusip et al., 2020). This process is carried out to remove contaminants from the soil. According to Irawati et al. (2017), biosorption and bioaccumulation are two of the many ways bacteria develop resistance to heavy metals. The biosorption process occurs when metal ions interact with bacterial cell walls through a physicochemical process. The bioaccumulation process occurs when bacteria accumulate heavy metals in their cells. (Kurniawan & Ekowati, 2016).

Several studies have reported that most indigenous microorganisms can tolerate heavy metals at high concentrations and play an essential role in the biological remediation of soil. Therefore, studying the diversity of microorganisms in areas contaminated with heavy metals is very important. Local microorganisms isolated under unfavorable conditions at industrial sites can provide new insights into their diversity (Singh & Hiranmai, 2021).

This research aims to isolate and identify native bacteria from coal waste that can reduce heavy metals or bioaccumulators. Hopefully, this research can show how well native microbes from coal waste can degrade heavy metals.

Method

Sample

Coal waste samples were obtained from PLTU Tanjung Jati B Jepara. The criteria for samples taken are those in the landfill for approximately five years. The materials needed for this research are NA media, planting media, chili seeds, CDCl2, PbCl2, and Aquades. The equipment required for this research includes chemical glassware, pH measuring instruments, Petri dishes, incubators, laminar air flow cabinets (LAF), and autoclaves.

Microbial Isolation

Multilevel dilution and the spread plate method are two techniques to isolate bacteria from coal waste samples. After one gram of sample was added to a test tube containing nine milliliters of sterile distilled water, the mixture was homogenized (dilution 6), and the sample was then subjected to serial dilution to a concentration of ten to the sixth power. The spread plate technique was used to transfer 100 microliters of sample from tubes 3 to 6 into a solid layer of Nutrient Agar (NA) media. After that, the inoculum was incubated at 37°C and spread evenly throughout the culture. The resulting colonies can be seen approximately 24 hours later.

Purification of Mycorrha

Colonies that are too thick need to be filtered before being cultivated purely. After a colony of bacteria has been carefully combined with sterile water, the mixture is transferred to a non-aqueous medium using a streak plate technique. The mixture was then incubated at 37°C for 24 hours. After that, a single thick column was applied, and a streak plate approach was used to transfer the column to a new NA medium. A pure culture is achieved after a gradual separation process occurs.

Microscopic observation

Microscopic observations were carried out by preparing a glass object smeared with bacterial isolate and adding a drop of crystal violet. Then, the bacterial isolate was left for one minute and washed with running water. Then add one drop of iodine, go for one minute, and wash with running water. Finally, the bacterial isolate was added with 95% ethyl alcohol for 30 seconds and washed with a flowing stream. Observations are made if the color shows purple, then the bacteria are gram-positive; if the color is red, it is gram-positive bacteria.

Analysis of heavy metal content using bacterial isolates

Research experiments were carried out by adding coal waste (FABA) with planting media and microbial isolates for plant growth. Bioremediation agent microbial testing was carried out on coal waste, mixed with planting media, then inoculated with microbial suspension and stirred evenly. Treatment details are as follows:
L1= 50 gram FABA + 150 gram planting medium + 5 ml/gram bacterial isolate,
L2= 100 gram FABA + 100 gram planting medium + 10 ml/gram bacterial isolate, and
L3= 150 gram FABA + 50 gram planting medium + 15 ml/gram bacterial isolate.

The experiment was repeated three times. Heavy metal content (Cd and Pb). Heavy metal analysis was carried out using an atomic absorption spectrophotometer. The parameters observed included soil pH and Cd and Pb content.

Data analysis

The data obtained was then analyzed descriptively with a quantitative approach.

Result

Isolation and Purification of Bacteria

Samples taken in the PLTU area were then isolated using the 10^{-1} to 10^{-6} dilution method. Microbial isolation was performed at dilutions of 10^{-3} to 10^{-6} using the spread plate method on NA media, which was incubated at 37°C for 24 hours. The results obtained can be seen in Figure 1.

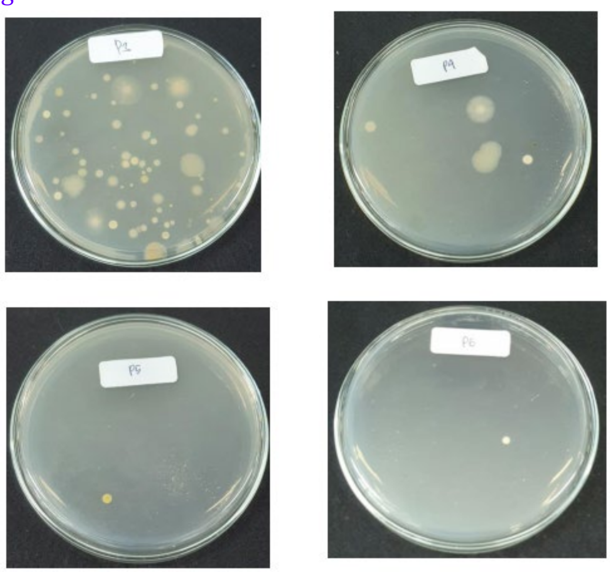

Figure 1. Results of isolation using the dilution method, which was incubated for 24 hours

The isolation results will then be taken from different isolates to be purified on NA solid media using a sterile toothpick. The results of bacterial purification can be seen in

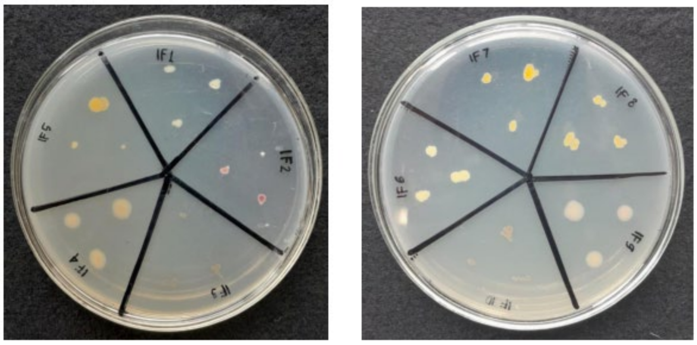

Figure 2. Results of microbial purification in NA media incubated for 24 hours

Indigenous bacteria grown on NA media are purified in Petri dishes containing NA media using the streak plate technique (Pakaya et al., 2022). The ever-increasing bacterial colonies are separated based on color, size, and shape and purified by growing them in the same media until they become pure colonies (Wondal et al., 2019). LAF lowered the air temperature to 37°C for one twenty-four-hour period and produced pure colonies to reduce air contamination.

Microscopic Observation

The ten indigenous bacterial isolates found were then subjected to gram staining. The microscopic observations using Gram staining showed that the ten isolates were Gram-positive bacteria with a bacillus form with a streptobacillus structure (Figure 3).

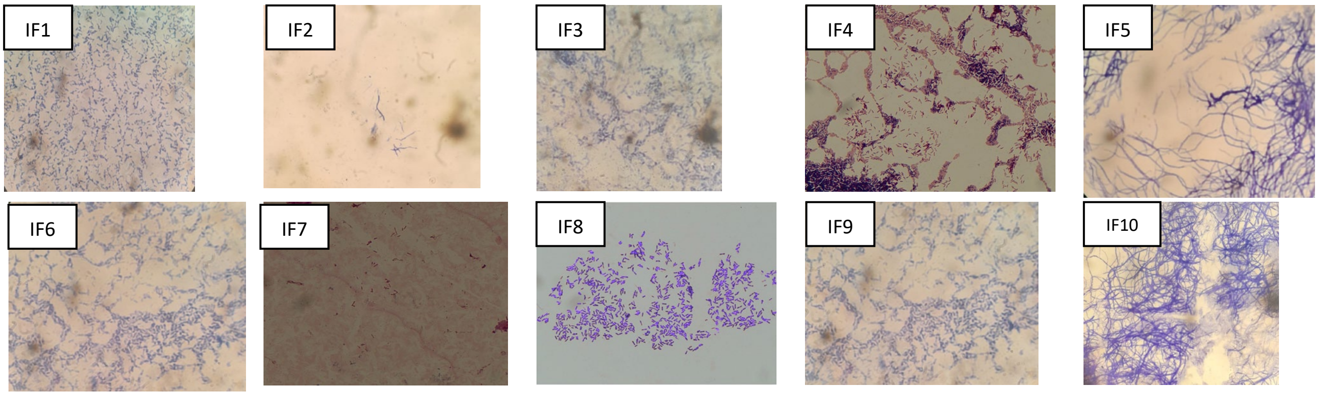

Figure 3. Observation of microscopic results with gram staining at 100x magnification

Analysis of Heavy Metal Content

This analysis was done by applying a microbial suspension to coal waste mixed with planting media for plant growth. The parameters observed included soil pH, Cd, and Pb content. The Abalisa results table can be seen in Table 1. To see the difference with the AAS method, where heavy metal content testing is carried out by checking heavy metals before and after treatment.

Table 1. Test results for heavy metal content

Sample	Parameter		
	Cd (Mg/Kg)	**Pb (Mg/Kg)**	**pH**
Beginning	<0.005	0.050	6.5
L1	<0.005	0.036	6.7
L2	<0.005	0.038	7.0
L3	<0.005	0.050	6.7

Discussion

Isolation is carried out using the culture method to obtain a pure culture. This procedure produces ten bacterial colonies obtained from the cultivation process. The resulting colonies were purified during the isolation process, which produced ten different colonies. The four-way streak coating technique is used to achieve this isolation procedure. As proposed by Rahadi et al. (2019), it is essential to observe the vitality of bacterial cells, investigate sample diversity, differentiate between different types of cells to produce cell cultures at lower costs, and understand the characteristics of colonies. For a colony to be isolated, the individual cells must be spread over the surface of the medium. The sample is placed on a solid medium in a petri dish in the form of a series of "scratches" during the process. The basic principle of the line plate or scratch plate isolation method is to divide the plate into four parts to obtain pure colonies (Pakaya et al., 2022).

After that, the ability of the isolate as a bioremedial agent against heavy metals such as Cd and Pb was observed under a microscope. The word "bioremediation" refers to the biological process of removing soil particles containing pollutants through the use of living creatures, which can include small and large-scale organisms (plants, microflora, and microfauna) (Sarie, 2020). Based on the results of microscopic characterization, it was shown that the ten bacterial isolates were Gram-positive bacteria. This is in line with the research of Christina et al. (2018), which succeeded in finding 15 isolates of Gram-positive bacteria isolated from former nickel mining land in East Halmahera, which could degrade heavy metals. According to Pulungan & Tumangger (2018), gram-positive bacteria dominate polluted environments by absorbing and removing heavy metals from cell fluids. This is natural because its thicker peptidoglycan and teichoic acid cell walls make it more resistant to extreme conditions. Table 1 shows decreased heavy metal Pb in L2 and L3. Rahadi et al. (2019) described how bacteria clean heavy metals through an ion exchange. These mechanisms fall into three categories: based on cell metabolism, which is divided above; metabolism-related and metabolism-unrelated processes; and based on the position of heavy metals found through extracellular accumulation (precipitation), intracellular accumulation, and metal absorption by cell membranes. The cell membrane (extracellular) and plasma membrane (intracellular) are responsible for initiating the metabolic process known as heavy metal metabolism (Rosita & Mustika, 2019). During the bioremediation process, enzymes produced by microorganisms undergo structural changes, turning into simple metabolites that no longer pollute the environment.

Bacteria can destroy Pb through intracellular or extracellular processes (Jarosławiecka & Piotrowska-Seget, 2014). The Pb degradation mechanism mainly reduces the toxicity of heavy metals so that they do not interfere with the biological functions of microorganisms. In Pb (II), toxicity can be reduced through extracellular degradation mechanisms present in the environment. Binding with extracellular polysaccharides or producing polyphosphate or polymer deposits are natural components of bacterial cell walls. The external degradation process aims to reduce the amount of heavy metals that can enter bacterial cell walls (Agustina & Lisdiana, 2023). Cadmium (Cd) analysis revealed variation in the results, indicating that the results in the control group remained unchanged. This variation is visible in the findings. However, potassium chloride does cause the soil pH to become alkaline, which is an ideal environment for plant development (Febriana et al., 2021). This applies even though potassium chloride does not affect reducing Cd concentrations.

Due to the ability of these bacteria to live in environments with high concentrations of cadmium or even utilize cadmium as a resource in their life cycle, this native bacterial consortium can reduce cadmium levels in the atmosphere. Because bacteria can produce Cd-binding proteins in their cells, a bactericidal mechanism against cadmium has been proposed (Wijayanti, 2017). Certain bacteria may also carry out metallothionine mechanisms, such as intracellular protein-mediated reduction of the logarithm of positive charge (Parhusip et al., 2020). Other mechanisms include a substitution of the positively charged logarithm with the logarithm of Cd in solution or the anionic logarithm with an anionic solution.

Conclusions

The microbial consortium isolated from PLTU coal waste showed a decrease in the heavy metal Pb, namely in treatment L1 to 0.036 Mg/Kg and treatment L2 to 0.038 Mg/Kg.

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Declaration statement

The authors reported no potential conflict of interest.

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