



Combination of Andisol Soil-Bioball-*Bacillus* sp. For Cadmium Removal Application

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ABSTRACT. Industrial growth in Indonesia, especially the metal industry, has the potential to produce waste that can pollute the environment and hurt humans, animals, and plants. One of these pollutants is heavy metal cadmium (Cd). Besides that, cadmium is very toxic and will cause serious illness for humans. *Bacillus* sp. is a potential bacterium that can remove harmful substances so that it can be used for bioremediation. However, the ability of *Bacillus* sp. to absorb cadmium has not been reported. The research aims to study the effectiveness of optimal pH conditions in absorbing heavy metal cadmium. An adsorbent with composition andisol soil: bioball: *Bacillus* sp. 2:0:0; 1.5:1:4.3×10⁵; 1:2:8.2×10⁵; 0.5:3:1.21×10⁶; and 0:4:1.58×10⁶ (g:piece:(cell/mL)) put into a beaker containing 100 mL of 6 ppm Cd solution with various pH 1, 2, 3, 4, 5, and 6. The aerator was added at a constant speed for 60 minutes. After 60 minutes, the Cd content was analyzed using Atomic Absorption Spectroscopy (AAS). The combination of andisol, bioball, and *Bacillus* sp. can absorb cadmium at the optimum conditions of pH 5, with composition andisol: Bioball soil: *Bacillus* sp. 1.5:1:4.3×10⁵ (g:piece:(cell/mL)), and contact time of 120 minutes with total removal of 75.6%.

INTRODUCTION

Water is the most abundant chemical compound in nature. However, along with the increase in the standard of living of humans, the need for water also increases, and recently, water has become an "expensive" item. In big cities, it is not easy to get a source of clean water that is used as raw material for clean water that is free from pollution because the water is sucked in by industrial activities that require a certain amount of water to support its production (Mategaonkar, 2021). On the other hand, land, which is a water drain, has been closed for various purposes, such as housing and industry, without regard to the land's function for storing water for the future (Nan *et al.*, 2020). Water is not scarce, but water that is fit for use is not necessarily available in various places, for example, water that comes from former rice fields, water that comes from industrial factory waste (Yang *et al.*, 2021), and water that comes from household waste (Zhang *et al.*, 2021).

Various kinds of industrial and technological activities that exist today, if not accompanied by a good waste management program, will cause water pollution to occur either directly or indirectly (Valiente *et al.*, 2020). Waste materials and wastewater originating from industrial activities are the main causes of water pollution (Wardhana, 2004). Industrial growth in Indonesia, especially in the metal industry, has the potential to produce waste that can pollute the environment and have a negative impact on humans, animals, and plants (Chu *et al.*, 2021). One of these pollutants is heavy metals. Heavy metal pollution in the environment is closely related to humans' use of these heavy metals (Pranoto *et al.*, 2018a). Heavy metals that often pollute the environment are Pb, Cu, Hg, Cd, Zn, and As (Chandler *et al.*, 2021; Mategaonkar, 2021; Valiente *et al.*, 2020; Zhang *et al.*, 2021). This waste will cause serious pollution to the environment if its heavy metal content exceeds the threshold, has hazardous toxic properties, and causes serious illness for humans if it accumulates in the body.

The accumulation of heavy metals in the human body will cause disruptions in physiological functions and the emergence of several diseases. Some research results state that there are several types of cancer in humans due to food contaminated with heavy metals. Heavy metals entering the human body will combine with several

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types of proteins or enzymes, especially those containing sulfur. When enzymes combine with heavy metals, the properties of proteins or enzymes will be converted into compounds that can cause cancer (Layer *et al.*, 2010; Robinson, 2015).

Cadmium (Cd) is an element with a 0.1 mg/kg distribution in the earth's crust. High concentrations of cadmium are found in sulfide rocks. Inorganic compounds, such as chloride, sulfate, and acetate solutions, are soluble in water, but their oxides and sulfides have low solubility (Basu and Guha, 2022). Cadmium is usually found with zinc. High consumption of metal cadmium can cause stomach irritation, vomiting, and diarrhea (Teng *et al.*, 2022).

Andisol soils in Java are located on slopes at an altitude of 700 - 1,500 meters above sea level, with areas having slightly cooler and wetter climatic conditions than in the lowlands. At high altitudes, climatic conditions are unsuitable for mineral crystallization. Therefore, andisols are often found with allophane and amorphous materials. Annual rainfall in the area varies from 2,000 - 7,000 mm, with annual temperatures between 18–22 °C (Case *et al.*, 2007).

Pranoto *et al.* (2018) explained that andisol is a dark black soil, very porous, containing organic matter and amorphous type clay, especially allophane, and a little silica and alumina or iron hydroxide, the water binding capacity is very high; if covered by vegetation, it is always saturated with water, very dense, loose but has a high degree of structural resistance so that it is easy to process.

Sulieman *et al.* (2020) said that the binding or sorption of P in andisol soils was influenced by several factors, namely: (1) The high Al oxide content in andisols, so that the adsorption power of phosphate ions was greater. (2) The degree of crystal, namely, the higher the degree of crystallization of the minerals that make up the soil, the lower the phosphorus adsorption capacity will be. The amorphous oxides in andisol have a low degree of crystallinity and a very large surface area, so they have high adsorption capacity. (3) The mineral composition of andisol, which is oxide, has high adsorption capacity. Soil with allophane minerals contains high Al oxide, which will absorb P in the form of Al-P; thus, the availability of P in the soil is low.

Andisol soils generally have low P fertilization efficiency because some of the given P will react with Al to form Al-P due to high levels of Al dissolved in the soil solution (Huang *et al.*, 2016). Regarding the problem of absorption of P nutrients, efforts that can be made to overcome high P uptake include developing plants that are tolerant of low P availability, improving soil P constraints, especially through P management, and a combination of the two methods. The vegetation found in Andisol soils is as abundant as in soils in the alluvial zone and humid climate areas, but the growth of agricultural crops in these areas is relatively poor due to the low nutrient content of plants, especially P and some micronutrients (Valiente *et al.*, 2020).

Bacillus sp. is a potential bacterium that can remove toxic substances so that it can be used in bioremediation. Pramono *et al.* (2014) showed that *Bacillus* sp. reduced Cr (VI) metal in cell growth and resting conditions up to 100% and 51% within 18 hours. In addition, Supriyanto (2018) showed that *Bacillus* sp. has the ability to reduce nicotine in tobacco solid waste. The use of bacteria requires a medium. Bioball is a filtration media that can filter large dirt, spread water in the filtration system, and become a place for good bacteria to break down dirt. Bioball can be used as a bacterial medium. Bioball has a function as a place for bacteria to live to maintain water quality (Said, 2005). In addition, the use of bioballs can balance expenses (Filliazati, 2013; Said, 2005). This study aims to test the ability of andisol, bioball, and *Bacillus* sp. on the removal of heavy metal cadmium (Cd).

Previous research by Pranoto *et al.* (2018), Pranoto *et al.* (2019); and Sulieman *et al.* (2020) on the use of andisol soil from Mount Lawu as a natural adsorbent for domestic waste. Research by Supriyanto (2018) on the use of activated sludge waste as an adsorbent for heavy metals Pb (II) and Cu (II). Research by Dharmawan *et al.* (2018) on the combined effectiveness of activated allophane and effective microorganisms attaching bioballs to reduce phenol concentration in water. Research by Sari *et al.* (2012) on the study of the effectiveness of the use of drinking water using clays/andisol adsorbents in the heavy metal cadmium (Cd) and pathogenic bacteria.

The novelty of this research compared to previous studies is the incorporation of andisol-bioball-*Bacillus* sp. to be used as an adsorbent for Cd metal, while previous studies only used one material to be used as an adsorbent. This study aims to determine the optimum conditions of pH, composition of andisol: bioball: *Bacillus* sp., and contact time in absorbing cadmium (Cd) metal.

METHODS

The tools used in this research are Atomic Absorption Spectroscopy (AAS) type AA-6650 F Shimadzu Quantachrome Surface Area Analyzer (SAA) Nova 1200e, Fourier Transform-Infrared (FT-IR) tools type FT-IR-8201 PC Shimadzu, Scanning Electron Microscope (SEM) JEOL JED-2300, and Olympus CX21 microscope.

The materials used in this research are andisol soil (from Mount Lawu Central Java), Bioball (from the Depok fish market, Surakarta, Central Java), LB medium (10 g peptone, 5 g yeast extract, 5 g NaCl and 15 g agarose), Cd mother liquor 1000 ppm (Merck), NaOH powder (Merck), 65% HNO₃ solution (Merck), 37% HCl solution (Merck), KCl powder (Merck), C₆H₈O₇ powder (Merck), Na₃C₆H₅O₇ powder (Merck), CH₃COOH solution (Merck), CH₃COONa powder (Merck), NaF powder (Merck), Ammonia Solution (Merck).

Andisol Soil Preparation and Activation

The andisol soil was washed with water and dried, and then the andisol soil was grounded. Next, the andisol soil was sieved through a 150-mesh sieve. The powder that passed 150 mesh was immersed in distilled water and filtered, then dried at 105 °C for 4 hours. A quantity of 50 grams of andisol soil was mixed with 250 mL of 3 M NaOH solution. Then, the mixture was stirred at 70 °C for 5 hours. After cooling, the mixture was filtered and washed with distilled water until neutral. After that, the andisol soil was dried in an oven for 4 hours at 105 °C.

The specific total acidity test of andisol soil was carried out using the ammonia sorption method. About 0.5 grams of Andisol soil was placed in a porcelain crucible, then weighed and placed in a desiccator, in the center of which was placed a small disk containing ammonia. The desiccator was closed tightly and left for 24 hours. The desiccator lid was opened and left for 2 hours so that the ammonia vapor that was not absorbed could evaporate into the air. The porcelain crucible was weighed to obtain the weight of the base adsorbed on the surface of the andisol soil.

Preparation of Liquid Inoculum for *Bacillus sp.*

The LB medium was put into a test tube under sterile conditions. After being sterile, *Bacillus sp.* isolate was added into a test tube containing LB agar medium and incubated for 2 × 24 hours. After 2 × 24 hours, the culture of *Bacillus sp.* was transferred to an erlenmeyer containing 100 mL of LB medium and shaken for 2 × 24 hours. After 2 × 24 hours, the inoculum of *Bacillus sp.* was transferred to an erlenmeyer containing 1L of LB medium and shaken until *Bacillus sp.*'s density/number of cells was as much as 10⁶ cells/mL. The density/number of cells of *Bacillus sp.* was calculated every 24 hours using the direct calculation method using a hemocytometer.

The calculation of cell density/number was carried out by taking 1 mL of *Bacillus sp.* and was diluted 100 times. The bacteria was dripped into the hemocytometer as much as one drop and was viewed using a microscope with a magnification of 10 times. The number of cells on the hemocytometer was then counted. The composition of Andisol:Bioball:*Bacillus sp.* carried out with variations in the composition of Andisol Soil: Bioball: *Bacillus sp.* (g:piece:(cell/mL)) 2:0:0; 1.5:1:3.9 × 10⁵; 1:2:7.8 × 10⁵; 0.5:3:1.17 × 10⁶; and 0:4: 1.56 × 10⁶.

Determination of Adsorption Isotherms

Make a Cd solution from a standard solution of Cd 1000 ppm with a concentration of 0, 0.2, 0.5, 1; 2, 4, 6, 8, and 10 ppm, with the addition of diluent. The absorbance of the solution was measured with Atomic Absorption Spectroscopy (AAS), and then a curve of the relationship between the absorbance and the concentration of metal ions was made. Andisol soil composition: bioball: *Bacillus sp.* (g : piece : cell/mL) 1:2:8.2 × 10⁵ put into a beaker containing 100 mL of 6 ppm Cd solution with various pH buffers 1, 2, 3, 4, 5, and 6. The aerator was added at a constant speed for 60 minutes for each beaker. After 60 minutes, the solution was diluted five times. The diluted solution was then tested using AAS.

Data Collection and Analysis Techniques

Qualitative data collection techniques using NaF solution were used to determine the pH of the andisol soil so that it could be known whether the andisol soil contained allophane. Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX) analysis was carried out to analyze the andisol soil's morphology and the elemental content in the andisol soil. The optimum conditions for adsorption and bioremediation of Cd metal solution were determined by comparing the ability of Andisol:Bioball:*Bacillus sp.* soil with various pH and using andisol soil composition variations: Bioball: *Bacillus sp.* 2:0:0; 1.5:1:4.3 × 10⁵; 1:2:8.2 × 10⁵; 0.5:3:1.21 × 10⁶; and 0:4:1.58

$\times 10^6$ (g:piece:(cell/mL)), and contact time of 30, 60, 90, 120, and 150 minute. AAS analyzed the solution to determine the concentration of Cd metal before and after the adsorption and bioremediation processes. Specific total acidity data was obtained using the ammonia vapor adsorption method which was used to see the difference in acidity between each adsorbent variation carried out with maximum absorption capacity. Specific total acidity can be determined by comparing the sample's weight before and after ammonia adsorption.

RESULTS AND DISCUSSION

Identification, Activation, and Characterization of Andisol Soil

The andisol in this research is the same material as previous research and contains allophane minerals (Pranoto *et al.*, 2018b). The presence of allophane minerals in the soil can be identified by performing the NaF pH test. If allophane is dispersed in NaF solution, OH⁻ ions will be exchanged with F⁻ ions and the formation of fluoaluminate. The soil survey identification method states that the NaF pH test with a value of 9.4 strongly indicates the presence of amorphous material in the soil, such as allophane. The results of the NaF pH analysis were carried out on andisol soil before activation, and the NaF pH value was 11.3.

Based on the pH value of NaF, it shows that the andisol soil contains allophane minerals, as indicated by a NaF pH test determining the allophane content of volcanic soils in Mexico, which showed a pH of 11.1. The NaF test was used as a field investigation for allophane minerals in the soil because the F⁻ anion was the strongest in absorbing allophane compared to other anions. NaF solution can react rapidly when added to andisol soil containing allophane minerals, where fluorine ion (F⁻) will react with Al metal as shown in Equation 1.



Determination of the pH of NaF is based on the release of OH⁻ ions in a solution where there is an exchange between OH⁻ ions and F⁻ ions. The exchange of OH⁻ ions with F⁻ ions can occur in soils dominated by amorphous minerals such as allophane. The more OH⁻ ions released, the more alkaline the soil's pH value is, indicating the presence of allophane mineral content. The number of released OH⁻ ions will be proportional to the amount of active Al in the soil. More analysis was performed to determine the acidity properties of andisol with the ammonia sorption method. The results of the acidity measurements can be seen in Table 1.

Table 1. Specific Total Acidity Test Results.

Specimen	Acidity (mmol/g)
Natural Andisol Soil	0,49
Activated Andisol Soil	1,26

Based on Table 1, the andisol soil experienced an increase in the acidity number of 0.77 mmol/g. The increase in acidity was due to the increase in the acidic sites of activated andisol soils. It states that the acid site is formed due to the presence of H⁺ cations from activation, which functions as a counterweight to the negative charge in andisol soils. In addition, the substitution was due to protonation in the Si–O–Si and Al–O–Al bonds to form Si–OH and Al–OH.

Characterization of Andisol Soil

SEM analysis was used to determine the surface morphology of the andisol adsorbent before and after activation. The results of SEM characterization are shown in Figure 1.

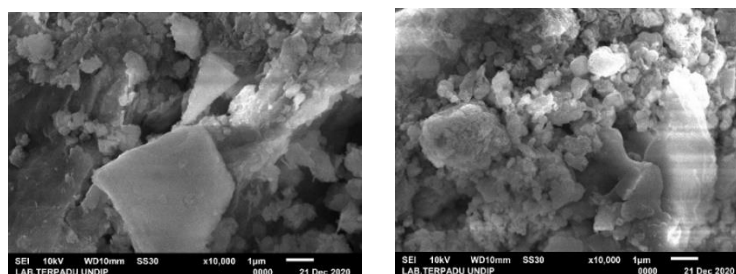


Figure 1. (a) Soil Morphology of Andisols Before Activation and (b) After Activation.

Before activation, the adsorbent pores are still mostly closed. After activation, more and more adsorbent pores are opened, as shown in the [Figure 1 \(b\)](#). This was confirmed by the analysis of the adsorbent before and after activation. After activation, the pore size is larger. The larger the pore size of the adsorbent, the more pollutants are adsorbed.

Test the density/number of *Bacillus* sp. cells before and after bioremediation

The density/number of cells in *Bacillus* sp. was calculated using a direct calculation method and observed using a microscope. The test results of the density/number of bacterial cells of *Bacillus* sp. are shown in [Table 2](#).

Table 2. Test Results for *Bacillus* sp.

Specimen	Total (cell/mL)
Cells prior to bioremediation	8×10^6
Cell after bioremediation	2.5×10^6

Based on [Table 2](#), the number of *Bacillus* sp. cells after bioremediation became smaller. The difference in the number of *Bacillus* sp. cells before and after the bioremediation process became smaller because most of the *Bacillus* sp. cells died after the bioremediation process. The growth phase of bacteria can be divided into 4 phases: the lag phase, the logarithmic (exponential) phase, the stationary phase, and the death phase. The lag phase is the adaptation phase of bacteria to the new environment. The exponential phase is characterized by a period of rapid growth. The stationary phase is when the rate of bacterial growth is equal to the rate of death, so the total number of bacteria will remain constant. This stationary phase is followed by a death phase which is characterized by an increase in the death rate that exceeds the growth rate. This shows that the number of *Bacillus* sp. cells is reduced because they have experienced a death phase.

Effect of bioball on the performance of *Bacillus* sp.

The effect of bioball media on *Bacillus* sp. in the bioremediation process can be seen in [Figure 2](#). [Figure 2](#) shows that the use of bioball media on *Bacillus* sp. resulted in a greater percentage of Cd reduction with a difference of 1.58% than without using bioball. This concludes that the addition of bioball media is able to give maximum results to *Bacillus* sp. in the bioremediation process. According to [Wang *et al.* \(2009\)](#), giving bioball can reduce death and increase cell ability so that it can provide maximum results.

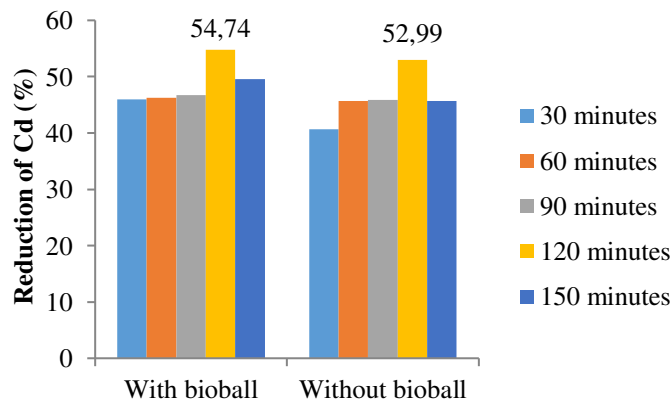


Figure 2. Performance of *Bacillus* sp. with and without bioball.

Effect of Varying pH Conditions

One of the factors that influence adsorption and bioremediation is pH. Variations in pH were carried out to determine the optimum pH conditions for adsorption and bioremediation. Determination of the optimum pH was carried out under pH conditions of 1 – 6 for 60 minutes with the composition of the soil andisol: bioball: *Bacillus* sp. 1:2: 8.2×10^5 (gr:piece:(cell/mL)). The results of the pH optimization can be seen in [Figure 3](#).

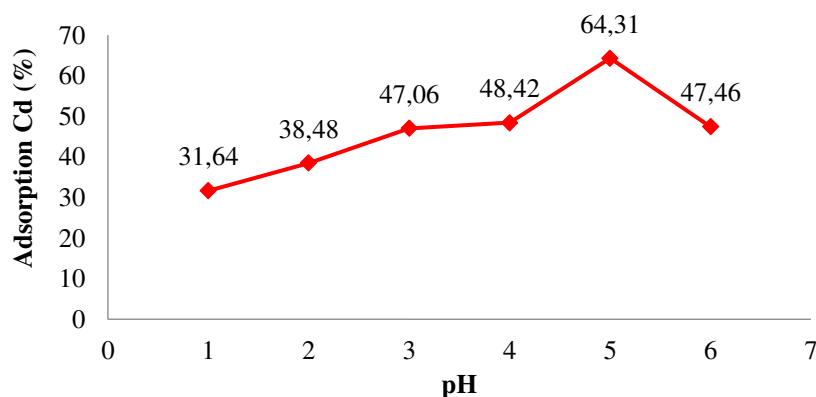


Figure 3. Effect of pH toward adsorption and bioremediation of Cd metals.

Based on [Figure 3](#), it can be seen that the optimum adsorption percentage occurred at pH 5. [Figure 3](#) also shows that at pH 1 – 5, the adsorption percentage decreased. This is because at conditions of pH <5 protonation will occur, which results in the formation of H_3O^+ so that there will be competition for H^+ ions with metals ([Mochalski et al., 2019](#)). The lower the pH, the more H^+ ions formed, so the adsorption percentage is lower. Meanwhile, at pH >5, hydroxide ions will be formed so that Cd will precipitate and form hydroxide precipitates, and the percentage will decrease ([Memon et al., 2007](#)). Based on the calculations, $Cd(OH)_2$ begins to form at pH 5.41. At pH 5, a deprotonation process occurs in andisol soil, forming a negative site for OH^- ions to have free electrons that are effective for binding Cd metal. Meanwhile, in *Bacillus* sp., a deprotonation process occurs in the cell wall, producing a maximum reductase enzyme at pH 5.

Suppose the pH of the andisol is more alkaline. In that case, the pores will also be larger, as in the following data, which shows that at pH 11, the andisol structural framework is almost intact, and only a small amount of silicate polymer is lost. In contrast, at pH > 11, the local structure of imogolite is destroyed due to desilication and dealumination at the edge of the pores, which is accompanied by amorphous formation. Then, at pH 12.5, the pore size is larger, which makes it easier for nitrogen to enter. The hollow spherical structure of andisol was damaged at pH 13, although there were still some unbroken andisol particles or only mild damage.

CONCLUSION

This research used andisol, bioball, and *Bacillus* sp. to adsorb Cd metal. The adsorption was conducted by variations of pH, composition, contact time, and initial concentration of Cd. The percentage of Cd reduction increases with increasing pH until the optimum conditions are obtained. Andisol, bioball, and *Bacillus* sp. soil can absorb Cadmium (Cd) with the best conditions at pH 5, with ratio pf andisol: Bioball soil: *Bacillus* sp. 1.5:1:4.3 $\times 10^5$ (gr:piece:(cell/mL)), and contact time of 120 minutes with a percentage removal of Cd of 75.6%. Combining three components shows good synergy in removing cadmium metal and can be developed in large-scale applications.

CONFLICT OF INTEREST

There is no conflict of interest in this article.

AUTHOR CONTRIBUTION

All authors conceived and designed the study. RTW: Experiment conduction, Data analysis, Manuscript preparation; PP: Supervision, Manuscript editing; EP: Supervision, Manuscript editing. All authors contributed to manuscript revisions. All authors approved the final version of the manuscript and agree to be held accountable for the content therein.

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