Short Communication:  
**The utilization and effectiveness test of andisol soil-bioball-\textit{Agrobacterium} sp. toward heavy metal chrome removal**

**PRANOTO\textsuperscript{1,*}, RETNO ROSARIASTUTI\textsuperscript{2}, ALFIAN PRIHANDOKO\textsuperscript{3}**

\textsuperscript{1}Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia. Tel./fax.: +62-271-663375, *email: pakpranotomi@gmail.com\textsuperscript{a}  
\textsuperscript{2}Department of Soil, Faculty of Agriculture, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia

Manuscript received: 31 August 2018. Revision accepted: 29 September 2018.

Abstract. Pranoto, Rosariastuti R, Prihandoko A. 2018. Short Communication: The utilization and effectiveness test of andisol soil-bioball-\textit{Agrobacterium} sp. toward heavy metal chrome removal. Biodiversitas 19: 1955-1959. This study was aimed to determine chrome metal adsorption and bioremediation ability using andisol soil, bioball, and \textit{Agrobacterium} sp. The andisol soil characterization was performed by Fourier Transform Infrared Analyzer (FTIR), X-Ray Diffraction (XRD), and Surface Area Analyzer (SAA) while the cell quantity of \textit{Agrobacterium} sp. was calculated by hemocytometer method. Determination of adsorption and bioremediation effectiveness was done using several parameters such as effect of pH solution, ratio variation of andisol soil-bio ball-\textit{Agrobacterium} sp, and contact time. The pH variation was ranged from 1-6, while the composition variation of andisol-bio-ball-\textit{Agrobacterium} sp. were 2: 0: 0, 1, 5: 1: 5, 1: 2: 10, 0, 5: 3: 15, and 0: 4: 20 (gr. item: mL), and contact time of 30,60,90,120, and 150 minutes. The result of this research shows that the optimum adsorption and bioremediation of Cr metal at pH 5, while the optimum ratio of andisol soil-bioball-\textit{Agrobacterium} sp was 1: 5: 1: 5 (gr. item: mL), with the contact time for 120 minutes with Cr decrease percentage of 71.3%. The adsorption isotherm followed Langmuir and Freundlich isotherm.

Keywords: Adsorption, \textit{Agrobacterium}, bioball, bioremediation, chrome, soil andisol

**INTRODUCTION**

Rapid industrial growth in Indonesia on one side has a positive effect to country, but it also has a negative impact on the environment. The increasing industrial residua especially waste can be accumulated and can pollute the environment. Heavy metals are among these harmful chemicals. Most industries such as the electroplating industry, metallurgy, melting, batik, and others contribute to spreading heavy metals to the environment (Baidho et al. 2013).

One of the heavy metals is chrome that harmful to health and the environment. If chrome is used excessively, it will cause acute poisoning. Other impacts, these heavy metals could be mutagenic and carcinogenic which lead to the serious disease such as lung cancer, kidney failure, anemia, skin allergies, asthma and stomach (Kaszycki et al. 2005, Palar 2008). According to the Ministry of Environment Decree KEP-03/MENLH/I/2010 the quality standard of industrial wastewater for maximum total chrome parameters is 1 mg/L.

Many method has been reported by previous researchers to treat chrome metals in industrial wastewater such as chemical precipitation (Gheju and Balcu 2011), ion exchange (Rafati et al. 2010), electrochemical precipitation (Animes et al. 2011), coagulation-flocculation (Haydar and Aziz 2009), solvent extraction (Elbagermi et al. 2013), membrane separation (Kumar et al. 2015), electrolysis (Wu et al. 2013), bioremediation (Iye 2015) and adsorption (Mthombeni et al. 2015). Bioremediation is the use of biological materials (e.g. microorganism) in the removal of toxic compounds from the environment such as the heavy metals which are considered more cost-effective and environmentally friendly (Iye 2015). Adsorption is also heavy metal removing method, and it is efficient, environmentally friendly, cost-effective and easy treatment method (Mthombeni et al. 2015).

Andisol soil is one type of soil that can be used as an adsorbent. In andisol, allophane minerals that have high specific surface area, porosity, and ion exchange capacity are usually found. It could be applied to wastewater treatment (Pranoto et al. 2013). The Andisol surface has properties such as the exchange of cations and anions, sorption of organic and inorganic compounds, and the acidity derived from silanol (Si-OH) and aluminol (Al-OH and AlOH\textsubscript{2}° - OH and single coordination - OH/monodental) functional groups (Sukmawati 2011).

\textit{Agrobacterium} sp. is a potential bacterium that can remove toxic substances so that it can be used in bioremediation. Pramono (2013) showed that \textit{Agrobacterium} sp. was able to reduced Cr (VI) in both cell growth and rest conditions up to 100% and 51% within 18 hours. Moreover, Wang (2009) showed that \textit{Agrobacterium} sp. has the ability to decrease nicotine in tobacco solid waste. The use of bacteria requires a medium. Bioball can be used as a bacterial medium. The bioball has a function as a place of living bacteria to maintain water quality (Said 2005). Furthermore, the use of bioball can balance the
expenses (Yang 2003). In this research, the ability of andisol soil, bioball, and *Agrobacterium sp.* on heavy metal (Cr metal) removal was studied.

**MATERIALS AND METHODS**

**Materials and instrumentation**

The materials used in this research were andisol soil (Cemoro Kandang, Lawu Mount, Indonesia), Bioball (Depok Fish Market, Surakarta, Indonesia), isolate of *Agrobacterium sp.* (collection to UNS Central Laboratory, Surakarta, Indonesia), Agar LB medium, distilled water, 1000 ppm of Cr standard solution (E-Merk), NaOH (E-Merk), HNO₃ p.a (E-Merk), HCl p.a (E-Merk), KCl (E-Merk), Ca₃(H₂O)₅ (E-Merk), Na₃C₆H₅O₇ (E-Merk), CH₃COONa (E-Merk), and CH₃COOH (E-Merk).

The instruments and apparatus used in this research were atomic adsorption spectrometer (AAS, Perkin Elmer Analyst 700), Fourier transform infrared spectroscopy (FTIR, Shimadzu 8201 PC), X-ray diffraction (XRD, Shimadzu 6000), Surface Area Analyzer (SAA, Nova 1200e), Hemocytometer (Assistant), Microscope (Olympus CX21), analytical balance (Sartorius BP 110), pH meter (Eutech Instrument pH 700), shaker (Mitamura Riken), and glass tools (Pyrex and Duran).

**Preparation of andisol soil and *Agrobacterium sp.***

Andisol soil was cleaned to remove impurities, washed with water and dried with aerated in the open air. Afterward, Andisol was crushed until smooth. Andisol soil was then sieved with a 150 mesh sieve. The powder that passed 150 meshes were soaked in distilled water and filtered, which was then dried at a temperature of 105°C for 4 hours. The subsequent andisol soil was mixed in 250 mL of 3 M sodium hydroxide solution for 5 h at 70°C. It was then washed with distilled water until neutral condition and calcined for 3 h at 400°C. The final product was then characterized using FTIR, XRD, and SAA.

Agar LB medium was placed into a sterile reaction tube. Then *Agrobacterium sp.* isolate was inserted into the test tube containing agar LB medium and incubated for 2x24 hours. After 2x24 hours, the culture of *Agrobacterium sp.* was transferred into a reaction tube containing 100 mL agar LB medium and incubated for 2x24 hours. After 2x24 hours, the culture of *Agrobacterium sp.* was transferred into a reaction tube containing 1L agar LB medium and incubated for 2x24 hours. The cell number of the final product was calculated by hemocytometer method.

**Optimization of pH solution**

Andisol soil: bioball: *Agrobacterium sp.* (gr: item: mL) 1: 2: 10 were placed into beaker containing 100 mL of 6 ppm Cr solution with buffer pH variation of 1, 2, 3, 4, 5, and 6. The solution was given an aerator and shaken for 60 minutes. After 60 minutes, the solution was diluted 5x and analyzed using AAS.

**Optimization of ratio and contact time**

Andisol soil: bioball: *Agrobacterium sp.* at ratio of 2: 0: 1, 1.5: 1: 5: 1: 2: 10, 0.5: 3: 15: 0: 4: 20 (gr: item: mL) and 20 mL *Agrobacterium sp.* without bioball were placed into beaker containing 100 mL of 6 ppm Cr solution (pH optimum). The solution was given an aerator, and shaken for 30, 60, 90, 120 and 150 minutes. Afterward, the solution was diluted 5x and analyzed using AAS.

**Adsorption isotherm**

Andisol soil: Bioball-*Agrobacterium sp.* ratios at best conditions were added to 10 mL of varied Cr solution (2, 4, 6, 8, 10 and 12 ppm). The solution was given an aerator and shaken at optimum contact time. Afterward, the solution was diluted 5x and analyzed using AAS.

**RESULTS AND DISCUSSION**

**Characterization of andisol soil**

**FTIR Analysis**

The IR spectra of natural andisol soil and active andisol soil was shown in Figure 1. It can be observed that Si-OH/Al-OH peak at 3445 cm⁻¹, H-OH peak at 1642 cm⁻¹, Si-O-Si peak at 1004 cm⁻¹, and Si-O/Al-O peak at 573-473 cm⁻¹ was found in natural andisol soil. The found to peak at 2305 cm⁻¹ was the peak of impurities contained in andisol soil (Silverstein and Webster 2005). Moreover, Si-OH/Al-OH peak at 3427 cm⁻¹, H-OH peak at 1654 cm⁻¹, Si-O-Si peak at 1002 cm⁻¹, and Si-O/Al-O peak at 557-465 cm⁻¹ was found in active andisol soil. The active andisol soil did not reach the peak at 2305 cm⁻¹ because the activation process is not capable for removing impurities. The loss of impurities after the activation process caused pores of andisol soil surface was opened.

**XRD analysis**

The XRD diffractograms of natural andisol soil and active andisol soil are shown in Figure 2. This figure presents allophane minerals appeared at (2θ) 8, 17.5, 23.0, 46.5, 52.0, 57.3, 63.5, 64.5, 78.8, and 82.0 degrees. There are also other minerals such as montmorillonite at 10.0, 21.9, 29.3, 35.5, 46.8, 57.3, 63.5, 78.8, and 82.0 degrees, as well as feldspar at 23.8, 34.4, and 41.0. The comparison of diffractogram after activation indicates that some peak shifted and the intensity, as well as appearance of a new peak, decreased. Structural damage at soil minerals andisol resulted in the decreased intensity of the diffractogram.

**SAA analysis**

The SAA analysis of natural andisol soil and active andisol soil was shown in Table 1. It shows that the surface area of andisol soil increases after the activation process. The impurity on the andisol soil surface has been dissolved during the activation process so that the opening of pores of the andisol soil and the value of the surface area increased. Adsorbents with larger surface area will provide more active sites (Hartopo 2014). The surface area will give the area of exposure to the surface of the andisol soil in the adsorption process to the chrome metal.
Identifications of Agrobacterium sp.

Test cell of Agrobacterium sp. before and after bioremediation

The quantity of Agrobacterium sp. cells after bioremediation became less (Table 2). Differences in the quantity of Agrobacterium sp. cells before and after bioremediation process was less because after the bioremediation process. Most of the Agrobacterium sp. cells died. Bacterial growth phase could be categorized into 4 phases, i.e., lag phase, logarithmic phase (exponential), stationary phase and death phase. The lag phase is the phase of bacteria to adjust to the new environment. The exponential phase is the process of a period of rapid growth of bacteria. The stationary phase is when the rate of bacterial growth is equal to the rate of death, so the quantity of bacterial will remain. This stationary phase is followed by a death phase that increases the rate of death than the rate of growth (Volk and Wheeler 1988). This indicates that the quantity of Agrobacterium sp. cells is reduced due to the death phase.

Effect of bioball in Agrobacterium sp.

The effect of bioball in Agrobacterium sp. is shown in Figure 3. The use of bioball medium in Agrobacterium sp. caused a larger percentage of Cr decrease, which is 54.74% than without bioball (Figure 3). This indicates that the addition of bioball medium is able to give maximum result to Agrobacterium sp. in bioremediation process. The giving bioball could reduce the cells death and increase the ability of cells to give maximum results (Ng et al. 2011).

Determination of optimum conditions

Effect of pH

The test results of the pH effect toward adsorption and bioremediation of Cr metal were presented in Figure 4. Figure 4 shows that at pH conditions 1-5, the percentage of Cr adsorption increased. However, at pH 6 conditions, the percentage of Cr adsorption decreased. At low pH (<5), the protonation will occur resulting in the formation of H₃O⁺. This will cause the competition between H₃O⁺ and metals. The lower of pH, the more H⁺ ions are formed so that the lower percentage of Cr decreased. While at pH>5, hydroxide ion will be formed so that the deposition of Cr occurred lead to the formation of precipitated hydroxide causing their percentages decreased. At pH 5, a process of deprotonation on andisol soil occurred so that a negative site of OH⁻ ions was formed to have effective free electrons to bind Cr metals. While on Agrobacterium sp., it will produce maximum reductase enzyme at pH 5.
The test result of the ratio and contact time effect toward adsorption and bioremediation of Cr metal was presented in Figure 5. It can be seen in Figure 5 that the optimum condition of ratio of andisol soil: Bioball: *Agrobacterium* sp. (gr: item: mL) was 1.5: 1: 5 at contact time for 120 minutes with the percentage of 71.3%. At that ratio the active site of soil andisol possibly had ability to absorb Cr metal greatly. The reductase enzyme produced by *Agrobacterium* sp. Could also help andisol soil in absorbing Cr metal.

The optimum contact time occurred at 120 minutes. When the contact time absorbing Cr metal was longer, the number of absorbed Cr metal will also greater until the optimum conditions. However, the concentration of Cr metal absorbed will decrease when it has passed the optimum contact time. At that condition, the saturation point, which means that Cr metal is no longer acceptable by andisol soil and *Agrobacterium* sp. and the absorbed Cr will be released back to the solution.

### Table 3. Langmuir and Freundlich Isotherm Value

<table>
<thead>
<tr>
<th>Isotherm adsorption</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Langmuir</strong></td>
<td></td>
</tr>
<tr>
<td>Adsorption capacity (mg/g)</td>
<td>0.2146</td>
</tr>
<tr>
<td>$K_l$</td>
<td>2.9298</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9896</td>
</tr>
<tr>
<td><strong>Freundlich</strong></td>
<td></td>
</tr>
<tr>
<td>Adsorption capacity (mg/g)</td>
<td>0.1651</td>
</tr>
<tr>
<td>$N$</td>
<td>8.2713</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9915</td>
</tr>
</tbody>
</table>

**Effect of ratio and contact time**

Langmuir and Freundlich isotherm graphics for Cr metal can be seen in Figure 6 and 7. Langmuir isotherms show that the adsorption process between adsorbent and adsorbate chemically occurred to form a monolayer (Bentahar et al. 2016). From Figure 6, the value of $R^2$ close to 1, precisely the $R^2$ value is 0.9896 indicating that the adsorbent active group interacts with Cr metal through chemical bond. While at Freundlich plot graph (Figure 7), the value of $R^2$ close to 1 by 0.9915. This indicates that the adsorption of Cr metal also physically happened through Van der Waals forces. When the Van Der Waals force occurs, the surface of the electronegative adsorbent interacts with the electrolytic Cr metal, although the interaction was weak. This weak repulsive force caused the adsorbate to move from one point of adsorbent surface to another surface forming a multilayer (Pranoto et al. 2013).

Based on the calculation at Table 3, the adsorption capacity of adsorbent on Langmuir isotherm was 0.2146 mg/g and Freundlich isotherms of 0.1651 mg/g in the Cr solution concentration ranged of Cr2-10 ppm.

In conclusion, soil andisol, bioball and *Agrobacterium* sp. could absorb the best chrome (Cr) metal at pH 5, with ratio soil andisol: Bioball: *Agrobacterium* sp. (gr: item: mL) 1.5: 1: 5, and contact time for 120 minutes with Cr decrease percentage of 71.3%. Type of adsorption isotherm
against chrome metal (Cr) solution was following by isotherms Langmuir and Freundlich.

REFERENCES


Hartopo. 2014. Kajian efektivitas campuran lempung bekonang dan andisol gunung lawu sebagai penjerap logam berat mangan (Mn) untuk peningkatan kualitas air. [Tesis]. Program Ilmu Lingkungan, Universitas Sebelas Maret, Surakarta. [Indonesian]


Palar H 2008. Pencernaan dan Toksikologi Logam Berat. PT. Rineka Cipta, Jakarta. [Indonesian]


