

Potency of Indigenous Bacteria of Mt. Merapi, *Arthrobacter chlorophenolicus* for Chromium (VI) Bioremediation

by Fina Binazir Maziya

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PREFACE

It is my great pleasure to grace all of your presence to the 6th ICSBE 2021 virtually. The International Conference on Sustainable Built Environment is bi-annual conference started from 2010 till now. At first, the 6th ICSBE was scheduled in 2020 however, the COVID-19 pandemic altered all of our life order including this conference that have to be postponed into 2021 and conducted virtually. This conference is held for 2 days in 19 – 20 October 2021 conducted in Faculty of Civil and Planning Engineering, Universitas Islam Indonesia, Yogyakarta-Indonesia.

The main theme of the 6th ICSBE 2021 is "sustainable infrastructure and environment for the smart cities". This theme was dedicated to discuss and deliver an implementable solution to address the issues of urban planning, transport systems, water and sanitation, waste management, disaster risk reduction, access information, education, and capacity-building. Therefore, it is important to mapping, planning and designing a sustainable environment and infrastructure that may greatly encounter and reduce those issues above to reach the sustainable development goals. Based on this, this conference focuses the following topics but not limited to livable rural and urban environment, green infrastructure, sustainable resource management, sustainable city and disaster management.

In this conference, we received more than 80 papers at the beginning, which were covering in five topics. All the submitted papers were reviewed by peer to peer and double blind with two reviewers. The 6th ICSBE highly applied strong and accurate review process prior to be published in the IOP conference proceeding indexed Scopus in the series of Earth Environmental Science (EES). Finally, we are accepting 50 papers to be presented in this conference. The conference model is plenary session and parallel session which is held for 2 days. In the plenary session, there are 3 invited speakers for the 1st day and 2nd day. Each invited speakers are allowed to give presentation for 30 minutes. The question answer is given after all invited speakers finished their presentation for 40 minutes. In the parallel session, there are 5 subtopics delivered in 2 days. In the 1st day, 3 subtopics: Green Infrastructure, Sustainable Resource Management, and Sustainable City are delivered. There are 26 presenters delivered their paper. In the 2nd day, 2 subtopics: Disaster Management, and Livable Rural and Urban Environment, are delivered. There 24 presenters delivered their paper. Each presenters have 12 minutes to give presentation and 3 minutes for question and answer. All the conference presentation is delivered by using zoom technology belongs to Universitas Islam Indonesia with 1000 capacity of participants. This conference is participated by audience from Indonesia, Turkey, Japan, USA, Malaysia, and Germany.

The 6th ICSBE 2021 is organized by the faculty of Civil Engineering and Planning, Universitas Islam Indonesia and supported by partner universities; Gifu University (Japan), University of Malaya (Malaysia), University of Rhode Island (USA), National Cheng Kung University (Taiwan), University of Boras (Sweden), University Hawaii at Manoa (USA), Hokkaido University (Japan), Alanya Alaaddin Kaykubat University (Turkey), Behaus-Universitaet Weimar (Germany), National University of Singapore (Singapore), and other international institutes,



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Last but not least, I would express our sincere gratitude to the keynote speaker: The Governor of Jakarta, Anies Rasyid Baswedan, Ph.D, to the invited speakers Prof. Nobouto Nojima, Prof. Noor Choliz Idham, Prof. Frank Eckardt, Asso. Prof. Anita Petterson, Assoc. Prof. Anita Pettersson, and Prof. NG How Yong, and as well as all participants for joining and sharing of idea, knowledge, and friendship in a relaxing environment. And, the most important, I would to sincere and great appreciation to the organizing committee, editors, scientific committee, and reviewers for all their solidity, harmony, and synergy work. We do hope all participants are going to enjoy the conference in terms of both its academic and social atmosphere.

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Table of contents

Volume 933

2021

[◀ Previous issue](#) [Next issue ▶](#)

6th International Conference on Sustainable Built Environment (ICSBE) 19-20 October 2021, Yogyakarta, Indonesia (Virtual)

Accepted papers received: 11 November 2021

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Preface

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Green Infrastructure

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An Overview of High-Rise Buildings in Jakarta since 1967 to 2020

M F Effendi, I F Ridzqo and S W Dharmatanna

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Swelling Characteristics of Compacted Claystone-Bentonite Mixtures

Y F Arifin, M Arsyad and H Muslim

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Comparative Study of Marshall Properties and Durability of Superpave and AC-WC Pavement by Using Starbit E-55 and Pen 60/70

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The Use of Steel Slag as Substitution of Coarse Aggregate on Indirect Tensile Strength and Marshall Properties of AC-WC

A A Nugraha, M Fauziah and Subarkah

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OPEN ACCESS 012005

Preliminary Study on Hand-cast Lightweight Concrete Block using Raw Rice Husk as Aggregate

S Winamo

+ Open abstract  View article  PDF

OPEN ACCESS 012006

An Overview of Behaviour of Concrete with Granulated Blast Furnace Slag as Partial Cement Replacement

R A T Cahyani and Y Rusdianto

+ Open abstract  View article  PDF

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Greenhouse Gases (GHG) Emissions during the Construction Stage of a Precast Building in Indonesia

K N R Putri, M I Tjandrawira and T N Handayani

+ Open abstract  View article  PDF

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Response analysis of a multi-story building structure with a variety of horizontal irregularities and shear wall geometries

A H Prathama, M Teguh and F Saleh

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Comparative study of structural response on multi-story buildings with shear wall and bracing systems

M A Rahman, M Teguh and F Saleh

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Resource Management

OPEN ACCESS 012010

The effect of land-use change on river watershed quality (case study: Cimahi Watershed, West Java, Indonesia)

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A N Lathifah, A A Asmara, F A N Alfisyahr and F B Maziya

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012012

Resilience Thinking Approach to Protect Marine Biodiversity in Small Islands: A Case of Gili Trawangan, Indonesia

LAA Bakti, Marjono, G Ciptadi and F Putra

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012013

The Effect of the COVID-19 Pandemic on Ambient Air Quality in Yogyakarta Urban Area Parameters SO₂, CO and, NO₂ with Inverse Distance Weighting (IDW)

E R Sihayuardhi, W Brontowiyono, F B Maziya and L Hakim

[+ Open abstract](#) [View article](#) [PDF](#)

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012014

The Influence of Aluminum Slag Ash for Paving Block Production

N Muzayyanah, F B Maziya, Y Yuriandala and Kasam

[+ Open abstract](#) [View article](#) [PDF](#)

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012015

Application of a hydrological method for determining monthly environmental flow in Code River, Indonesia

Adam Rus Nugroho

[+ Open abstract](#) [View article](#) [PDF](#)

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012016

Study of Solid Waste Management in the Buildings Area of Integrated Campus of Universitas Islam Indonesia

F M Iresha, Kasam, F Muhammad and A Rahmat

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012017

Physical Parameters of Compost Made from Cattle Farming Waste Using Vermicomposting Method

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Kasam, F M Iresha, F Muhammad, A Rahmat, W S Ramadhani and M Nurtanto



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Sustainable City

OPEN ACCESS 012018

Comparison Study Of Physical And Mechanical Properties Of AC-WC Mixtures And Porous Asphalt Mixtures With Variations Of Binding Materials

M D Hafidz, M Fauziah and Subarkah

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS 012019

Evaluation of Post-Construction Communal Wastewater Treatment Plants (WWTPs) in Sleman District, Special Region of Yogyakarta

A Yulianto, A F Pramudita, N I Wantoputri and S Rahmawati

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS 012020

Sustainable Architectural Investigations on Bugis Vernacular House: Case Study of Tenun Tourism Village, Samarinda Seberang, East Kalimantan, Indonesia

Iga Nur Ramdhani and Sugini

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS 012021

Sustainable Socio-cultural Aspect Within Green Building User Behavior in Bali, Indonesia

N W Meidayanti Mustika, N P Sueca, N K Acwin Dwijendra and I D G Agung Diasana Putra

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS 012022

Changes in CO2 Emissions in Online Student Learning Activities Due to the COVID-19 Pandemic in Indonesia and Japan

D P J Salamena, Y Cong, F Lin, F Ardisa, U Novella, T Kurosawa, M Sako, Y Ohtani and I Fujiyama

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS 012023

Awareness Before and During Pandemic toward Food Waste: "Comparison between Indonesia and Japanese Students"

Christine, D Aliefia, G E Syaputra, U Novella, A Yamazaki, K Nakatsuka and I Fujiyama

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Assessing the Potential of Solar PV Installation based on Urban Land Cover Analysis

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012025

Outlining Smart Kampung Indicators: Preference Study in Kampung Terban Yogyakarta

I F Maharika, S A Permana, F Nugraheni and M Böhlen

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Disaster Management

OPEN ACCESS

012026

Riverbed Erosion Analysis of Winongo River Using HEC-RAS 5.0.7

P Harsanto, Nursetiawan, B P Kamiel and I Cahyani

[+ Open abstract](#) [View article](#) [PDF](#)

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012027

Landscapes Vulnerability on Climate Change in Yogyakarta Province, Indonesia

D Wacano, N D Latifah, H Bishop, H Gutama, N A I Hasanah, A Yulianto, S Rahmawati, A Nurmiyanto,

H P Putra, F B Maziya *et al*

[+ Open abstract](#) [View article](#) [PDF](#)

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012028

A Study of the Hazard Level and Vulnerability of the Society Against Debris Flow of Merapi in the Putih River Watershed, Indonesia

J Ikhsan, R Faizah, A Hairani and P T Sutawiguna

[+ Open abstract](#) [View article](#) [PDF](#)

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012029

Evaluation of fire safety maintenance of an educational laboratory facility

E R Novanandini, O C Dewi and Y S Nugroho

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012030

The development of structure in the *limasan-* and *joglo-style* vernacular houses after the 2006 Yogyakarta earthquake

J A Rini and N C Idham

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012031

The Spatio-temporal Tectonic Condition and Microzonation Map of Malang Region after the 2021 M6.1 Malang Earthquake for Disaster Risk Mitigation

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Y Muntafi and N Nojima

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012032

Study on the Contribution of Normalization to Reducing Flood Risk in the Ciliwung River, Tebet District, Jakarta

M Yatsrib, A N Harman, S R Taufik, T N A Kesuma, D Saputra, M S B Kusuma, M Farid and A A Kuntoro

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012033

Equivalency of the diagonal shear strength of plastered and non-plastered lightweight brick walls to the strength of red brick walls

Sarwidi and Jafar

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012034

Risk analysis of landslide as a basis of mitigation plan: a case study of Mangunan Village, Bantul, Indonesia

S Aminatun, Jafar and A U Jamal

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012035

Directing Housing Developments for Achieving Earthquake Disasters Safety in Indonesia

N C Idham

[+ Open abstract](#) [View article](#) [PDF](#)

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012036

Physical Distancing Concept of Public Space Placemaking in Post Covid-19: A Study Precedent

S P Anggraini

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012037

Implementation of occupational safety and health management system (OSHMS) on work-related accident rate in the manufacturing industry, Indonesia

A U Abidin, E M Nurmaya, W Hariyono and A H Sutomo

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012038

Hybridity Culture: The Adaptive Reuse Concept for an Escape Place. Study Case: Gedung Antara, Pasar Baru, Jakarta

A A S Fajarwati, O SC Rombe, L Henry, I Rachmayanti and S Meliana

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012039

A Comparison Review of Kevin Lynch's Urban Theory with the Chinese Cosmography of Feng Shui in the Heritage Area, Case Study: Pasar Baru, Jakarta

S Meliana, O SC Rombe, L Henry, A AS Fajarwati and I Rachmayanti

[+](#) Open abstract [View article](#) [PDF](#)

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012040

Liveable Muslim Settlements: A Systematic Literature Review to Understand Current Factors and Challenges

N G Yuli, R Firmansyah and S Haningsih

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012041

Children's Creativity and Creative Environment's impact on Children Quality of Life. Case Study: Kampung Jogoyudan, Yogyakarta.

M Ifitroni

[+](#) Open abstract [View article](#) [PDF](#)

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012042

Coastal Settlement Behavior Setting in Kampung Blok Empang, North Jakarta

A D Tohjiwa, R Q Pramantha and P R E Paksi

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012043

Induced Bank Filtration: Hydraulic Testing of Pilot Filters

M Schelling, T B Boving and K Patil

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012044

Saussure Theory to Determine the Relationship between Sacred Space and Multicultural Community Node. Case Study: *Gereja Ayam*, Pasar Baru, Jakarta

I Rachmayanti, O SC Rombe, L Henry, S Meliana and A A S Fajarwati

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012045

A Spatiotemporal Analysis of Water Quality and Land Use in Tambora Bayan River, Yogyakarta

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A Putri, R Jana, A S Florensia, A A Asmara, A Yulianto and W Brontowiyono

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012046

Risk level mapping of organophosphate pesticides application in agricultural area of Cangkringan Subdistrict, Yogyakarta, Indonesia

S Rahmawati, D Wacano, M Erpinda and A Juliani

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012047

Groundwater Mapping of Total Coliform Contamination in Sleman, Yogyakarta, Indonesia

F D Ekarini, S Rafsanjani, S Rahmawati and A A Asmara

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Potency of Indigenous Bacteria of Mt. Merapi, *Arthrobacter chlorophenolicus* for Chromium (VI) Bioremediation

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Potency of Indigenous Bacteria of Mt. Merapi, *Arthrobacter chlorophenolicus* for Chromium (VI) Bioremediation

A N Lathifah^{1,2}, A A Asmara^{1,3}, F A N Alfisyahr¹ and F B Maziya^{1,4}

¹ Environmental Engineering Department, Faculty of Civil Engineering and Planning, Universitas Islam Indonesia, Yogyakarta, 55584, Indonesia

² Environmental Biotechnology Laboratory, Universitas Islam Indonesia, Yogyakarta, 55584, Indonesia

³ Centre for Environmental Studies, Universitas Islam Indonesia, Yogyakarta, 55584, Indonesia

⁴ Solid and Hazardous Waste Laboratory, Universitas Islam Indonesia, Yogyakarta, 55584, Indonesia

e-mail: annisa.lathifah@uii.ac.id

Abstract. Chromium (VI) in the production process, such as textile, tannery, and electroplating industry, produce hazardous waste when disposed of directly into the aquatic environment. Several chromium pollutions cases, not only in water but also in the aquatic organism, occurred in some regions in Indonesia. Various methods can reduce the Chromium (VI) waste. One of them is the biological method by employing such kinds of bacteria. *Arthrobacter chlorophenolicus* is a pioneer bacterium of Mt. Merapi, which can survive in the minimum conditions of the bacterial primary nutrients, carbon, and nitrogen. This study aims to investigate the ability of *A. chlorophenolicus* to remove Cr (VI) at various concentrations. The research was carried out by growing the *A. chlorophenolicus* into two nutrient media conditions, minimal and rich-nutrient media containing different concentrations of Cr (VI) (5, 10, 20 ppm) for eight days. The results showed that the *A. chlorophenolicus* were grown on both minimal and rich-nutrient media. The *A. chlorophenolicus* could reduce for about 80% of 10 and 20 ppm chromium in eight days. Our results indicate that *A. chlorophenolicus*, the pioneer bacteria of Mt. Merapi, has a great promise for use in Cr (VI) remediation even under minimum nutrients conditions.

1. Introduction

Chromium (Cr) is naturally abundant in Earth's mantle and released by weathering process into water or soil. It can also be produced by industrial processes, such as textile, electroplating, tannery industry, and many so on. Chemically, chromium is one heavy metal that exposes a complex element. Marina [1] explained that Cr in water mainly exists in two oxidation states, i.e., Cr (III) and Cr (VI), depending on pH, potential redox value, and other reducing agents. As well as Cr (III) and Cr (IV) present different chemical, toxicological, and epidemiological characteristics [2]. Chromium hexavalent toxicity is known as highly toxic, while Cr (III) is less mobile toxic. Cr (VI) pollution in the environment is attracting attention since it is widespread worldwide, with high levels in water and soil as a result of natural and anthropogenic processes [3,4]. Furthermore, the poisonous Cr (IV) also affects organisms and microorganisms. The risks to human health range from skin irritation to DNA damage and cancer formation, depending on the dose, degree, and duration of exposure [1].

Among environmental problems, heavy metal pollution in developing countries still becomes a considerable challenge to prevent and solve. For supplying basic requirements of domestic and maintaining economic growth, numerous industries are built and operated. Nevertheless, most of them tend to be careless and have minimum treatment for both solid and wastewater. Specifically, chromium



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is mainly identified in the textile, tannery, and electroplating industries in Indonesian cases. Asmadi [5] explained tannery industry in Semarang city use a colouring agent containing chromium metal. A study by Murniati [6] showed that the production of Batik, an intangible heritage craft awarded by UNESCO, resulted in wastewater containing heavy metals such as chromium. Besides, Agustina *et al.* [7] reported that the electroplating industry in West Java resulted in wastewater containing chromium hexavalent up to 750 ppm. The improper utilizing and treatment of wastewater containing Cr (VI) is the leading cause of artificial chromium pollution in the environment.

Unfortunately, several chromium pollutions cases, not only in water but also in the aquatic organism, occurred in some regions in Indonesia. In Jember, East Java, an electroplating industry reported directly throwing their wastewater in the water stream without treating accomplishment. Bedadung River around the industry-accepted discharge water resulted in the water river quality over the allowed standard. Chromium concentration reached 0.15 mg/L exceed the maximum permitted standard by the government, which is 0.05 mg/L [8]. Even though chromium concentration in Winongo River Yogyakarta was below the maximum standard, chromium inside Red Tilapia (*Oreochromis* sp.) edible fish tissue nurtured in the fishing net surpassed the National Agency of Drug and Food Standard. The limitation allowed to be consumed by a human is 2.5 mg/Kg, but in Tilapia, muscles pointed on 10.2265 mg/Kg, 9.8107 mg/Kg, and 9.2245 mg/Kg in Station 1 to 3, respectively [9]. A higher concentration of chromium in fish tissue than in water exposes the heavy metal possessed bioaccumulation characteristic due to high solubility of Cr (VI) as the toxic ion is transferred quickly through the food chain. River and fish contaminated by chromium hexavalent as the source of water and food can be dangerous for human health. Cr (IV) causing liver and kidney damage, internal bleeding, and breathing problems, so that the International Agency for Research on Cancer has classified it as carcinogenic to humans (Group I) [10, 11].

Some strategies are applied to treat and remediate toxic Cr (VI) include chemical reduction, precipitation, adsorption, ion exchange, electrocoagulation, or biological reduction [1]. Each method has advantages and disadvantages. By comparing chemical and biological methods for removing Cr (VI), the most suitable strategy is based on a requirement of case, site, and the initial evaluation. According to Majone [12] and Linkov [13], especially for in-situ interventions, which necessitate a complete understanding of contaminated matrix geochemistry, hydrogeology, microbiology, and ecology. Moreover, cost-benefit analysis is compiled frequently and in conjunction with comparison risk assessment.

Based on research history in Yogyakarta, to mitigate chromium pollution, a combination of phytoremediation and adsorption was employed to treat tannery Cr-wastewater [14] by selecting chromium reduction bacteria in the waste of tanning leather industries by ozone method [15] and bio-sorption using biomass of *Fusarium* sp. and *Aspergillus niger* for reducing bearing tannery wastewater [16]. A new approach is to remove Cr (VI) contamination using the bioremediation method that employs indigenous bacteria isolated from the Mt. Merapi volcanic deposit after two years of eruption. The bacteria were identified as pioneer bacteria that can survive in the minimum condition of the primary nutrients, Carbon (C) and Nitrogen (N), namely *Arthrobacter chlorophenolicus* [17]. Other studies investigated the potency of *Arthrobacter* sp. to mitigate chromium hexavalent from wastewater, for example, Molokwane [18] using *Arthrobacter* sp., which was able to reduce Cr (VI) up to 94.3% from the initial concentration of 100 mg/L in 24 hours of incubation. In addition, Dey and Paul [19], using the *Arthrobacter* sp. SUK 1201 as a mediator showed a 67% reduction in 2 mM Chromate within seven days of incubation. Thus, this study aims to investigate the ability of indigenous bacteria of Mt. Merapi, *Arthrobacter chlorophenolicus*, to remove Cr (VI) at various concentrations and to observe the effect of incubation time on the ability to reduce Cr (VI) in wastewater. Hence, this study is an early stage of applying that microorganism on bioreactor in the later studies to reduce heavy metals in wastewater and by releasing them to mitigate Cr (VI)-contamination in water resources.

2. Materials and methods

2.1 Sample Collection

Bacterial isolates, *Arthrobacter chlorophenolicus*, were isolated from the volcanic deposit of Mount Merapi, Special Region of Yogyakarta Province, as identified by Lathifah *et al.* [17]. The source of Cr (VI) utilized in this study is the $K_2Cr_2O_7$ compound named synthetic wastewater. The synthetic wastewater receipt is referred to from Rcheulishvili [20]. Some various treatments are occupied, including the incubation time and Cr (IV) concentration. The time incubation series are 24, 120, and 168 hours; also, the Cr (IV) concentration is in the range of 5, 10, 20 ppm. Some nutrition, such as NaCl (Sodium Chloride), KH_2PO_4 (Monopotassium Phosphate), Na_2HPO_4 (Disodium phosphate), NH_4Cl (Ammonium Chloride), $MgSO_4$ (Magnesium Phosphate), $CaCl_2$ (Calcium Chloride), and D-Glucose are added in the medium, and as control of the treatment is no additional nutrition.

2.2 Isolate preparation and acclimatization

Arthrobacter chlorophenolicus isolated from the previous research of Lathifah *et al.*, [17] was inoculated into Nutrient Broth media at 30°C for 48 hours in a shaker incubator. The minimum populations of cell numbers are notified by 10^9 CFU/ml or equal to 0.4 A [21]. Furthermore, the isolate was acclimatized in 5 ml of media containing five ppm Cr (VI), ready for the running stage [22].

2.3 Running stage

For testing the ability of *Arthrobacter chlorophenolicus* to remove Cr (VI) was carried out on a laboratory scale by analysing its growth ability in media containing chromium hexavalent in various concentrations (5, 10, 20 ppm) using the batch culture method [23]. In this study, the growth media used were specific mineral media. The composition of the media (in each g/L) is Na_2HPO_4 (1.264); KH_2PO_4 (0.326); NH_4Cl (1); $MgSO_4$ (0.098); $CaCl_2$ (0.044); and Glucose (0.1). The media was added by $K_2Cr_2O_7$ solution as a source of Cr^{6+} [24]. More detailed procedures are explained as follows:

(1) Media preparation

Specific mineral media added with $K_2Cr_2O_7$ reagent were prepared to obtain a specific media solution with a concentration of 5, 10, 20 ppm of Cr (VI). The various $K_2Cr_2O_7$ solutions were prepared by diluting 20 ppm of $K_2Cr_2O_7$ solution to get ten ppm and five ppm [24].

(2) Inoculation of *Arthrobacter chlorophenolicus* in growth media

An *Arthrobacter chlorophenolicus* of 5 ml was prepared in growing media + Cr (VI) at various concentrations on a batch reactor illustrated by Fig. 1. After that, it is incubated with a shaker using an orbital shaker at 30°C for 192 hours or eight days. At the 0, 24, 120, and 192 hours, every solution sample was taken to analyse bacterial growth using an OD spectrophotometer. Then, at 0 and 192 hours, the Total Plate Count (TPC) was taken, followed by pH and temperature measurements. The concentration of media containing Cr (VI) was known initially so that the test was run only for 192 hours or the last day of the running step [20]. As a control, the same media was used without the addition of *Arthrobacter chlorophenolicus*. Blank samples and treatments are evaluated in duplicate with each set of samples.

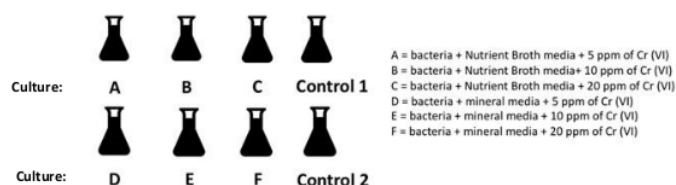


Figure 1. Batch culture with various treatment condition of Cr (VI) removal. Control 1: control for treatments in the rich media; Control 2: control for treatments in the minimal media.

(3) Analysing of microorganism growth in specific media containing Cr (IV)

The capability of bacteria growth on media testing was observed OD spectrophotometer. Briefly, in the OD spectrophotometer method, as much as 3 mL of liquid media from the growth media, including Cr (VI), was inserted into the cuvette. Then, the absorbance value was measured using a spectrophotometer with a wavelength of 600 nm. Likewise, the colony OD test was calculated at an amount of 10^9 CFU/ml or equal to 0.4A measured by OD spectrophotometer [22].

(4) Cr (VI) Reducing Analysing

Analysis of Cr (VI) reduction was accomplished by using a UV-Vis spectrophotometer. Test samples at 0; 24; 120; and 192 hours were observed for Cr ions removal with a wavelength of 535.9 nm [20].

2.4 Data analysis

2.4.1 Removal efficiency of Cr (VI)

The Chromium removal efficiency was obtained after testing through the UV-Vis spectrophotometer before bacterial cultured and after the running stage. Based on the test, the metal removal efficiency can be calculated according to spectrophotometer analysis. The following is a formula for calculating the efficiency of metal removal [26]:

$$\text{Removal Percentage \%} = \frac{C_0 - C}{C_0} \times 100\% \quad (1)$$

$$\text{With } C_0 = \text{Initial concentration (mg/L)} \text{ and } C = \text{Later concentration (mg/L)} \quad (2)$$

2.4.2 Graph analysing

Bacterial growth can be determined by using OD Spectrophotometer. The bacterial growth can be recognized by an increase of OD on the tested media. In addition, the metal reduction can be detected by using a spectrophotometer with a concentration ratio between incubation time and the growth of bacteria. The decreased Cr (VI) concentration employing *A. chlorophenolicus* is estimated by more than 60% removal efficiency percentages.

3. Results and discussions

3.1. Results

Growth of *Arthrobacter chlorophenolicus* (*A. chlorophenolicus*), indicated by the increasing of OD, as well as hexavalent chromium (as $K_2Cr_2O_7$) reduction, was monitored at different initial chromium concentrations of 5, 10, 20 ppm both in Nutrient Broth (NB) media and specific mineral media during 192 hours incubation. The *A. chlorophenolicus* growth and Cr (VI) reduction was evaluated at 0, 24, 120, 192 h. Cr (VI) reduction was found to increase proportionally with the increase in bacterial growth indicated by the increase of OD of the culture. Chromate reduction occurred rapidly in NB (**Fig. 2a**) and mineral media (**Fig. 2b**). In NB media, the percentage removal of chromium by *A. chlorophenolicus* in culture A (5 ppm Cr), culture B (10 ppm Cr), and culture C (20 ppm Cr) was 40%, 30%, 25%, respectively, after 24 hours incubation. With further incubation by 120 hours, about 50% of the Cr (VI) added in each tested culture were reduced. After 192 hours of incubation, the added Cr (VI) was removed

for 64%, 80% and 83% in cultures A, B, and C, respectively (**Fig. 2a**). The percentage removal of chromium results in minimal media was not much different from the results of the NB one. The removal of chromium occurred after 24 hours incubation and reached about 50% after 120 hours incubation. With further incubation, 192 hours incubation, the percentage removal of chromium by *A. chlorophenolicus* in the culture D (5 ppm Cr), culture E (10 ppm Cr), and culture F (20 ppm Cr) was 60%, 74%, 79%, respectively (**Fig. 2b**).

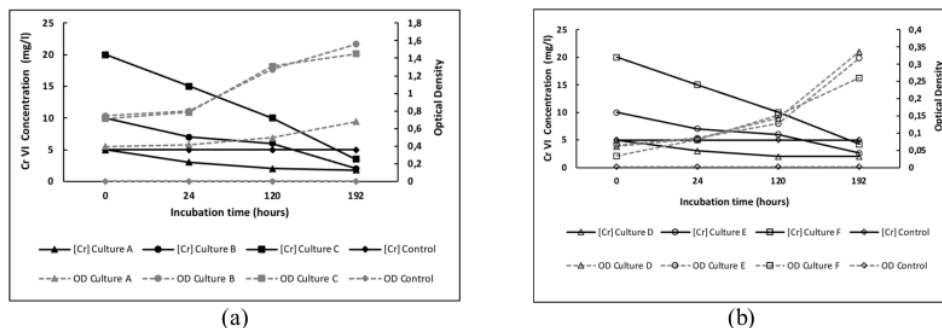


Figure 2. Growth and reduction of hexavalent chromium (Cr VI) by isolate *Arthrobacter chlorophenolicus* in (a) Nutrient Broth (NB) media and (b) specific mineral media under batch culture.

During the growth of *A. chlorophenolicus* in reducing chromium, each culture's pH and temperature were observed at 0 and 192 hours incubation. The results showed that the pH value at 0 h was recorded for 5-6 and increased to seven after 192 hours incubation in all the tested cultures (**Fig. 3a**). The temperature also was observed for all the tested cultures at the same time. The results showed that the temperature was constantly at 26-27°C in all the tested cultures (**Fig. 3b**).

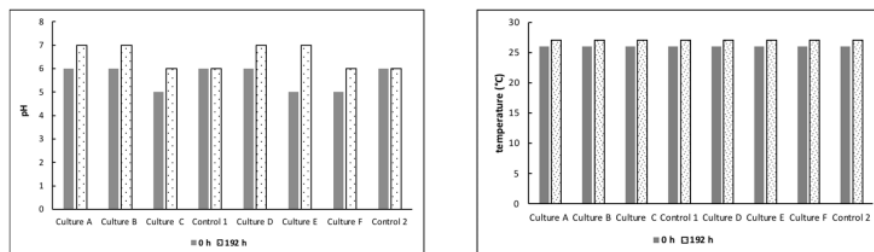


Figure 3. (a) pH value and (b) temperature of each culture after 192 hours incubation

3.2 Discussion

Bioremediation of heavy metal pollution is an attractive solution that is cost-effective and environmentally friendly. Several studies have been reported on chromate reduction by *Arthrobacter* spp., members of actinomycetes genus isolated from soil contaminated with tannery effluent [27], basalt rocks [28], activated sludge [18], and landfarming process soil sample [29]. These studies also reported its application in bioremediation of Cr pollutant in culture scale. Further, the following study by Dey *et al.* [21] in their recent study [19] reported optimizing cultural conditions for the growth of *Arthrobacter* spp. to evaluate their removal efficacy. However, the study of *Arthrobacter* spp. isolated from the volcanic area, specifically from volcanic deposit and evaluation of its potential for bioremediation of chromium has not yet been reported before. This study using *Arthrobacter* isolate from the previous study by Lathifah *et al.* [17], which is characterized to be able to grow in the minimum conditions of the

primary bacterial nutrients, Carbon (C) and Nitrogen (N). Thus, it is a beneficial advantage when the isolate will be applied for bioremediation on a large scale because the addition of C and N sources in large amounts is not needed in maintaining the culture.

In this study, we differentiate the growth of *A. chlorophenolicus* in rich media, namely Nutrient Broth (**Fig. 2a**) and minimal nutrient media, namely mineral media (**Fig. 2b**), which both containing Chromium in various concentrations (5, 10, 20 ppm). The results showed that *A. chlorophenolicus* grew in the minimal nutrient media, as can be seen, based on the increase of the OD value. When comparing with the growth of the isolate in the NB media, rich nutrient media, the growth was comparable even though the growth in NB media seems relatively higher than in the minimal one. It is expected to occur because the growth rate of bacteria depends on environmental conditions. If the environmental conditions have low nutrition, the general growth will be slower than the growth of bacteria in the nutrient-rich medium [31].

The percentage removal of chromium results indicated that *A. chlorophenolicus* showed about 80% reduction of 10 and 20 ppm chromium in 8 days (**Fig. 2a and Fig. 2b**). The result suggested that the isolate could reduce chromium hexavalent almost completely even under minimal nutrient conditions (**Fig. 2b**). Meanwhile, in the concentration of 5 ppm chromium, the *A. chlorophenolicus* showed about 60% reduction in both NB and mineral media. When observing the growth of *A. chlorophenolicus* in the media containing five ppm chromium, the growth rate is not as rapid as the isolate's growth in the media containing ten ppm and 20 ppm chromium (**Fig. 2a**). It might indicate that the amount of the bacterial cell in each culture during the incubation is different, resulting in a lower percentage of chromium removal in culture A and culture D. only use the energy. When compared with the previous study of hexavalent chromium remediation by *Arthrobacter* spp., these results seem nearly identical with the recent study by Dey *et al.* [19], which resulted in the reduction of 0.5 mM Cr (VI). Thus, the isolate could be promised bacteria for use in the bioremediation of chromium pollution.

In this study, the pH and temperature were observed during the running cultures. The reduction of Cr (VI) by *A. chlorophenolicus* is quite good when the initial pH of the media is maintained at pH 4 to 8 [21] [32]. The *A. chlorophenolicus* effectively reduced chromite in a narrow range of pH with an optimum of pH 7 (Fig. 3a), similar to those results of Camargo *et al.* [32] and Dey *et al.* [21]. In addition, the parameters temperature plays a vital role in the growth of *A. chlorophenolicus*, which will grow optimally at a temperature of 20-40°C, characteristic of mesophilic microorganisms [21]. In this study, the temperature of 26-27°C could be maintained in all the tested cultures until the final run of culture at 192 hours incubation (**Fig. 3b**).

The study by Molokwane *et al.* [18] using *Arthrobacter* isolated from dried activated sludge while Dey *et al.* [12] [19] using *Arthrobacter* sp. isolated from chromite mining in their study. In other words, that *Arthrobacter* spp. isolated from such heterotrophic conditions, ordinary Carbon and Nitrogen contents. Meanwhile, our study using *Arthrobacter* isolated from such oligotrophic conditions, mostly zero Carbon and Nitrogen contents, which imply the ability of the isolates to survive in such harsh conditions. Thus, our finding might have such a positive impact when the isolate is applied for bioremediation on a large scale because adding C and N sources in large amounts is not needed to maintain the culture.

4. Conclusions

Our results indicate that *A. chlorophenolicus*, the pioneer bacteria of Mt. Merapi, has a grand promise for use in Cr (VI) remediation even under minimum nutrients conditions. Further studies in optimizing the culture condition to improve the chromium removal efficacy are now in progress.

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