



THE REDUCTION OF Cr(VI) IN SOIL BY *Microbacterium* sp. STRAIN SpR3 IN VERMICOMPOST CARRIER

Reduksi Cr(VI) pada Tanah Menggunakan *Microbacterium* sp. Strain SpR3 dengan Bahan Pembawa Vermikompos

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ABSTRAK

Kromium hexavalen [Cr(VI)] adalah polutan yang berasal dari kegiatan industri. *Microbacterium* sp. strain SpR3 dapat digunakan sebagai agen bioremediasi Cr(VI). Kemampuan agen biologi untuk mereduksi Cr(VI) umumnya meningkat bila diinokulasi dalam media pembawa. Tujuan penelitian ini adalah untuk menganalisis kemampuan *Microbacterium* sp. strain SpR3 dalam mengurangi Cr(VI) serta membandingkannya dengan kondisi ketika diinokulasi dalam media pembawa vermicompos. Pengamatan dilakukan selama tujuh hari pada tiga perlakuan yang berbeda, yakni *Microbacterium* sp. strain SpR3 dengan dan tanpa media pembawa vermicompos yang diinokulasi pada tanah steril yang mengandung 50 ppm Cr(VI), dan tanah steril tanpa inokulasi bakteri yang mengandung 50 ppm Cr(VI). Variabel yang diamati adalah jumlah bakteri, konsentrasi, dan kecepatan reduksi Cr(VI) oleh bakteri tersebut di dalam tanah pada T0 (hari ke-0) dan T7 (hari ke-7). Hasil penelitian ini menyimpulkan bahwa vermicompos bisa digunakan sebagai media pembawa *Microbacterium* sp. strain SpR3 karena dapat meningkatkan jumlah bakteri sampai 2×10^{10} CFU g⁻¹ di dalam tanah dan dapat mengurangi Cr(VI) dengan kecepatan 0,095 mg L⁻¹ jam⁻¹.

Kata kunci: bioremediasi, kromium hexavalen, *Microbacterium* sp., reduksi, vermicompos

ABSTRACT

Hexavalent chromium [Cr(VI)] is a pollutant originated from industrial activities. *Microbacterium* sp. strain SpR3 can be used as Cr(VI) bioremediation agent. The ability of bioagent to reduce Cr(VI) usually improves when inoculated in a carrier. This research aimed to assess the ability of *Microbacterium* sp. strain SpR3 to reduce Cr(VI) in soil and compare its ability when inoculated in vermicompost carrier. Observations were carried out for seven days on three different treatments, namely *Microbacterium* sp. strain SpR3 with and without vermicompost inoculated in sterile soils containing 50 ppm Cr(VI), and sterile soils containing 50 ppm Cr(VI) without bacterial inoculation. The observed variables were the number of bacteria, the concentration of Cr(VI) and the rate of Cr(VI) reduction by these bacteria in the soil at T0 (day 0) and T7 (day 7). It was concluded that vermicompost could be used as a carrier of *Microbacterium* sp. strain SpR3 as it could increase the number of the bacteria to 2×10^{10} CFU g⁻¹ in soil and could reduce Cr(VI) at the rate of 0.095 mg L⁻¹ h⁻¹.

Keywords: bioremediation, hexavalent chromium, *Microbacterium* sp., reduction, vermicompost

INTRODUCTION

Hexavalent chromium [Cr(VI)] is one of the most abundant compounds found on earth (Oliveira 2012). The increasing amount of chromium which resulted from industrial and anthropogenic activities, poses hazards to the environment and soil (Oliveira 2012). Industrial activities that produce Cr(VI) waste are leather tanning industry, ink manufacture, metal and alloy industry, textile industry, metal finishing, stainless steel production, chrome plating, and copy machine toner (Saha et al. 2011). In Indonesia, the industrial waste of Cr(VI) also polluted the soil environment in many different regions. Soil pollution by Cr(VI) in several Java areas can be seen in Table 1.

The presence of Cr(VI) in agricultural soil could contaminate plants that grow on it. Sukarjo et al. (2019) showed that rice plants, which grew on chrome-contaminated soil, can grow normally. However the chrome was accumulated in the plants and the rice may contain the chrome residue and when these plants are consumed by humans, Cr(VI) will be accumulated in the digestive organs and can cause cancer. In laboratory experiments, Cr(VI) can also induce mutation in vitro and in vivo and sensitize the respiratory tract (Saha et al. 2011).

Heavy metal contamination in soil is usually remediated by physical and chemical methods, such as soil replacement, thermal desorption, soil leaching, and chemical fixation. These chemical and physical methods consume much energy, time, money, and inefficient to be used in vast areas and they can also change the bioavailability of fixed heavy metals, environmental conditions, soil structures, and affect microbes in soil (Qayyum et al. 2020). Therefore, more environmentally friendly solutions need to be identified to solve this problem and the use of microorganisms for the Cr bioremediation process is one of the ideal solutions for this purpose (Satyanarayana et al. 2012). Bioremediation is one of the waste and pollutant remediation techniques by using microorganisms such as bacteria or fungi. These microorganisms are specifically selected, cultured, and grown in a polluted environment to lower the concentrations of wastes or pollutants to an acceptable level (Priadie 2021). Bioremediation by microorganisms occurs

due to enzyme production by the microorganism and this enzyme changes the toxic chemicals into non-toxic chemicals (Priadie 2012). Isolate SpR3 is one of the potential microorganisms that can be used in the Cr(VI) bioremediation process. The bacteria, which are isolated from soil contaminated with activated sludge of textile industry, had the ability to reduce Cr(VI) by 90.85 ppm (Meitiniarti et al. 2012). The highest ability of bacteria to reduce Cr(VI) was achieved when the bacteria growth reached the exponential phase into the stationary phase. Meitiniarti et al. (2014) have identified 100% similarity of rRNA structure between *Microbacterium* spp. and SpR3, known as *Microbacterium* sp. strain SpR3.

Bioremediation of Cr(VI) in soil directly using *Microbacterium* sp. strain SpR3 Cr(VI) can decrease the viability of bacteria and its ability to reduce Cr(VI) (Nugroho et al. 2015). One method for maintaining the number of bacteria is by inoculating bacteria with a carrier. The carrier is a temporary habitat for microbes before applying into the soil (Rajasekar et al. 2012). The carrier plays an important role in nutrition supply and the viability of bacteria so that the number of bacteria maintained (Nurdika and Nurcahyanti 2019).

Vermicompost is compost that results from the decomposition of organic materials by earthworms (Hazra 2018). Vermicompost is a good carrier for this purpose as it is abundant and easily found, cheap, environmentally friendly, and it is an excellent soil fertilizer (Packialakshmi and Riswana 2014).

Compared with compost and peat, vermicompost has a better water holding capacity, contains more organic compounds (included carbon source), and has neutral pH (7.14). Therefore, vermicompost has excellent properties for maintaining the number of microorganisms. The research by Packialakshmi and Riswana (2014) showed that the number of *Azotobacter* sp. (1.3×10^7 CFU mL⁻¹) which incubated in vermicompost for 60 days, increased to 1.6×10^7 CFU g⁻¹. *Azotobacter* sp. (10^{11} CFU mL⁻¹) in vermicompost supplied with nutrients (22% Trypton Soya Broth), can maintain the number of *Azotobacter* sp. for 180 days constant at 10^{11} CFU g⁻¹ (Larasati et al. 2012). The purpose of this research is to

Table 1. Concentration of Cr(VI) contaminant in soil in several Java regions

No	Region	Sample Site	Cr(VI) Concentration (mg kg ⁻¹)	Reference
1	Kaligarang River, Central Java	Soil sample taken from Kaligarang River sediment	11.61–16.91	Susanti et al. 2014
2	Trimulyo River, Central Java	Soil sample taken from Trimulyo River sediment	32.55–45.78	Tri Nuraini et al. 2017
3	Pati Regency, Central Java	Paddy field soil polluted by electroplating industrial waste	6.0–27.7	Pramono et al. 2012
4	Selayar island, West Java	Soil sample taken from Selayar Island	10.9–144.98	Irzon 2018

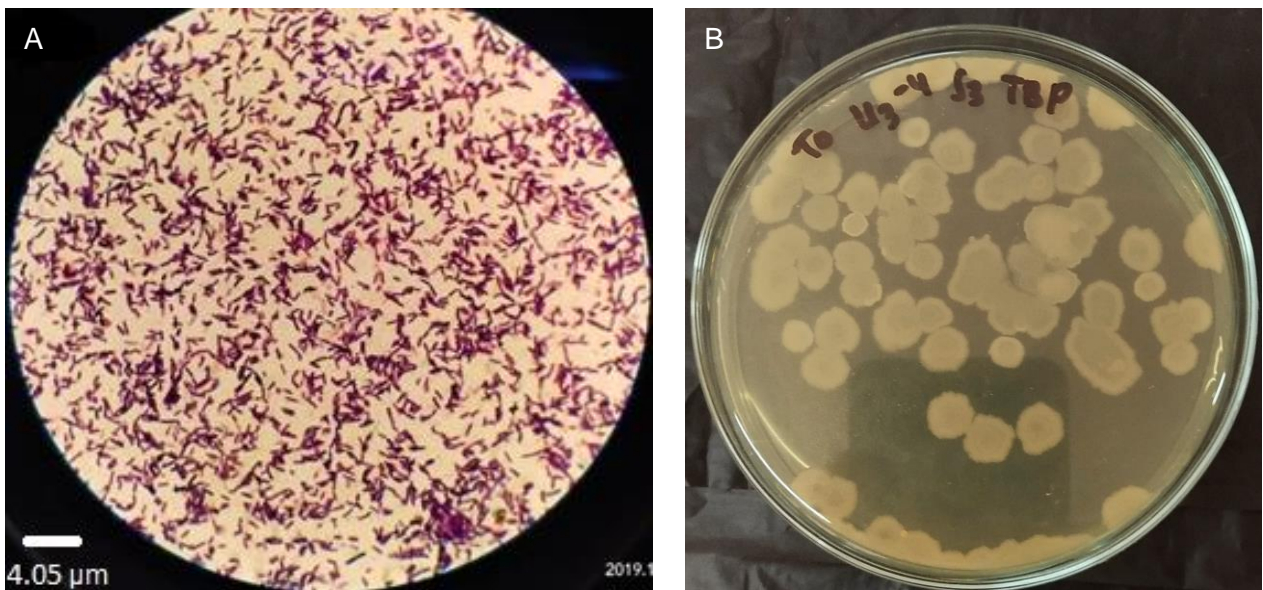


Figure 1. *Microbacterium* sp. strain SpR3 isolate in LB medium added with 50 ppm of Cr(VI): A rod cell shape and gram-positive characteristic of *Microbacterium* sp. strain SpR3 (1000x cell magnification image) (A) and a colony shape of *Microbacterium* sp. strain SpR3 in LB plate medium (B)



Figure 2. Soil (A) and vermicompost (B) that had been sieved with 2 mm soil sieve

analyze the ability of *Microbacterium* sp. strain SpR3 inoculated in vermicompost carrier to reduce Cr(VI) in soil.

MATERIALS AND METHODS

Location and time

This research was conducted in the Laboratories of Microbiology and Biochemistry and Molecular Biology at Satya Wacana Christian University, Salatiga, Central Java from November 2019 to April 2020.

Bacteria isolate, vermicompost, and soil

Microbacterium sp. strain SpR3, which was used in this research, was obtained from the Microbiology laboratory at Faculty of Biology, Satya Wacana Christian University, Central Java. *Microbacterium* sp. strain SpR3 are gram-positive, rod-shaped, and non-spore forming bacteria. Nugroho et al (2015) showed that this bacteria could reduce Cr(VI) at 32.63 ppm in the soil for eight days. Cells and colony form of *Microbacterium* sp. strain SpR3 can be seen in Figure 1. The bacteria were maintained in an LB slant agar medium containing 50 ppm Cr(VI) and incubated at 37 °C for two days. Vermicompost as carrier and soil as the treatment medium was obtained from Bioflora store, in Imam Bonjol Street KM 2 Kecandran, Kec Sidomukti, Kota Salatiga, Central Java (S 7° 19' 11.085", W 110° 28' 54.604"). The pH of vermicompost was 7.14, whereas the soil was from 7.00 to 7.30 and it has 54.7% water content.

Sterilization of vermicompost, soil, and pot

Before being used, both vermicompost and the soil were first sieved using a 2 mm diameter soil sieve (Figure 2) and then were sterilized separately in an autoclave at 121 °C for 60 minutes. Sterilization was conducted twice, 24 hours apart between the first sterilization and second sterilization. Besides that, cylindrical pots with a height of 5 cm and a diameter of 4.5 cm were also sterilized by immersing in a weak acid solution overnight and rinsed with distilled water before being used in the treatment. Sterilized vermicompost was used as a carrier, while sterilized soil and pots were used as treatment mediums.

Medium preparations and cell production

For the treatment medium preparation, as much as 80 g of soil was poured into a pot and then was mixed with Cr(IV) solution so that there were 50 ppm Cr(IV) in the soil. For bacteria cell production, 48 hours old of LB slant agar culture of *Microbacterium* sp. strain SpR3 was collected using loops and inoculated into a flask that containing 100 mL sterile LB broth medium with 50 ppm Cr(VI) within. This culture was incubated on a shaker at 120 rpm for 24 to 48 hours or until the culture reached OD 0.9 at 600 nm wavelength. After that, the culture was harvested using a centrifuge (9,900 rpm, 5 minutes) and concentrated to OD 600nm 2.0 (equivalent to 16×10^8 CFU mL⁻¹). Then the bacteria culture was ready to be used in the treatments. The culture *Microbacterium* sp. strain SpR3 in LB Broth medium which contained Cr(VI) can be seen in Figure 3.

Incorporation of cell suspension

Five mL of bacterial cell suspension with concentration of 16×10^8 CFU mL⁻¹ was inoculated into 10 g of sterile vermicompost that had been prepared before. This mixture of cell suspension and vermicompost was homogenized and incubated for seven days. The number of bacteria was determined at T0 and T7 to analyze their ability to adapt and survive in carriers.

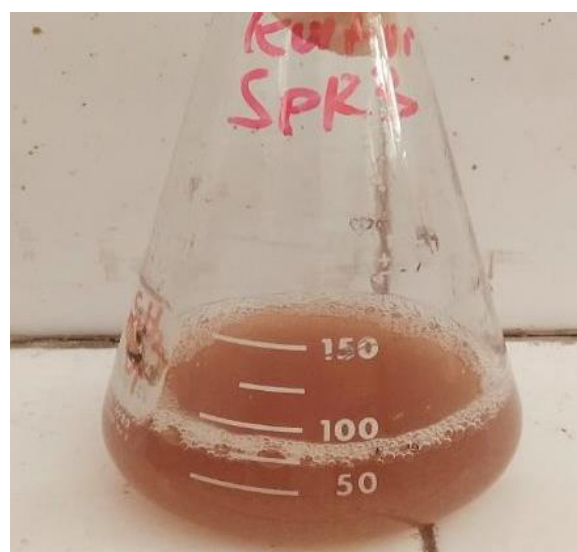


Figure 3. The culture of *Microbacterium* sp. strain SpR3 with OD value 0.9 in LB broth medium which contains Cr(VI) of 50 ppm concentration

Experimental design

Treatment 1 was the inoculation of *Microbacterium* sp. strain SpR3 in a carrier into the soil, Treatment 2 was the inoculation of *Microbacterium* sp. strain SpR3 without the carrier, and treatment 3 was the sterile soils containing Cr(VI) without inoculation, to ensure that the soil used was sterile and did not contain bacteria. Furthermore, this third treatment was called the treatment media without bacterial inoculation (only sterile soil + Cr(VI) solution). Each treatment was replicated three times. Sampling was carried out twice, namely on day 0 (T0) and day 7 (T7). Figure 4 shows the soil which had been treated with three different treatments. The parameters observed were the number of bacteria, the concentration of Cr(VI) in the soil, and the reduction rate of Cr(VI).

Analyses of variables

The number of bacteria in the samples was determined by pouring plate method (Sanders 2012). Cr(VI) concentration in the soil was determined by the spectrophotometric method using Diphenylcarbazide reagent (Walter 1961). Cr(VI) concentration in the soil was extracted using potassium dihydrogen phosphate (KH₂PO₄) + dipotassium phosphate (K₂HPO₄) solution as described by Gheju et al. (2009). The reduction rate of Cr(VI) was determined using the formula as described in Walter (1961):

$$\text{Reduction rate of Cr(VI)} (\text{mg L}^{-1} \text{ h}^{-1}) = \frac{A - B}{T}$$

Note:

A = Cr(VI) concentration at T0 (mg L⁻¹)

B = Cr(VI) concentration at T7 (mg L⁻¹)

T = 7 × 24 hours = 168 hours

RESULTS AND DISCUSSION

During 7 days of incubation in vermicompost carrier, the number of bacteria cell increased (Table 2). The increasing of the cell number of *Microbacterium* sp. strain SpR3 showed that vermicompost could maintain bacterial cells and support bacterial growth. Table 2 shows the number of *Microbacterium* sp. strain SpR3 in vermicompost carrier at T0 and T7.

The number of *Microbacterium* sp. strain SpR3 in vermicompost carrier increased on day seven. This increase was due to the neutral pH of vermicompost and it has high contents of organic materials and nutrients to support the sustainability of *Microbacterium* sp. strain SpR3. This assumption is in accordance with the result of Rajasekar et al. (2012) that claimed the growth of bacteria was due to the pH level of the carrier at 7.14 (neutral) and the composition of the organic materials was 58.96% to support the sustainability of the targeted bacteria. According to Yan et al. (2015), *Microbacterium* sp. reached optimum growth at pH 7.00. Another factor that influenced the growth of *Microbacterium* sp. strain SpR3 was the availability and sufficiency of organic materials in vermicompost carrier. The presence of

Table 2. The Number of *Microbacterium* sp. strain SpR3 in Vermicompost Carrier

Observation Period (days)	Σ SpR3 in Vermicompost (CFU g ⁻¹ Vermicompost)
T0	(4 ± 0.6) × 10 ⁶
T7	(7 ± 0.4) × 10 ¹⁰



Figure 4. Soil which contained 50 ppm Cr(VI) was inoculated with *Microbacterium* sp strain SpR3, experiment setting was conducted in accordance with a completely randomized design

organic matter in this media was needed by bacteria as a source of nitrogen for energy synthesis and carbon for cell growth (Tays et al. 2018).

During seven days of incubation, the number of *Microbacterium* sp. strain SpR3 in the soil increased for soil + inoculant with carrier and without carrier treatments (Table 3). The growth of the bacteria in soil + inoculant in carrier treatment might be due to the ability of *Microbacterium* sp. strain SpR3 using organic materials in vermicompost + sterile soil. This assumption is in accordance with Rohmah et al. (2016), which stated that the carrier can maintain the number of bacteria during incubation in the carrier and when applied to the soil the bacteria can adapt in the soil. Consequently, *Microbacterium* sp. strain SpR3 can multiply

faster when they were in the soil. On the other hand, the bacteria used organic materials from soil only in soil + inoculant treatment. Therefore, the bacteria grew slower with this treatment. This assumption is in agreement with Djaenuddin et al. (2018), which claimed that the number of bacteria should increase more slightly owing to the absence of the carrier which can support faster bacterial growth.

The number of bacteria in the soil after 7 days of incubation showed a bigger increase in the soil treatment + inoculant in a carrier than that without a carrier. On the contrary, there were no bacteria in sterile soil treatment without inoculation because there were no other bacteria which can survive from Cr(VI) contaminant except *Microbacterium* sp. strain SpR3.

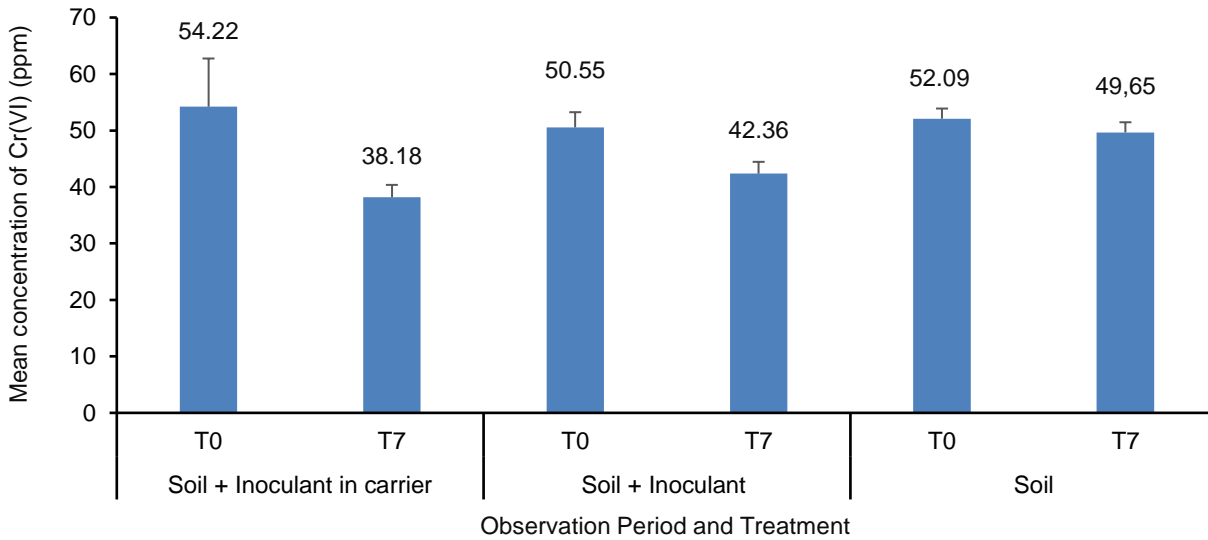


Figure 5. The average concentration of Cr(VI) in the soil in three different treatments

Table 3. The number of *Microbacterium* sp. strain SpR3 in the soil media

Observation Period (days)	Σ SpR3 Bacteria (CFU g ⁻¹ soil) in Soil		
	Treatment 1	Treatment 2	Treatment 3
T0	$(2 \pm 0.3) \times 10^7$	$(2 \pm 0.3) \times 10^7$	0
T7	$(2 \pm 0.1) \times 10^{10}$	2×10^8	0

Note:

Treatment 1: Soil + Inoculant in Carrier

Treatment 2: Soil + Inoculant

Treatment 3: Soil

Table 4. Reduced concentration of Cr(VI) by *Microbacterium* sp. strain SpR3 in the soil media

Observation Period (hours)	Cr(VI) Concentration (mg L ⁻¹) in Soil			Reduction Rate Cr(VI) (mg L ⁻¹ h ⁻¹) in Soil		
	Treatment 1	Treatment 2	Treatment 3	Treatment 1	Treatment 2	Treatment 3
T0	54.22 ± 8.53	50.55 ± 2.68	52.09 ± 1.80	0.095	0.049	0.015
T7	38.18 ± 2.19	42.36 ± 2.08	49.65 ± 1.81			

The presence of Cr(VI) in the soil was suspected to be one of the factors that inhibited the growth of *Microbacterium* sp. strain SpR3. According to Baldiris et al. (2018), the presence of Cr(VI) caused the bacteria to switch some of their energy, which is normally used for cell multiplication, to form the enzyme chromate reductase to reduce the toxic effect of Cr(VI). Therefore, in the soil treatment + inoculant in the carrier, bacteria gained more benefit from the existence of organic materials in the carrier and in soil. Thus, bacteria obtained more energy from organic materials, which was used for bacterial reproduction. Conversely, in the treatment of soil + inoculant, bacteria reproduced slower because they only obtained organic materials from the soil.

The differences in the concentration and reduction rates of Cr(VI) in the soil in different treatments might be due to the difference in the number of bacteria in the treatment of soil + inoculant with carrier and without carrier. This assumption was supported by Nugroho et al. (2015) experiment, which claimed that reduction of Cr(VI) by *Microbacterium* sp. strain SpR3 decreased proportionally with the number of bacteria in the soil. Table 4 shows the reduction rate and concentration of Cr(VI), which has been inoculated by *Microbacterium* sp. strain SpR3 with or without a carrier at T0 and T7. On the Treatment 1, there was a reduction of Cr(VI) concentration from 54.22 mg L⁻¹ to 38.18 mg L⁻¹. On the Treatment 2, Cr(VI) concentration reduced from 50.22 mg L⁻¹ to 42.36 mg L⁻¹ and the Treatment 3 from 52.09 mg L⁻¹ to 49.65 mg L⁻¹. Figure 5 showed the decline of Cr(VI) concentration in the soil by *Microbacterium* sp. strain SpR3 in three different treatments.

Table 4 also showed a slight reduction of Cr(VI) concentration in the soil with sterile soil without inoculation. This slight decrease might be due to the natural chemical activities in the soil. According to Saidy and Badruzsaufari (2009), high organic carbon content in the soil, can decrease the Cr(VI) concentration. Cr(VI) reduction in this case was mainly caused by the rise of electron quantity level and organic carbon in the soil, which triggered chemical reaction of Cr(VI) reduction to Cr(III). Based on the above results and discussion, the benefit of this research is that the integration of bacteria and

carrier materials can be used and developed as an agent to remediate heavy metals. In addition, this study can be used to grow *Mesorhizobium* which can produce Indole Acetic Acid (IAA) and can maintain Nitrogen (N) in the soil.

CONCLUSION

Based on this research, it can be concluded that vermicompost can be used as a carrier of *Microbacterium* sp. strain SpR3 because the bacteria can grow from 4 × 10⁶ CFU g⁻¹ to 7 × 10¹⁰ CFU g⁻¹ for 7 days. Treatment 1 showed that the amount of *Microbacterium* sp. strain SpR3 increased to 2 × 10¹⁰ CFU g⁻¹ in soil and can reduce Cr(VI) at a rate of 0.095 mg L⁻¹ h⁻¹.

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