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Bacillus Resistance and Potensial as Chromium (Cr) Bioremoval

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Abstract. Chromium is one of heavy metal that encountered environment from industrial waste so it can contaminate the environment. Chromium resistance bacteria can transform Cr(VI) to Cr(III). The aim of this research is to find out chromium resistance and bioremoval potential of Chromium by *Bacillus*. This research was started with resistance assay of 6 isolates *Bacillus* to determine growth ability of isolates on medium contained Chromium. Range finding test were done to determine heavy metal concentration that used in research and determine 3 isolates that more resistance than others. Growth curves were measured by OD using spectrophotometer UV-Vis. Chromium bioremoval was measured by Atomic Absorption Spectroscophy method. Viability assay was done by pour plate method. *Bacillus* S1, DA11 and SS19 resistance to nutrient agar-chromium 150 mg/L. *Bacillus* DA11 got highest bioremoval efficiency in the amount of 76.8% at concentration 38.8 mg/L, while at concentration 1.9 mg/L the amount of bioremoval efficiency were same for all isolates. Chromium concentration were significantly influence the amount of bioremoval efficiency, while type of isolates were not significantly influence

Keywords: *Bacillus*, bioremoval, chromium, resistance.

Introduction

Chromium (Cr) is one of heavy metal that is toxic and can't be degraded naturally, although in very low concentrations [1]. Cr is widely used in industrial activities, such as on paint, tanneries, electroplating and textiles [2]. Wider using of chromium will cause environmental pollution if it's not managed properly.

Chromium in nature generally are in the form of trivalent (Cr III) and hexavalent (Cr VI). Cr VI is easily soluble in water and mobile, so it can accumulate in the human body through the food chain. Cr VI is more dangerous than Cr III because of its carcinogenic properties [3]. *Bacillus* is one of genus of bacteria that are resistant to Cr through its Cr VI reduction mechanism to Cr III aerobically [4], so that the Cr toxicity is reduced.

Bacillus S1, DA11 and SS19 are isolated from Kalimas Surabaya and are known resistant to Hg, Cd, Pb and Cu [5], but the resistance and Cr bioremoval efficiency of those isolates are unknown. From this research, *Bacillus* S1, SS19 and DA11 are expected to be developed as bioremediation agent for Cr polluted land.

Material and Method

Isolates Resistance To Chromium (Cr)

Bacillus S1, SS19 and DA11 were used in this research. Resistance assay was done by growing the isolates on agar medium containing $K_2Cr_2O_7$ by continue streak plate method aseptically. $K_2Cr_2O_7$ concentration that used in this research were 5, 25, 50, 100, 150 and 200 mg/L. Cultures were incubated for 24 hours at room temperature. Resistant of isolates were characterized by the growth of colonies on agar medium containing $K_2Cr_2O_7$.

Chromium (Cr) Bioremoval Assay

45 ml culture of *Bacillus* in liquid medium (nutrient broth) were added to 180 ml of nutrient broth, then the suspension were incubated until μ hours (obtained from the growth curve). Before the metal exposure to the isolates in liquid medium, cell density on μ hours were counted using haemocytometer. Cell density used in this research was 10^6. 50 ml of μ-hour-old cultures in nutrient broth

were exposed to $K_2Cr_2O_7$ concentration 100 and 150 mg/L. Nutrient broth medium containing $K_2Cr_2O_7$ with no isolates was used as control. Cultures were incubated for 24 hours on rotary shaker with 100 rpm speed. 50 ml of 24-hour-old cultures that exposed by $K_2Cr_2O_7$ were centrifuged with 400 rpm speed for 20 minutes. Supernatants were separated from bacterial pellet and were inserted to the test tube. 10 drops of HNO_3 were added to the test tube and were heated for 10 minutes. Remaining chromium concentration were measured by Atomic Absorption Spectroscophy (AAS) on 357.9 nm wave length. Chromium concentration that removed by *Bacillus* and its bioremoval efficiency were counted using following formula.

$$R = K_0 - K_a \qquad E = (R/K_0) \times 100\%$$

R : $K_2Cr_2O_7$ concentration removed by *Bacillus*
K_0 : initial $K_2Cr_2O_7$ concentration in medium without *Bacillus*
K_a : final $K_2Cr_2O_7$ concentration in *supernatant*
E : $K_2Cr_2O_7$ bioremoval efficiency

Viability of *Bacillus* After Exposed by $K_2Cr_2O_7$

100 µL of *Bacillus* which have been exposed by $K_2Cr_2O_7$ were taken and were inoculated using pour plate method on nutrient agar medium containing no $K_2Cr_2O_7$. Growing colonies were colonies that viable after $K_2Cr_2O_7$ exposure and were counted using Colony Forming Units (CFU) method.

Result and Discussion

Isolates Resistance To Chromium (Cr)

Bacillus S1, SS19 and DA11 were resistant to chromium because they grew well on medium containing $K_2Cr_2O_7$ at concentration ≤ 100 mg/L. The growth ability of *Bacillus* colonies were decreased with increasing concentration of Cr in the nutrient agar medium. *Bacillus* colonies grew moderate at $K_2Cr_2O_7$ concentration 150 mg/L and grew poorly at concentration 200 mg/L (Table 1 and Fig. 1)

Table 1. *Bacillus* Resistance to Chromium in nutrient agar containing $K_2Cr_2O_7$

Bacillus Isolates	Growth of Isolates on Medium Containing $K_2Cr_2O_7$ (mg/L)						
	1	5	25	50	100	150	200
DA11	+++	+++	+++	+++	+++	++	+
S1	+++	+++	+++	+++	+++	++	+
SS19	+++	+++	+++	+++	+++	++	+
B. cereus ATCC1178*	+++	+++	+++	+++	+++	++	+

(+++ good, ++ moderate, + poor, - not row)

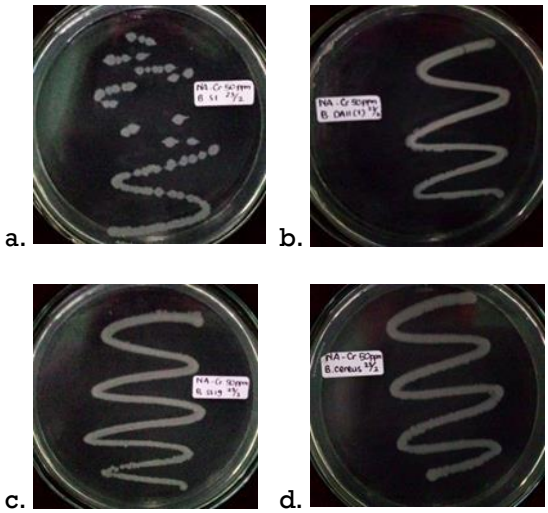

Figure 1. Resistance of *Bacillus* to 50 mg/L $K_2Cr_2O_7$ in medium nutrient agar (a. *Bacillus* S1, b. *Bacillus* DA11, c. Bacillus SS19 dan d. *Bacillus cereus* ATCC1178)

The mechanism of hexavalent chromium (Cr^{6+}) entering bacterial cell is through sulfate (SO_4^{2-}) absorption pathway, because Cr^{6+} has the same structure with sulfate (SO_4^{2-}) so that Cr^{6+} can easily enter the cell membrane, replacing the sulfate (SO_4^{2-}) [6]. If there is intracellular chromate reductase enzyme in bacterial cell, Cr^{6+} will reduced to Cr^{3+}. But, if not, Cr^{6+} will accumulate in cell and will induce *chr* operon, so that chromate efflux pump encoded by *chrA* will active. Therefore, bacterial cell will be protected from Cr^{6+} toxicity because Cr^{6+} will pumped out of cell through chromate efflux pump [7].

Chromium Bioremoval

Initial concentration of chromium in medium without inoculum after measured by AAS were decreased to 1.9 mg/L and 38.8 mg/L, so that those concentrations were used as

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initial concentration for bioremoval assay. Decreasing of chromium concentration was happened because of chromium chelation to nutrient broth medium composition, one of the composition is protein, so when the protein were ionized it will chelate to the metal [8]. Protein binding to chromium will caused the formation of metalloprotein [9]. This chelation causing chromium concentration in the media was decreased.

All *Bacillus* isolates were capable to remove almost all of 1.9 mg/L concentration with bioremoval efficiency in the amount of 99.7%. At concentration 38.8 mg/L, *Bacillus* S1 was capable to remove chromium in the amount of concentration 20.7 mg/L, *Bacillus* DA11 was in the amount of 29.8 mg/L and *Bacillus* SS19 was in the amount of 29.1 mg/L, with the bioremoval efficiency as much as 53.3%, 76.8% and 75% (Table 2).

Table 2. Chromium Bioremoval and Bioremoval Efficiency by *Bacillus*

Bacillus Isolates	Treatment Concentration (mg/L)	AAS measured concentration (mg/L)	Chromium concentration remaining (mg/L)	Removed chromium concentration (mg/L)	Chromium efficiency removal (%)
S1	100	1.9	< 0.0051	1.89	99.7
	150	38.8	18.1	20.7	53.3
DA11	100	1.9	< 0.0051	1.89	99.7
	150	38.8	9	29.8	76.8
SS19	100	1.9	< 0.0051	1.89	99.7
	150	38.8	9.7	29.1	75

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Bacillus is one of microbes that can reduce Cr VI to Cr III aerobically [4]. This reduction process needs oxygen as acceptor electron and NAD(P)H as donor electron, which involved NADH-dehydrogenease in periplasm [10].

Bacillus Viability after Exposed by Chromium

All of *Bacillus* isolates, S1, SS19 and DA11 have growth ability on nutrient agar medium that containing $K_2Cr_2O_7$ after chromium exposed for 24 hours (Table 3). *Bacillus* DA11 has higher viability than *Bacillus* S1 and SS19.

Table 3. *Bacillus* Viability after Exposed by $K_2Cr_2O_7$ for 24 hours

Bacillus Isolates	Viability (on CFU) after exposed by $K_2Cr_2O_7$ (mg/L) for 24 hours	
	100	150
S1	148	0
SS19	300	0
DA11	54	39

Conclusion

Bacillus S1, DA11 and SS19 were resistant to chromium and were capable to conduct chromium bioremoval. *Bacillus* DA11 has higher chromium bioremoval potential than other isolates with bioremoval efficiency in the amount of 76.8%.

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