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Bioremediation of Chromium(VI) to Chromium(III)

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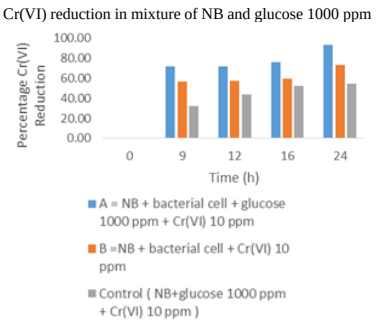
GRAPHICAL ABSTRACT



ABSTRACT

Wastewater discharged from industrial processes contains a lot of hazardous materials, of which Cr(VI) is categorized as carcinogenic and mutagenic, hence harmful towards human and living things. In this sense, industrial wastewater must be treated before discharged to the environment. Previous studies using mesophilic bacteria showed reduction of Cr(VI) to Cr(III) in industrial effluents, but the strain could not survive at higher temperatures. Hence, reduction of Cr(VI) to Cr(III) by using thermophilic bacteria have been studied as this bacteria can withstand higher temperature. This study reports on the reduction of Cr(VI) to Cr(III) by *Geobacillus caldoxylosilyticus* UTM 6 (GenBank Acession No.KR867680) under optimized conditions. The effect of glucose (1000 ppm and 2000 ppm), sucrose (2000 ppm) and acetate (2000 ppm) on growth of *G. caldoxylosilyticus* was studied. *G. caldoxylosilyticus* showed the highest growth in mixture of NB and glucose at concentration of 1000 ppm with OD_{600} (0.817) compared to NB alone, OD_{600} (0.798) after 13 h incubation. The maximum growth for *G. caldoxylosilyticus* in mixture of salt minimal medium and glucose was at 8 h incubation with OD_{600} of 0.079. *G. caldoxylosilyticus* shows the ability to utilize NH_4^+ from basal salts with addition of glucose for metabolism. *G. caldoxylosilyticus* showed the highest reduction capacity of (92.79%) for 10 ppm Cr(VI) in mixture of NB and glucose after 24 h incubation compared to the control. *G. caldoxylosilyticus* was successfully shown to reduce Cr(VI) and can be used to treat Cr(VI) laden industrial effluent where the temperature may reach up to 60 ºC.

Keywords: *Reduction; Geobacillus; Cr(VI)*

1. INTRODUCTION

Increasing industrial development and urbanization have resulted in the generation of toxic substances in large quantities which are introduced into the environment without proper treatment. The presence of toxic heavy metal contaminants such as chromium, lead, copper, cadmium and zinc in aquatic streams arising from release of metal containing effluents into water bodies [1].

From the industrial processes which includes metal finishing industry, petroleum refining, leather tanning, iron and steel industries, inorganic chemicals production, textile manufacturing and pulp producing process, a large number of chromium is discharged to the environment. U.S. EPA reported that approximately 10,000 lb per day of chromium has been found in groundwater [2]. This human-caused Cr(VI) contamination has recently been regarded as a critical issue because of the carcinogenicity and mutagenicity of Cr(VI). In addition, Cr(VI) is soluble in water thus, it can be mobile in the environment.

Therefore, the reduction of Cr (VI) to Cr(III) serves as an important process for removal of Cr(VI) contaminated water and wastewater. Recently, many researches have focused on the development of biological methods for the treatment of Cr (VI) in industrial effluents. Several bacteria have been recognized for their tendency to reduce Cr(VI) due to its resistance mechanism. The common bacteria used for reduction of Cr(VI) in effluents are the mesophilics [3]. However, these mesophilic bacteria show inefficiency in reducing Cr(VI) at high temperature as the optimum temperature for mesophilic bacteria ranges between 20˚C-40˚C [4]. The present study was carried out to study the efficiency of a thermophilic bacteria to reduce Cr(VI).

2. EXPERIMENTAL

2.1. Bacteria

In this work, the bacteria used was isolated from hot spring located in Selayang, Selangor Malaysia. The bacteria was identified as *Geobacillus caldoxylosilyticus* UTM 6 (GenBank Acession No. KR867680) by 16S rRNA sequencing. The bacteria was maintained in nutrient agar (NA) plate incubated at 53˚C.

2.2. Preparation of bacterial cell suspension

The cells were harvested by centrifugation (SIGMA 2K-15, B. Braun) at 9000 rpm, 5 min and 0 ˚C. Pellet obtained was washed twice using 0.85 g NaCl/100 mL solution at 9000 rpm, 5 min and 0 ˚C. The pellet was resuspended in the same solution at 17.5% of the original volume of cell. Cell suspension of 5 mL, 4 mL, 3 mL, 2 mL, and 1 mL which mark up with distilled water in 5 mL test tube was filtered through hydrophobic-type 0.45 µm Whatman filter paper each and dry weight was

determined after overnight drying at 70-80 ˚C. The optical density at 600 nm using a UV-Vis Spectrophotometer (Hach DR 5000) was measured for each suspension.

2.3. Growth in mixtures of NB and carbon sources

Cells (10 mg dry weight) were added to 100 mL NB medium in 1 L Erlenmeyer flask. Then, 0.2% (w/v) from (5% w/v) glucose stock solution were transferred into 100 mL NB before incubated at 53 ˚C, 200 rpm for 48 h. The carbon source stock solution was filter-sterilized prior to use. Bacterial growth was determined at OD_{600}. The experiment was repeated with sucrose 0.2% (w/v), acetate 0.2% (w/v) and glucose 0.1% (w/v). Control experiments consisted of NB medium only and NB medium added with respective carbon sources minus the bacteria.

2.4. Growth in salts minimal medium and carbon sources

Cells (10 mg dry weight) were added to 100 mL basal salts in 1 L Erlenmeyer flask. Basal salts consist of 3 g/L $(NH_4)2SO_4$, 0.5 g/L $MgSO_4{\cdot}7H_2O$, 0.5 g/L K_2HPO_4 and 0.1 g KCl. Then, 0.1% (w/v) from (5% w/v) glucose stock solution was transferred into 100 mL NB before incubated at 53 ˚C, 200 rpm for 48 h. The carbon source stock solution was filter-sterilized prior to use. Bacterial growth was determined at OD_{600}. Control experiments consisted of saline solution only and saline solution added with respective carbon sources minus bacteria.

2.5. Cr(VI) reduction in mixtures of NB and carbon source

A 10 ppm of 1000 mg/L stock Cr(VI) solution is added into a series of 1 L Erlenmeyer flasks containing 100 mL NB medium for each flask. One of the carbon sources which give the highest bacterial growth was added which is 0.1% (w/v) from 5% (w/v) stock solution into 10 mg cell dry weight of *Geobacillus caldoxylosilycus*. The mixture was incubated at 53 ˚C, 200 rpm for 48 h. Cr(VI) reduction was determined using 1,5 – diphenylcarbazide (DPC) method while cell viability was determined using spread-plate method during 48 h incubation period.

2.6. Analytical method

The initial and residual concentrations of Cr(VI) were determined colorimetrically at 540 nm by reacting 1,5 – diphenylcarbazide (DPC). In a 10 mL volumetric flask, 1 mL of sample was mixed with 9 mL of 0.2 M H_2SO_4. Then, 0.2 mL of freshly prepared 0.25% (w/v) DPC in acetone was added to the volumetric flask. The mixture was then vortexed (Maxi Mix-II Thermolyne) for about 15-30 s and let to stand between 10 and 15 min for full colour development. The red-violet to purple colour formed was then measured at OD_{540} using distilled water as reference. Instrument used was calibrated using 0.4-2.0 mg/L Cr(VI) prepared from Cr(VI) stock solution (1000 mg/L).

3. RESULTS AND DISCUSSION

3.1 Growth Profile of Thermophilic Bacteria

Figure 1, depicts the pattern of growth profile for thermophilic bacteria in NB medium for 24 h. The growth profile of thermophiles was monitored based on the optical density measured at 600 nm (OD_{600}). Growth profile consists of four phases which are lag, log, stationary and death phase. At the lag phase, the OD_{600} reading for active culture was low which is around (0.2). During this phase, the thermophiles started to absorb nutrients, synthesize enzymes and prepares for cell division. Hence, there is no increase in bacterial number which corresponds to low viable cell count as shown in Table 1. During the log phase, rapid multiplication of cells occurs after 12 h incubation resulting in a dramatic increase in thermophile cell number. The OD_{600} starts to increase from (0.170) to (0.580) and the CFU after 12 h incubation also increased from too few to count to 4.65×10^7 CFU/ml. However, the cells multiplication began to slow down during the stationary phase as the nutrients in the media decrease and the toxic waste resulting from bacterial metabolism increase, hence number of dividing thermophile equals to the number of dead bacteria. The OD_{600} reading was (0.580) at 16 h, (0.593) at 20 h and (0.617) at 24 h incubation. Interestingly, increase in OD_{600} is correlated with increasing CFU as seen in Table 1.

3.2 Effect of Carbon Source Concentration in Geobacillus caldoxylosilyticus growth.

In this study, the effect of carbon sources concentration in *G. caldoxylosilyticus* growth was examined. Initially, several carbon sources such as glucose and sucrose with concentration of 2000 ppm were selected to assess their role as electron donor in growth of the thermophile. However, from the results obtained in Figure 2, the presence of glucose and sucrose leads to a decrease in growth of *G. caldoxylosilyticus* relative to the control. The control was shown to have the highest bacterial growth

which is OD_{600} (0.541) while bacterial cell added with glucose and sucrose showed lowest bacterial growth compared to the control with OD_{600} (0.215) and (0.287) after 25 h incubation. A similar finding where a high concentration of carbon sources was found to limit bacterial growth by inhibiting enzymatic activity. The cell's ability to breakdown and reincorporate proteinacious resources will be reduced and causes protein degradation which may limit the growth of bacterial populations in sugar solutions [5].

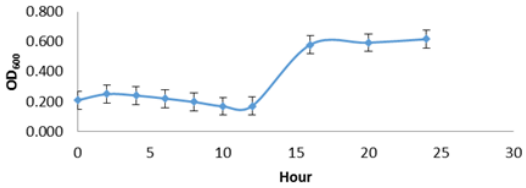


Fig. 1 Growth profile of Thermophilic bacteria

Table 1 Profile of viable cell count

Time (h)	OD_{600}	pH	DW(g)	CFU
0	0.209	6.920	0.0013	1.65×10^3
2	0.250	6.860	0.0018	2.25×10^3
4	0.240	6.830	0.0015	2.10×10^3
6	0.220	6.810	0.0011	1.95×10^3
8	0.197	6.800	0.0009	TFTC
10	0.168	6.830	0.0005	TFTC
12	0.170	6.820	0.0007	TFTC
16	0.580	6.440	0.0041	4.65×10^7
20	0.593	6.700	0.0049	5.50×10^7
24	0.617	6.810	0.0052	6.90×10^7

*TFTC : Too few to count

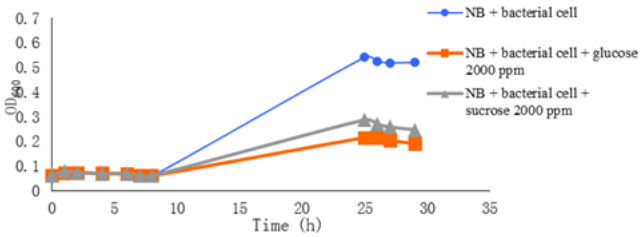


Fig. 2 Growth profile of *G. caldoxylosilyticus* in NB with glucose and sucrose

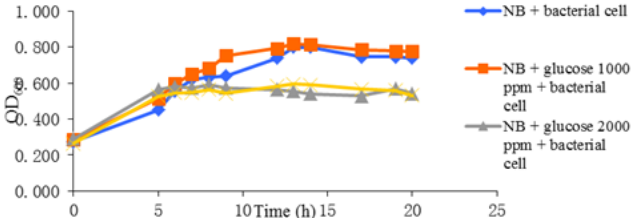


Fig. 3 Growth profile of *G. caldoxylosilyticus* in NB with glucose and acetate

The study shows that the growth of *G. caldoxylosilyticus* was the highest in the presence of glucose with concentration with concentration 1000 ppm with OD_{600} (0.817) when compared to the control after 13 h incubation (Figure 3). This was expected as glucose is the simplest carbohydrate for metabolism to supply energy. In glycolysis, the energy is produced by breaking down glucose which takes place in the cytoplasm of the bacterial cells [6]. The glucose shows a better substrate and major source of maintaining energy for cell metabolisms when compared to other carbon sources for production of poly-3-hydroxybutyrate (PHB) by *Geobacillus* strain [7]. However, the presence of high concentration of glucose showed the lowest bacterial growth with OD_{600} (0.554) after 13 h incubation. A possible explanation is that the addition of glucose and sucrose at concentration 2000 ppm could initially suppressed the growth of thermophile. Acetate did not affect *G. caldoxylosilyticus* growth as the OD_{600} value was similar (0.527) and (0.590) in the presence and absence of acetate after 20 h incubate.

3.3 Growth in salts minimal medium and glucose

Figure 4 depicts the growth profile in mixture of salts minimal medium and glucose. Salt minimal medium is used as chemically-defined media in this experiment where the exact chemical composition is known. For a medium to support microbial growth, it must provide carbon, nitrogen, sulphur, phosphorus and any other organic growth factors. Figure 4 shows the maximum growth for *G. caldoxylosilyticus* in mixtures of salt minimal medium and glucose after 8 h incubation (0.079) and started to decrease after 12 h incubation. The lowest value of OD_{600} was (0.063) at 20 h incubation probably due to nutrient deficiency and accumulation of toxic wastes.

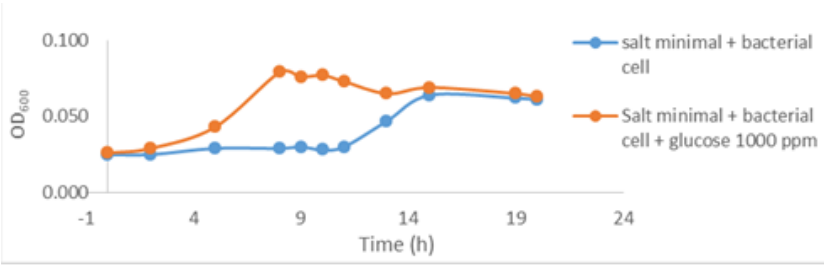


Fig. 4 Growth profile in mixture of salt minimal medium and glucose 1000 ppm.

It was observed that the value of OD_{600} in mixtures of salt minimal medium and glucose after 8 h incubation is higher compared to salt minimal medium only, indicating that *G. caldoxylosilyticus* was able to utilize glucose as carbon source and NH_4^+ from basal salts for metabolism. However, the concentration of nutrients in salt medium may not be sufficient to sustain bacterial growth. The growth of *G. caldoxylosilyticus* in salt minimal medium adding with glucose was not as high as growth in nutrient broth medium only. Hence, the presence of yeast extract and peptone should provide the N-requirement for the bacterial growth [4].

3.4 Cr(VI) reduction in mixtures of NB and glucose

The reduction of Cr(VI) by *G. caldoxylosilyticus* was studied and the results are shown in Figure 5. Based on Figure 5, the addition of 1000 ppm glucose in NB increased Cr(VI) reduction. The thermophile shows a higher Cr(VI) reduction of 92.79% compared to the thermophile grown in the absence of glucose 73.09% after 24 h incubation. Meanwhile, the control only shows the Cr(VI) reduction of 54.01% after 24 h incubation. This shows that the addition of glucose as extra carbon source for *G. caldoxylosilyticus* increase Cr(VI) reduction as addition of glucose can cause an increase in cell concentration (CFU/mL). However, Cr(VI) reduction capacity was likely difference due to number of cells present. The cell viability was decreased over the time after 24 h incubation as shown in Table 2. Similarly, findings were also reported for *A. baumannii* reduced Cr(VI) as the time cell incubation increased [8]. In short, at 16 and 24 h incubation, more bacterial cells would be present in the solution, thus higher Cr(VI) reduction was observed.

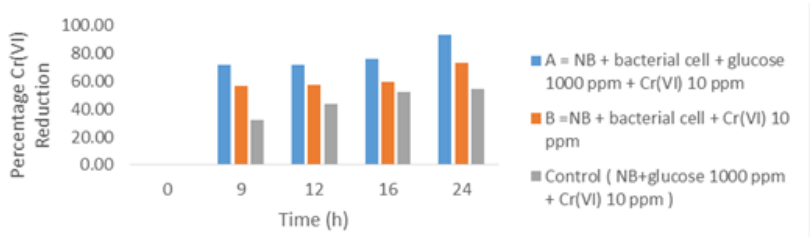


Fig. 5 Cr(VI) reduction in mixture of NB and glucose 1000 ppm.

Table 2 Viable cell count (CFU/mL) at Cr(VI) concentration, 10 ppm

	Hour, h	0	9	12	16	24
Flask	A	4.35×10^7	3.90×10^5	3.65×10^5	4.25×10^5	TFTC
	B	3.25×10^7	3.25×10^5	2.95×10^5	2.30×10^3	TFTC

*TFTC : Too few to count

4. CONCLUSION

As a conclusion, the thermophilic bacteria used in this study was shown to be a potential biological reducing agent for Cr(VI). From the results obtained, a strain of thermophilic bacteria, identified as *Geobacillus caldoxylosilycus* UTM 6 (GenBank Acession No.KR867680) was found to grow in 10 ppm of Cr(VI). The G. caldoxylosilyticus was able to reduce (92.79%) of 10 ppm Cr(VI) when grown in the presence of 1000 ppm glucose.

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