Removal of Chromium (VI) in Tannery Wastewater by *Bacillus cereus*

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Abstract: *Bacillus cereus* was used to remove Cr(Ⅵ) in tannery wastewater containing medium at different initial Cr(Ⅵ) concentration, temperature, pH and biomass to get better conditions. At the optimum condition, the Cr(Ⅵ) in tannery wastewater was treated by each component of the cells to detect the capacity of the components for the Cr(Ⅵ) reduction. Also, the *Bacillus cereus* was continuous cultured to test the durative ability for the Cr(Ⅵ) removal. After treating 20mg/L Cr(Ⅵ) in tannery wastewater for 48h, the *Bacillus cereus* was observed by SEM, with the strain grown in Luria broth for 48h as control. The results showed that the removal rate of Cr(Ⅵ) was the higher when 37℃, pH=7.0－9.0, 20g/L of the biomass and less than 50mg/L of the initial Cr(Ⅵ) concentration; at the optimum conditions, the removal rate of Cr(Ⅵ) for the cell-free extracts reached 92.70 %, nearly the same as the whole-cells(96.85 %), indicating the Cr(Ⅵ) reductase generated by the *Bacillus cereus* was mainly intracellular. In the process of continuous culture, the strain showed consecutive growth and removal ability of Cr(Ⅵ). Also, the SEM images showed that the *Bacillus cereus* treating Cr(Ⅵ) grew well and had the same size, compared with its grown in Luria broth. The experiments demonstrated that the *Bacillus cereus* could be selected as a new biomaterial to remove Cr(Ⅵ) in tannery wastewater.

Key words: tannery wastewater; *Bacillus cereus*; hexavalent chromium; reduction characteristic

1 Introduction

Hexavalent chromium (Cr(Ⅵ)) in tannery wastewater has been paid great attention with the increase of people's awareness for environmental protection and health. At present, conventional methods for removing dissolved Cr(Ⅵ) include chemical reduction, electrochemical treatment, chemical precipitation, ion exchange, etc. However, these high-technology processes have significant disadvantages, including requirements for expensive equipments and monitoring systems, or generation of other waste products. These problems can be solved by application of microbial cells at affordable costs. Microorganisms as biosorbents offer a potential alternative to existing methods for removing Cr(Ⅵ) in wastewater.[1-2]

In recent years, a lot of strains with high effect for the reduction of Cr(Ⅵ) have been isolated from wastewater or activated sludge containing Cr(Ⅵ), such as *Bacillus fusiformis* [3], *Bacillus subtilis* [4,5], *Bacillus cereus* [6], Sulfate-reducing bacteria(SRB) [7], *Escherichia coli* [8], and so on. Nevertheless until now there has not been any reports about removing Cr(Ⅵ) in tannery wastewater with strains which have high reduction efficiency for Cr(Ⅵ) such as *Bacillus cereus*.

In the experiments, the *Bacillus cereus* will be used to remove the Cr(Ⅵ) in tannery wastewater containing medium at different initial Cr(Ⅵ) concentration, temperature, pH and biomass to get better conditions. At the optimum condition, the Cr(Ⅵ) in tannery wastewater will be treated by each component of the cells to detect the reduction capacity of the components. Also, the *Bacillus cereus* will be continuous cultured to test the consecutive ability for the Cr(Ⅵ) removal. After treating Cr(Ⅵ) in tannery

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wastewater, the *Bacillus cereus* will be observed by SEM, with the strain grown in Luria broth as control.

2 Experimental

2.1 Materials

Yeast extract and peptone were biochemical level and respectively purchased from Beijing AoBoXing LEIVERSEEN BIO-TECH Co. Ltd (China) and Chengdu Changshou BIO-TECH Co. Ltd (China). Other reagents were research grade.

2.2 Bacillus cereus

Bacillus cereus in the experiments was conserved in our laboratory. Before used, it was cultured in Luria broth containing Cr(Ⅵ) to adapt for Cr(Ⅵ) in wastewater.

2.3 Tannery Wastewater Containing Chromium(Ⅵ) and Medium

The wastewater in the chrome tanning process was selected and the content of Cr(Ⅵ) was determined according to IUC 18[9]. Then, the wastewater was diluted 20 times and some nutrients were added to help the growth of the strain, ie, yeast extract, peptone and sodium lactate. Next, some of the potassium dichromate ($K_2Cr_2O_7$) was added to get different concentrations of Cr(Ⅵ) in the wastewater, according to the requirements of the experiments. Thus, the tannery wastewater containing Cr(Ⅵ) and medium was obtained.

The determination for the content of Cr(Ⅵ) was as follows. At first, the diphenylcarbazide solution was made: 1.0g 1,5-diphenyl-carbazide was dissolved in 100mL acetone and made acidic with one drop of glacial acetic acid; the solution was kept in a brown glass bottle at 4℃ for 14 days. Also, the phosphoric acid solution was made with 700mL o-phosphoric acid (d=1.71g/mL) and 300mL distilled water. After different volumes (0.5-20.0mL) of the Cr(Ⅵ) standard solution (1μg/mL) were pipetted into 50mL volumetric flasks, 1mL diphenylcarbazide solution and 1mL of phosphoric acid solution was added to each flask, and the distilled water was made up to the volume mark. When the substances in every flask were mixed well and stood for 15±5 min, the extinction of the solutions was measured in a 2cm cell at 540nm against the blank with a UV/VIS spectrophotometer (Hong Qiao High-tech Instrument Co., Shanghai, P.R. China). The blank did not contain the Cr(Ⅵ) standard solution, compared with the samples. Thus, the extinction-concentration calibration curve of Cr(Ⅵ) was plotted. The extinction of Cr(Ⅵ) in the wastewater was analyzed following the measure procedure as the above. Referring to the calibration curve of Cr(Ⅵ), the content of Cr(Ⅵ) was obtained.

2.4 Impact Factors for the Strain to Remove Cr(Ⅵ)

2.4.1 Initial Cr(Ⅵ) Concentration

250mL tannery wastewater which contained medium and different concentrations of Cr(Ⅵ) (5, 10, 50, 100, 200, 500 mg/L) at the pH value of 7.0 was respectively taken into a corresponding conical flask and sterilized at 121℃ for 20min. Then the flasks were inoculated 1.0g cells which were collected through centrifugating of the fresh culture suspension at 8000r/min for 5min at 4℃. After the flasks were incubated at 37℃ under gyratory shaking of 140r/min, samples were got at different time. The A_{600} for each sample was detected to draw the growth curve. Next, samples were centrifugated at 8000r/min for 5min at 4℃ and the supernatants were used to estimate the remaining Cr(Ⅵ) content.

2.4.2 Temperature

0.3g cells were inoculated to 50mL sterilized tannery wastewater which contained medium and 9.5mg/L Cr(Ⅵ) (pH＝7.0). Then, the flasks containing wastewater were oscillated at 27, 32, 37, 42 and 47℃ for 48h, respectively. Next, the samples were centrifugated at 4℃ and the remaining Cr(Ⅵ) content was estimated in the supernatants.

2.4.3 pH Value

Tannery wastewater containing medium and 20.0mg/L Cr(Ⅵ) was adjusted to different pH (4.0-9.0) with 0.1mol/L NaOH or 0.1mol/L HCl solution. Then, 50mL sterilized wastewater with different pH was taken into a corresponding conical flask and inoculated 0.25g cells. After the flasks were oscillated at 37℃ for 48h, the remaining Cr(Ⅵ) content was estimated. Meanwhile, the wastewater inoculated no strain at every pH was as control.

2.4.4 Biomass

The cells were respectively inoculated to conical flasks with 50mL sterilized tannery wastewater containing medium and 15.0mg/L Cr(Ⅵ) (pH＝7.0) to form different biomass (2.0, 10.0, 20.0, 100.0g/L). When the flasks were oscillated at 37℃ for 48h, the remaining Cr(Ⅵ) concentration was estimated.

2.5 Reduction Capacity of Cr(Ⅵ) with Components of the Cells

Cells grown 36h in Luria broth containing 5.0mg/L Cr(Ⅵ) were centrifuged at 8000 r/min for 5 min at 4℃. The supernatants were obtained as the extracellular sample. The centrifuged cell pellets were washed twice with 50 mmol/L Tris-HCl buffers (pH 7.0). Then, the washed cells were decided two samples: one was as the whole-cells, the other was resuspended with 4 mL of the same buffer. These resuspended cells were placed in ice bath and disrupted using an Ultrasonic Probe (UH-500B, China) with amplitude of 30% at 750w with 9 s pulses at 2 s intervals for 20 min. Sonicates thus obtained were then centrifuged at 8000 r/min for 5 min at 4℃. Thus the supernatants were as the cell-free extracts.

The samples, including the supernatants, the whole-cells and the cell-free extracts, were added to tannery wastewater containing medium and 10.0mg/L Cr(Ⅵ) (pH＝7.0). After oscillated at 37℃ for 24h, they were centrifugated at 4℃ and estimated for the remaining Cr(Ⅵ) concentration.

2.6 Repeated Reduction of Cr(Ⅵ) on Continuous Inputs

50mL tannery wastewater containing medium and 8.8mg/L Cr(Ⅵ) (pH＝7.0) was inoculated 1.0g cells and then oscillated at 37℃(140r/min). The nutritions and the Cr(Ⅵ) were continuously added after every 48h with the same as the culture condition of the first time. Estimations of the remaining Cr(Ⅵ) were made before and after every 48h.

2.7 Observation of the Strain by SEM

The strain was cultured for 48h respectively in tannery wastewater containing 20.0mg/L Cr(Ⅵ) and in Luria broth without Cr(Ⅵ). After centrifugated at 4℃, the cells were observed by a scanning electron microscope (SEM) (JSM-5900LV,Philips Co., Netherlands).

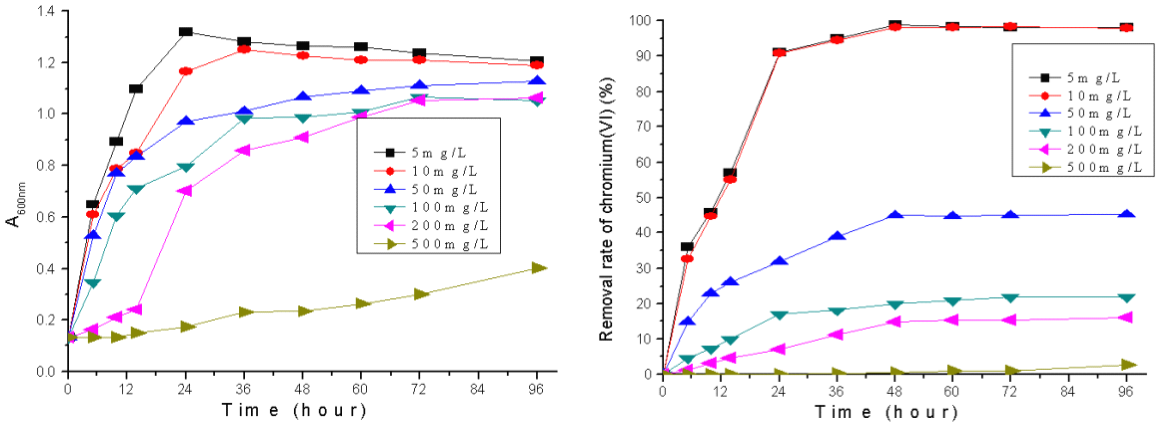
3 Results and discussion

3.1 Initial Cr(Ⅵ) Concentration

At higher initial Cr(Ⅵ) concentrations (more than 200mg/L) a decrease in growth rate is observed and the logarithmic phase is postponed, compared to the growth for the *Bacillus cereus* at no more than

100mg/L, as shown in Fig. 1(a).

With the increase of the Cr(Ⅵ) concentration, the ability to remove Cr(Ⅵ) is gradually weakened for the strain(Fig. 1(b)). However, the removal rate of Cr(Ⅵ) reaches more than 98% at 48h with lower initial Cr(Ⅵ) concentrations (5mg/L and 10mg/L), and the removal is mainly in 24h. Thus, the characteristic of the strain can be used to treat tannery wastewater which does not contain too much Cr(Ⅵ).



(a) **(b)**

Fig. 1 Growth curve of the *Bacillus cereus* (a) and its removal effects of Cr(Ⅵ) (b) at different initial Cr(Ⅵ) concentration

3.2 Temperature

Figure 2 shows that the Cr(Ⅵ) removal rate increases from 27℃ to 37℃. The Cr(Ⅵ) removal rate reaches the highest of 98.22% at 37℃. When the temperature is higher than 37℃, the Cr(Ⅵ) removal rate declines. Thus, 37℃ was selected as the optimum temperature to remove Cr(Ⅵ) in wastewater containing Cr(Ⅵ) in the following experiments.

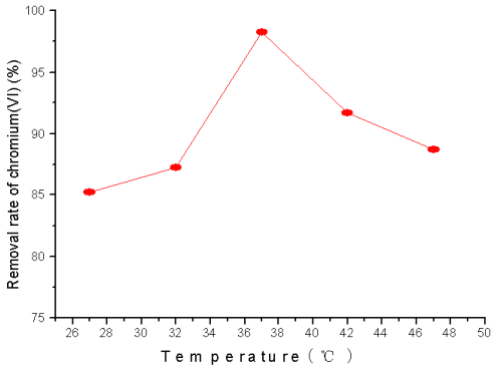


Fig. 2 The removal rate of the Cr(Ⅵ) for the *Bacillus cereus* at different temperatures

3.3 pH Value

When pH value was 4.0, the Cr(Ⅵ) content in the wastewater without treated by the *Bacillus cereus* reduced 11% after 48h. This might be because the Cr(Ⅵ) was reduced to the Cr(Ⅲ) with the reductant in the wastewater. However, when pH value was from 5.0 to 9.0, the Cr(Ⅵ) content in wastewater without treated by the *Bacillus cereus* has no more changes after 48h. (not shown in table or figure)

The removal rate of Cr(Ⅵ) removed by the *Bacillus cereus* reaches the highest of about 89% when the pH value is 7.0－9.0(Fig. 3), during the range of pH value (4.0-9.0). Therefore, it was suitable for its growth and reduction of Cr(Ⅵ) in neutral or micro alkaline environment (7.0－9.0).

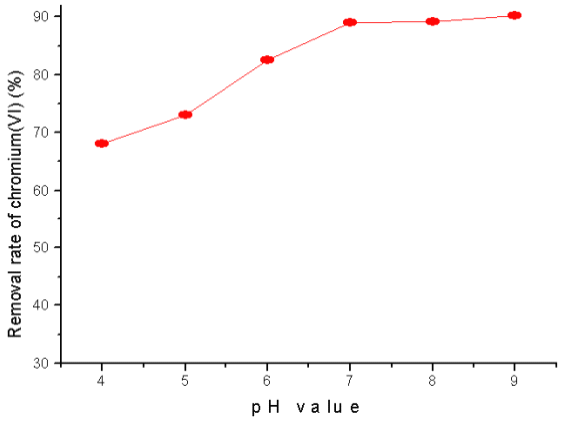


Fig. 3 The removal rate of the Cr(VI) for the *Bacillus cereus* at different pH value

3.4 Biomass

As can be seen from Fig. 4, the efficiency for the Cr(VI) removal increases from 2.0g/L to 20.0g/L of biomass. When the biomass is 20.0g/L, the removal rate of Cr(VI) reaches the highest of 97.37%. When the biomass is more than 20.0g/L, the Cr(VI) removal rate declines. This was because there were no enough nutritions for the growth when the biomass was too much and thus reduced the reduction ability of the *Bacillus cereus*. Therefore, 20.0g/L of the biomass was chosen as a reference amount for the following experiments.

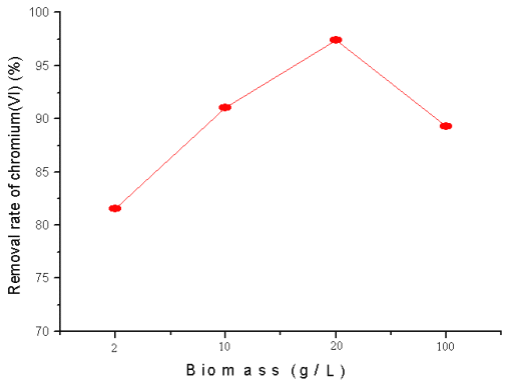


Fig. 4 The removal rate of the Cr(VI) for the *Bacillus cereus* with different biomass

3.5 Reduction Capacity of Cr(VI) with Each Component of the Cells

Tab. 1 Reduction capacity of Cr(VI) for each component of the cells

Cell components	Initial quality (mg)	End quality (mg)	Removal rate of Cr (VI) (%)
Whole-cells	0.1904	0.0060	96.85
Supernatants	0.1992*	0.1495	24.96
Cell-free extracts	0.1904	0.0139	92.70

Note: "*"— The initial Cr(VI) (0.1992mg) was more than 0.1904mg because there was Cr(VI) residue in the supernatants after the bacteria grew in Luria broth containing 5.0mg/L Cr(VI) for 36h.

The Cr(VI) reduction capacity for each component of the cells was different, see table 1. The

supernatant samples (extracellular) reduce only 24.96% of Cr(Ⅵ). However, the cell-free extracts is able to reduce 92.70% of Cr(Ⅵ), which is close to the reduction ability of the whole-cells(96.86%). This indicated that the Cr(Ⅵ) reductase generated by the *Bacillus cereus* might be mainly intracellular.

3.6 Repeated Reduction of Cr(Ⅵ) on Continuous Inputs

It is seen from Fig. 5 that initial 8.765 mg/L Cr(Ⅵ) is reduced near to zero within 48h by the *Bacillus cereus* at 37℃. Moreover, the *Bacillus cereus* exhibits reduction of the Cr(Ⅵ) up to four consecutive inputs. Therefore, the strain will exhibit an ability to repeatedly reduce the Cr(Ⅵ) if there are enough nutrients and sufficient electron donor, suggesting its potential application in continuous bioremediation of Cr(Ⅵ).

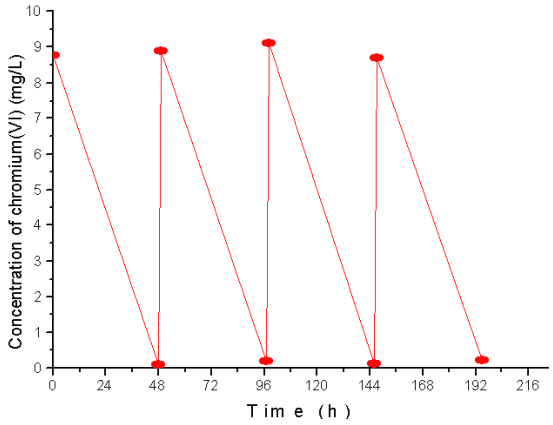
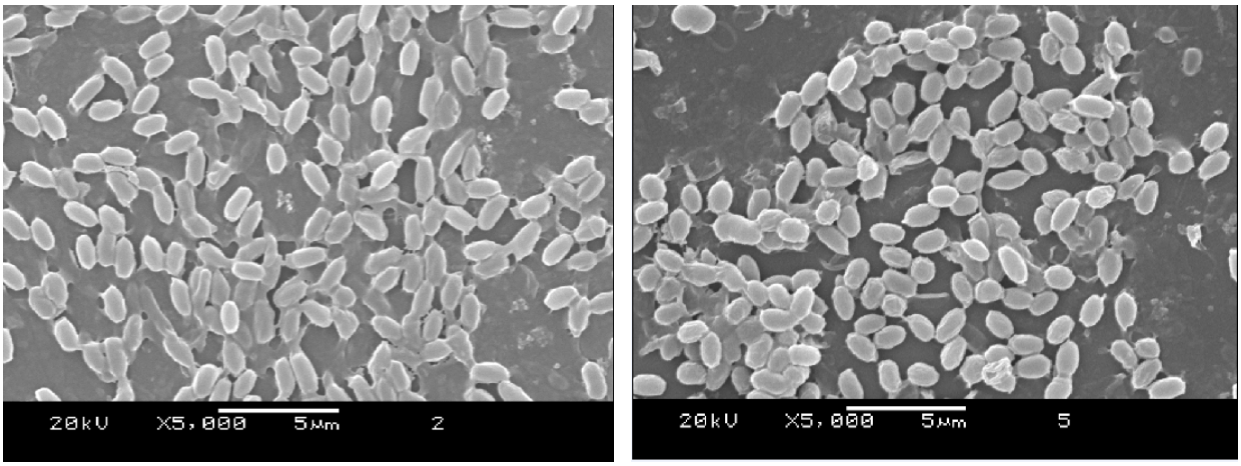


Fig. 5 Repeated detoxification of Cr(Ⅵ) by the *Bacillus cereus*

3.7 Observation by SEM



(a) in tannery wastewater containing 20mg/L Cr(Ⅵ) **(b) in Luria broth without Cr(Ⅵ)**

Fig. 6 SEM image of the *Bacillus cereus* (× 5000 times)

The SEM images shows that the *Bacillus cereus* treating Cr(Ⅵ) in tannery wastewater has a complete shape and an unanimous size after 48h(Fig. 6(a)), compared with the *Bacillus cereus* grown in Luria broth without Cr(Ⅵ) (Fig. 6 (b)). This indicated that the *Bacillus cereus* had no deformation or ill growth with the poison of the Cr(Ⅵ).

4 Conclusions

This study represents the fact that *Bacillus cereus* is rather effective to remove Cr (Ⅵ) at lower initial

Cr(Ⅵ) concentrations (less than 50mg/L) and exhibits an ability to repeatedly reduce Cr(Ⅵ) if there are sufficient electron donor and nutrients. It is expected that the results will contribute to application of the *Bacillus cereus* to the treatment of Cr(Ⅵ) in tannery wastewater.

Acknowledgements

The authors wish to thank the Ministry of Science and Technology of China for the financial support (Item No. 2006DFA41010).

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