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Detoxification of chromium slag by chromate resistant bacteria

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Abstract

This paper presented direct detoxification of chromium slag by using microorganisms. Our work showed that a bacterial consortium isolated from chromium slag can efficiently accelerate Cr(VI) leaching rate and remove it. The chromate resistant bacterial consortium consists of three strains. The detoxification efficiencies of the three respective strains follow the sequence: strain I > strain III > strain II. The detoxification capability of the bacterial consortium is far stronger than that of the respective strain, showing an excellent synergistic effect. The specific growth rates in pure and chromium-containing medium are 0.1475 and 0.1573 h^{-1}, respectively. The presence of Cr(VI) has little effect on growth rate of the bacterial consortium. Cr(VI) removal takes place in exponential growth period of the bacterial consortium; the detoxification time should be controlled in this phase for every run. The kinetics of detoxification process was studied and can be described as $\eta = 0.0615e^{0.1573t}$, which can be used to predict Cr(VI) removal efficiency.

Keywords: Chromium slag; Hexavalent chromium; Bacterial consortium; Detoxification

1. Introduction

Domestic metallurgical and chemical industries discharge 200–300 thousand tonnes of chromium slag per year and the accumulative total amount is not lower than 2 million tonnes [1]. Chromium slag from chromate plants is one of the most hazardous solid wastes because of its high content of dissolvable Cr(VI), which is known to be toxic to plants and animals, a strong oxidizing agent and potential carcinogen [2,3]. The continuous leaching out of the Cr(VI) in chromium slag can cause sustainable contamination to its near environment; some effective measures must be taken to remove it.

Chromium slag is usually treated using solidification and then safely filled after it reaches the filling standard [4]. The domestic method in general is comprehensive utilization after turning Cr(VI) into Cr(III) [5,6]. But this method can only consume a less amount as compared to the large amount of chromium slag. In addition, the treatment processes consume a great deal of energy, causing a high treatment cost. At present, it is still difficult to find an economic, safe, efficient and applicable technology for the detoxification of chromium slag.

On the other hand, the Cr(VI) in chromium-containing effluent can be efficiently removed by chromate resistant bacterial consortium [7–11]. Often biotechnological methods are cheaper and do not need application of chemical reagents to the environment as compared with physicochemical processes. The rate of Cr(VI) leaching out from the slag is controlled by its diffusion process; higher concentration gradient is beneficial to the enhancement of Cr(VI) leaching. The Cr(VI) present in the liquid phase, which is leached from chromium slag, can be continuously removed by biosorption and bioaccumulation of microorganisms [8,9]. This process helps to maintain a higher concentration gradient between the surface of chromium slag particles and the bulk liquid phase, thus facilitating the detoxification process controlled by diffusion. In this paper, we presented direct removal of Cr(VI) from chromium slag by chromate resistant bacteria and investigated the detoxification effect and kinetics.

2. Materials and methods

2.1. Chromium slag sample and pretreatment

Chromium slag samples were collected from Chongqing Minfeng Chemical group Company, Chongqing, China. The

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fresh chromium slag samples were dried in an oven at 80 °C, then ground in a ball mill and passed 200 mesh sieve. The treated chromium slag was sterilized in an oven for 2 h at 160 °C before use.

2.2. Isolation of Cr-resistant bacteria [9,12]

For isolation of Cr-resistant bacteria, the following procedure was used. Five grams of fresh chromium slag was added into 50 ml beef extract peptone medium in a 250 ml conical flask and the mixture was incubated on a shaker at 150 rpm for 96 h at 30 °C to select Cr-resistant bacteria. The culture medium consisted of beef extract 5 g/l, peptone 10 g/l, NaCl 5 g/l, pH 7.0. After the culture, 5 ml of medium was taken out and then transferred to a 50 ml beef extract peptone medium holding 20 g/l of the treated chromium slag and the mixture was incubated again at the above-mentioned conditions to acclimatize the selected bacteria. For subsequent acclimatization, the quantity of chromium slag in culture medium was gradually increased from 20, 30, 40 to 50 g/l, then the mixture was repeatedly incubated again at the same conditions. When the last acclimatization was over, 5 ml of the medium was transferred to 50 ml medium and incubated on a shaker at 150 rpm for 18 h at 30 °C. The obtained medium with Cr-resistant bacterial consortium was then stored at 4 °C for further use.

2.3. Observation of the Cr-resistant bacteria [13]

Seventeen to twenty grams per liter agar was added into the above-mentioned medium to form solid medium. Different colonies were isolated using dilution and streak plate method. The characteristics of the colonial strains and the respective strain were observed by microscopes.

2.4. Detoxification effects of respective strains and the bacterial consortium [9]

To compare detoxification effects, the isolates and Cr-resistant bacterial consortium were inoculated individually into 20 ml sterilized medium with 50 g/l pretreated chromium slag in 100 ml conical flasks and incubated at 30 °C at 150 rpm for 48 h. The detoxification systems were centrifuged at 1500 rpm for 5 min. The supernatants were used for determining Cr(VI) content in the culture medium. The detoxified chromium slag samples were washed two times with deionized water to remove the bacteria adhering to the slag particles. Every time the slag was separated by centrifuge. The washed slag samples were dried in an oven at 80 °C and used for the determination of Cr(VI). All the tests at the same condition were performed in duplicate, the Cr(VI) contents are the averaged values of two analytical results.

2.5. Orthogonal test

Orthogonal tests were carried out in this work to probe main factors affecting detoxification efficiency and optimize detoxification conditions. Temperature, pH and rotation speed were selected as the main factors. The levels of temperature are 20, 30 and 37 °C; the rotation speeds are 150, 200 and 250 rpm; pH levels are 5, 7 and 9. In each experiment, 2 ml of the Cr-resistant bacterial consortium medium was inoculated into 20 ml medium with 50 g/l chromium slag in a 100 ml conical flask and incubated for 48 h. The subsequent processing of the detoxification system was finished according to the procedure mentioned in Section 2.4. All the tests at the same conditions were performed in duplicate.

2.6. Growth curve of the bacterial consortium

Two milliliter of the Cr-resistant bacterial consortium medium was inoculated into 20 ml sterilized medium in a 100 ml conical flask and incubated at 30 °C at 150 rpm. A series of the incubation experiments was prepared according to this procedure, and every time two conical flasks were taken out at random every 2 h to determine the optical density of the culture medium at 660 nm [9], the growth curve was made according to the time and optical density.

In order to get the relationship between optical density and biomass (g/l), 15 incubation experiments was prepared according to the same procedure, and every time three conical flasks were taken out at random to determine the optical densities (OD) of the culture medium and biomass concentration X (g dry weight/l) by drying biomass to constant weight at 80 °C. And then the correlation result between averaged dry weights and averaged optical densities was obtained as: $X \approx 0.5927$ OD, in the range of 0–6.2 of optical densities, and the correlation coefficient $R^2 = 0.9981$, which shows that the dry weight of biomass is approximately proportional to the value of optical density.

2.7. Detoxification kinetics of the bacterial consortium

Two milliliter of the Cr-resistant bacterial consortium medium was inoculated into 20 ml culture medium with an amount of chromium slag in a 100 ml conical flask and incubated at 30 °C at 150 rpm. A series of detoxification systems was prepared according to this procedure, every time two conical flasks were taken out at random to be centrifuged at 1500 rpm for 5 min. Then the subsequent processing of every sample was finished according to the procedure mentioned in Section 2.4.

2.8. Cr(VI) analysis procedures

The culture medium sample was centrifuged at 10,000 rpm for 10 min and the supernatant was used to determine the concentration of Cr(VI) in the medium. The Cr(VI) contents in the detoxified chromium slag samples were first extracted for 30 min with 10% H_3PO_4 aqueous solution at 0.12–0.14 MPa, 121 °C. [16], the extract solution was used for analysis. The concentration of Cr(VI) in solution was determined by UV spectrophotometers at 540 nm using 1,5-diphenylcarbazide as a pink colored complex agent [8].

Table 1
Colonial characteristics of the three strains

Colonial characteristics	Strain I	Strain II	Strain III
Shape	Sphere	Sphere	Sphere
Diameter (mm)	1–2	1–2	3–5
Color	Milk white	Yellow	Milk white
Surface	Velvet	Velvet	Velvet
Edge	Regular	Regular	Irregular
Center	Thick	Thicker	Thicker

3. Results and discussion

3.1. Characteristics of chromate resistant isolates

A chromate resistant bacterial consortium was obtained after acclimatization. Three strains were isolated from the bacterial consortium and named strain I, strain II and strain III, respectively. Tables 1 and 2 show the characteristics of the colonial and the three respective strains. It is found from several observations that the colonial number of strain I:strain II:strain III is 50–58:2–5:1–3, which shows that the bacterial consortium was predominated by strain I. Srinath et al. [9] isolated 71 strains that are capable of bioaccumulating Cr(VI) from tannery effluent. Two strains, identified as *Bacillus circulans* and *Bacillus megaterium*, showed excellent bioaccumulation ability (34.5 and 32.0 mg/g dry weight, respectively). They have rodlike cell shape and are gram-positive. The minimum inhibitory concentration (MIC) for *B. circulans* and *B. megaterium* reached as high as 130 and 170 mg Cr(VI)/l, respectively. In this work, three chromate resistant strains were isolated from the bacterial consortium, two of them also have rodlike shape, but one is gram-negative. In addition, a strain with spherical shape was found.

3.2. Detoxification efficiency of the respective strain and bacterial consortium

Table 3 shows the results of detoxification efficiency of the respective strain and bacterial consortium. The Cr(VI) content in the raw chromium slag is 7.483 mg/g. It could be seen that the existence of the chromate resistant bacteria can effectively remove Cr(VI) in the medium and obviously accelerate the Cr(VI) transfer from the chromium slag to the medium as compared to comparison test. The detoxification efficiency of the three respective strains follows the sequence: strain I > strain III > strain II. The bacterial consortium exhibited much better detoxification efficiency than the three respective strains, showing a very strong synergistic effect.

Table 2
The characteristics of the three single strains

Characteristics	Strain I	Strain II	Strain III
Cell shape	Rod	Sphere	Rod
Size (μm)	0.3–0.5 × 1–3	1 × 1	1 × 2
Connection form	Single	Grape	Single
Gram stain	−	+	+
Spore	−	−	+

Microbial heavy metal accumulation often comprises of two phases, i.e. an initial rapid phase involving physical adsorption or ion exchange at cell surface and a subsequent slower phase involving active metabolism-dependent transport of metal into bacterial cells [14]. The adsorption is based on mechanisms such as complexation, ion exchange, coordination, adsorption, chelation and microprecipitation which may be synergistically or independently involved [15]. The bacterial consortium can provide more adsorption and metabolism pathways than the respective strain. Therefore, the strong synergistic effect of the bacterial consortium may be ascribed to the change of metabolic pathways. But the synergistic mechanism at molecular level remains unclear and needs to be studied in detail.

3.3. Orthogonal tests

The calculation of the orthogonal test results and the analysis of mean squares showed that temperature is the most important factor among the three factors (data not shown). The optimized parameters for removing Cr(VI) from chromium slag using the bacterial consortium are as follows: temperature 30 °C, pH 7.0, rotation speed 150 rpm.

3.4. The growth and detoxification curves of the bacterial consortium

Fig. 1 shows the growth curve of the bacterial consortium. It can be roughly seen that the exponential growth period should lie in 2–18 h, and the bacterial consortium grew very slowly from 18 to 38 h, which could be viewed as its steady growth period. After about 38 h, the bacterial consortium began to enter its death period which is considered not useful to the removal of Cr(VI) from liquid phase. This growth curve is very similar to that of *B. megaterium* reported by Srinath et al. [9], which MIC of Cr(VI) is the highest (150 mg/l) of the 71 strains screened by them. In order to know the more accurate exponential growth period, it is necessary to analyze the data shown in Fig. 1. It is well known that in exponential growth period of microorganisms, plotting $\ln(X/X_0)$ versus t should be a line, and the slope of the line is specific growth rate, μ (h^{-1}). Data processing indicated that in

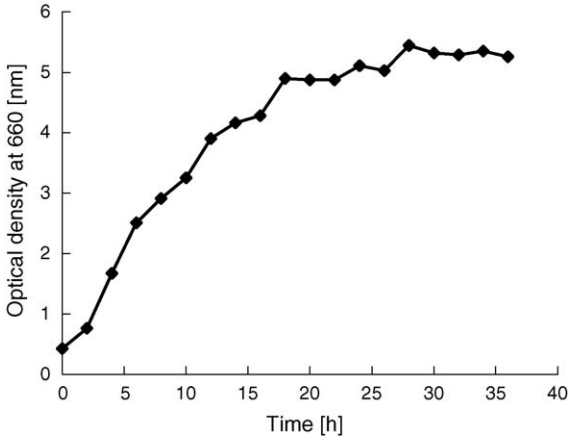

Fig. 1. Growth curve of the bacterial consortium.

Table 3
The detoxification efficiency of the respective strain and the bacterial consortium

Strains	Initial Cr(VI) (mg)[a]	Cr(VI) in medium (mg/l)	Cr(VI) in slag (mg/g)	Total residual Cr(VI) (mg)[b]	Cr(VI) removal efficiency (%)[c]	Leaching rate of Cr(VI) (mg/g day)[d]
Strain I	7.483	103.3	2.658	4.724	36.87	2.413
Strain II	7.483	119.5	3.936	6.35	15.14	1.774
Strain III	7.483	97.4	3.026	4.974	33.53	2.229
Bacterial consortium	7.483	0.6	2.896	2.908	61.14	2.294
Comparison (no strains)	7.483	123.9	4.682	7.16	4.32	1.401

[a] The initial Cr(VI) amount is calculated based on chromium slag 50 g/l, culture medium 20 ml and Cr(VI) content is 7.483 mg/g.
[b] The values are calculated based on chromium slag 50 g/l, culture medium 20 ml.
[c] The values are given by [initial Cr(VI) − total residual Cr(VI)]/initial Cr(VI).
[d] The values are given by [initial Cr(VI) − residual Cr(VI) in slag]/detoxification time.

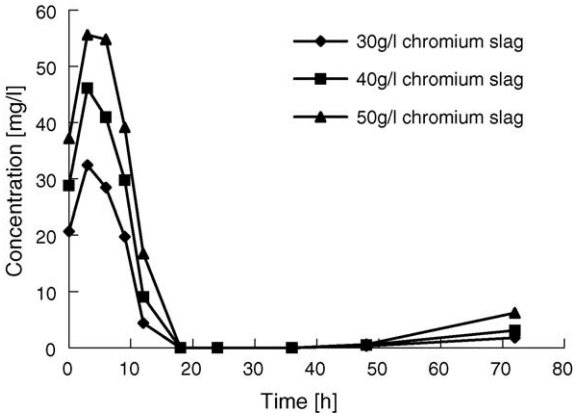

Fig. 2. Cr(VI) concentration in the medium.

the growth periods of 2–12 and 2–18 h, the μ values are 0.1475 and 0.1212 h^{-1} and the correlation coefficients R^2 are 0.9244 and 0.9056, respectively. Therefore, it seems that the more accurate exponential growth period of the bacterial consortium should be from 2 to 12 h. The growth of the bacterial consortium began to slow down from 12 to 18 h, which is a transitional phase between the exponential growth period and the steady growth period.

Figs. 2 and 3 show the changes of the Cr(VI) contents in the detoxification systems under the existence of the bacterial consortium. It could be seen that the biggest changes of Cr(VI) contents in the liquid and solid phase occurred during the exponential growth period and the steady growth period of the bacterial consortium (about 2–18 h), indicating that Cr(VI) removal is related to active metabolism in this phase. This experimental result is in accordance with the conclusion that the rate of bioaccumulation is controlled by active metabolism-dependent transport of metal into cells [14]. The residual Cr(VI) concentration was close to zero during the steady growth period (18–38 h), and the change of Cr(VI) content in the solid phase decreased slowly and then leveled off in this period. When the bacterial consortium went into its declining period (after about 38 h), the Cr(VI) concentration in the medium increased again, and the Cr(VI) content in the solid phase also began to decrease.

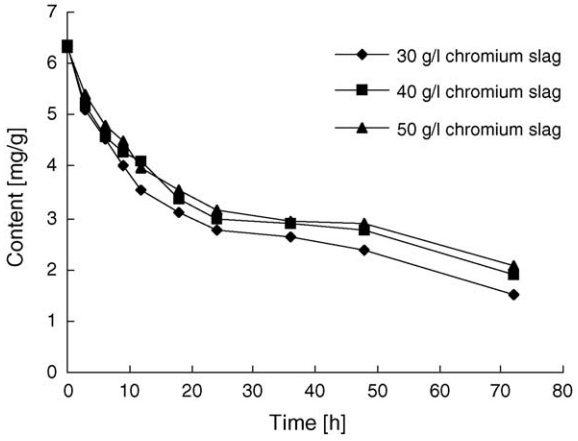

Fig. 3. Cr(VI) content in the chromium slag.

This change may be related to the process the bacterial consortium underwent in a batch operation mode. During the steady growth period, it seems that the leaching of Cr(VI) from the slag phase was stopped. This was caused probably due to the large amount of bacteria. The microorganisms could grow on the particle surface of chromium slag, and form a layer of microorganism phase. This bacterial layer may hinder Cr(VI) from diffusing into liquid phase. This speculation can be verified by the Cr(VI) content changes in the liquid and solid phase during 18–22 h, as shown in Figs. 2 and 3. In this period, the slower leaching of Cr(VI) from the slag did not lead to Cr(VI) rising of the liquid phase. This illustrated that the bacterial layer cannot only hinder Cr(VI) diffusion, but also absorbs the Cr(VI) leached. When the bacteria go into its declining period, they would die and drop off from the surface. The Cr(VI) contents in the liquid and solid phases begin to rise and drop again. Therefore, the optimum detoxification time should be controlled within exponential growth period for every run.

3.5. Kinetics of detoxification process

Figs. 2 and 3 are the kinetics of the Cr(VI) removal from the liquid and solid phases, respectively. In order to predict detoxification rate and analyze the effect of environmental conditions on the growth rate of the bacterial consortium, it is very necessary to establish a mathematical model. As mentioned before, the exponential growth period is the main phase of Cr(VI) removal. So the mathematical model of detoxification kinetics should be established based on this period.

It was known from many textbooks that during exponential growth period, the growth rate of microorganism follows the

Table 4
Specific growth rates and detoxification rate constants at different experimental conditions

Experimental conditions	Specific growth rate, μ (h^{-1})	Detoxification rate constant, β
$W = 30$ g/l, $C_{s0} = 6.348$ mg/g, $C_0 = 20.70$ mg/l	0.1508	0.0689
$W = 40$ g/l, $C_{s0} = 6.340$ mg/g, $C_0 = 28.80$ mg/l	0.1439	0.0542
$W = 50$ g/l, $C_{s0} = 6.323$ mg/g, $C_0 = 37.10$ mg/l	0.1771	0.0612
Comparison: pure medium	0.1475	

apparent first order kinetics model as follows:

$$X = X_0 e^{\mu t} \quad (1)$$

where t is time (h); X the concentration of the bacterial consortium (g/l); X_0 the initial concentration of the bacterial consortium (g/l); μ is the specific growth rate of the bacterial consortium (h^{-1}).

In order to obtain the μ in the detoxification system, it is supposed that the amount of Cr(VI) removal from the liquid is proportional to the concentration of the bacterial consortium because microbial heavy metal accumulation is controlled by the active metabolism-dependent transport of metal into cells [12], and a material balance was made as follows:

$$WC_{s0} + C_0 = WC_{st} + C_t + \alpha X \quad (2)$$

where W is the weight of the chromium slag per liter of culture medium (g/l); C_{s0} and C_{st} the contents of Cr(VI) in the chromium slag at the beginning and any time t, respectively (mg/g); C_0 and C_t the concentrations of Cr(VI) in the medium at the beginning and any time t, respectively (mg/l); α is the coefficient for the concentration of the bacterial consortium.

Substituting Eq. (1) into Eq. (2), then rearranging the Eq. (2) and taking a logarithmic form can get the following relationship:

$$\ln[W(C_{s0} - C_{st}) + (C_0 - C_t)] = \mu t + \ln(\alpha X_0) \quad (3)$$

It can be seen from Eq. (3) that plotting $\ln[W(C_{s0} - C_{st}) + (C_0 - C_t)]$ versus t can obtain a straight line, which slope is the specific growth rate of the bacterial consortium in the detoxification system, μ. Therefore, processing the experimental data obtained during the exponential growth period with this relationship can produce the corresponding μ values at different experimental conditions. As a comparison, the μ in pure medium was also calculated according to Eq. (1), by plotting $\ln(X/X_0)$ versus t or $\ln(OD/OD_0)$ versus t, because experiments showed that the concentration of the bacterial consortium is proportional to the optical density in experimental range. The μ values calculated at different experimental conditions are listed in Table 4.

It can be seen that the μ values in the detoxification system fluctuate between 0.1439 and 0.1771. Because there are some analytical errors in determining the Cr(VI) content in the chromium slag, the little fluctuation of the μ values could be neglected. The averaged value is 0.1573 h^{-1}, which is slightly higher than or close to that in pure medium. This illustrated that the growth of the bacterial consortium in the detoxification system was almost not affected by the Cr(VI) present in the liquid phase. The growth curve of *B. megaterium* remained almost unchanged when the initial Cr(VI) concentration was lower than 50 mg/l [9]. A similar result was obtained in this work.

From Eq. (2), the following relationship can be obtained:

$$1 - \frac{WC_{st} + C_t}{WC_{s0} + C_0} = \frac{\alpha X_0 e^{\mu t}}{WC_{s0} + C_0} \quad (4)$$

It can be seen that the right side of Eq. (4) represents the ratio of the total weight of Cr(VI) in the microorganism phase at any time to that of Cr(VI) at the beginning, left side is the ratio of the total Cr(VI) removed from the liquid phase to that of Cr(VI) at the beginning, they in fact represent the detoxification efficiency. Therefore, detoxification efficiency can be defined as follows:

$$\eta = \frac{\alpha X_0 e^{\mu t}}{WC_{s0} + C_0} \quad (5)$$

Further defining $\beta = \alpha X_0/(WC_{s0} + C_0)$ can get a simplified expression:

$$\eta = \beta e^{\mu t} \quad (6)$$

where β can be called detoxification rate constant. It can be noted from Eq. (4) that plotting the left side of the Eq. (4) versus $e^{\mu t}$ can obtain a straight line, the slope is β. Thus β values could be easily calculated by using the averaged specific growth rate $\mu = 0.1573\ h^{-1}$, as shown in Table 4, the averaged β is 0.0615. Thus we obtained the following mathematical expression of detoxification kinetics as follows:

$$\eta = 0.0615\, e^{0.1573t} \quad (7)$$

Fig. 4 shows the comparison of calculated data and experimental data. It can be seen that the experimental data basically fit the calculated values for the systems with 40 and 50 g/l chromium slag, but are slightly higher than the calculated values for the systems with 30 g/l chromium slag. This difference may

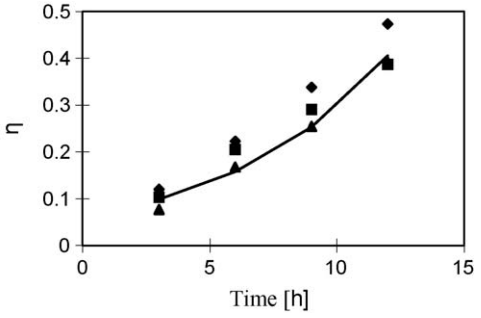

Fig. 4. The comparison of detoxification efficiencies. The line is theoretical values and the symbols represent experimental values. (♦) 30 g/l, (■) 40 g/l and (▲) 50 g/l.

be probably caused by the different Cr(VI) concentration gradients between the surface of chromium slag particles and the liquid phase. It could be seen from Figs. 2 and 3 that the Cr(VI) concentration in the liquid phase for the system with 30 g/l chromium slag is much lower than that for the other two systems, but the Cr(VI) concentrations in the chromium slag phases do not have big differences, especially in the earlier detoxification processes for the three systems. This means that the system with 30 g/l chromium slag has a higher Cr(VI) concentration gradient than the other two, thus leading to a quicker diffusion rate and higher η values. As a result, the kinetics model presented in this work can be applied to roughly estimate the detoxification efficiency in the exponential growth period (2–12 h).

4. Conclusions

Biosorption and bioaccumulation have been applied in Cr(VI) removal from chromium-containing effluents. This paper presented direct detoxification of chromium slag by using microorganisms. Our work showed that a bacterial consortium isolated from chromium slag can efficiently accelerate Cr(VI) leaching rate and remove it. The detoxification efficiency of the three respective strains follows the sequence: strain I > strain III > strain II. The chromate resistant bacterial consortium consists of three strains. The detoxification capability of the bacterial consortium is far stronger than that of the respective strain, showing an excellent synergistic effect. The specific growth rates in pure and chromium-containing medium are 0.1475 and 0.1573 h^{-1}, respectively. The presence of Cr(VI) has little effect on growth rate of the bacterial consortium. Cr(VI) removal takes place in exponential growth period of the bacterial consortium; the detoxification time should be controlled in this phase for every run. The kinetics of detoxification process was studied and can be described as $\eta = 0.0615e^{0.1573t}$, which can be used to predict Cr(VI) removal efficiency.

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