SUPPORTING INFORMATION

Bioremediation of Cr(VI) and immobilization as Cr(III) by *Ochrobactrum anthropi*

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1. The preliminary data related to the experimental design.

The amount of biomass, chromate concentration, the effect of contact time and pH should be considered before conducting the Cr(VI)-cell debris interaction experiments. In order to achieve the maximum reduction of Cr(VI) and the maximum immobilization of Cr(III), three series of experiments were carried out to optimize the conditions as follows.

1.1 The determination of the maximum immobilization capability of *O. anthropi*

For determining the maximum reducing and immobilizing capability of *O. anthropi*, about 0.1 g/mL cells at wet weight were contacted with 10, 50, 100, 200 and 400 mg/L Cr(VI) for 24 h respectively. As shown in Table S1, when the initial concentration of Cr(VI) is between 10-200 mg/L, almost all of the reduced Cr(III) could be immobilized. However, when the initial concentration of Cr(VI) is at 400 mg/L, there is still 168-42=126 mg/L of reduced Cr(III) remaining in the solution. This indicates the maximum immobilization was achieved at this amount of bacteria. We also calculated that the maximum immobilization capability of *O. anthropi* is 23.2 mg/g cell at dry weight.

Table S1. The reduction & immobilization situation of *O. anthropi* living cells *vs.* different initial concentrations of Cr(VI) after interacting for 24 h.

Initial concentration of Cr(VI) (mg/L)	Reduction ratio (%)	Immobilization ratio (%)	Residual Total Cr in supernatant (mg/L)	Residual Cr(VI) in supernatant (mg/L)
10	99.0	90.0	1.0 ±0.008[†]	0.1±0.006
50	99.6	97.6	1.2±0.014	0.2±0.012
100	99.6	98.7	1.3±0.015	0.4±0.016
200	99.9	98.1	3.4±0.018	0.3±0.01
400	89.5	58.0	168±0.017	42±0.015

*The final cell concentration of *O. anthropi* living cells is 0.1 g/mL at wet weight.

[†] The number that follows the ± sign is the standard deviation (s.d.), which was calculated with three times of independent experimental data in this table and following Table S4.

1.2 The determination of the amount of biomass in the reaction system

As showed in Table S2, when the initiation concentration of Cr(VI) was set at 100mg/L and the interaction time was set at 24 h, the reduced Cr(III) can not be effectively immobilized with the concentration of the cell debris is at 0.01, 0.05 or 0.08 g/mL at wet weight. However, once the concentration of the cell debris increases into 0.1 g/mL, almost 100% immobilization could be achieved. Thus for the well understanding of the competitive coordination effect of soluble small molecules to the cell debris, the concentration of cell debris was set at 0.1 g/mL at wet weight.

Table S2. The influences of the concentration of cell debris to the immobilization efficiency

Concentration of the cell debris (g/mL)	0.01	0.05	0.08	**0.1**			0.12
Contact time (h)	24	24	24	**6**	**12**	**24**	24
Reducing ratio* (%)	36.1	72.2	100	63.5	88.4	100	100
Immobilizing Ratio (%)	5.8	35.6	92.5	42.7	84.6	98.7	98.8

*The initial concentrations of Cr(VI) and pH are 100 mg/L and 7.4, respectively.

1.3 The influence of pH.

In order to investigate the influence of pH to the Cr(VI) reduction and Cr(III) immobilization capability of cell debris, experiments were done in the following conditions: pH ranged from 0 to 14, the initial concentrations of Cr(VI) was 200 mg/L, the amount of cell debris was 0.1 g/mL at wet weight and the interacting time was 24 h.

As showed in Fig. S1, the cell debris can be used to reduced Cr(VI) and immobilize Cr(III) at the wide range of pH (from 3.6 to 10), revealing that the *O. anthropi* could be applicable to treat with Cr(VI)-containing wastewater at a wide scope of situations.

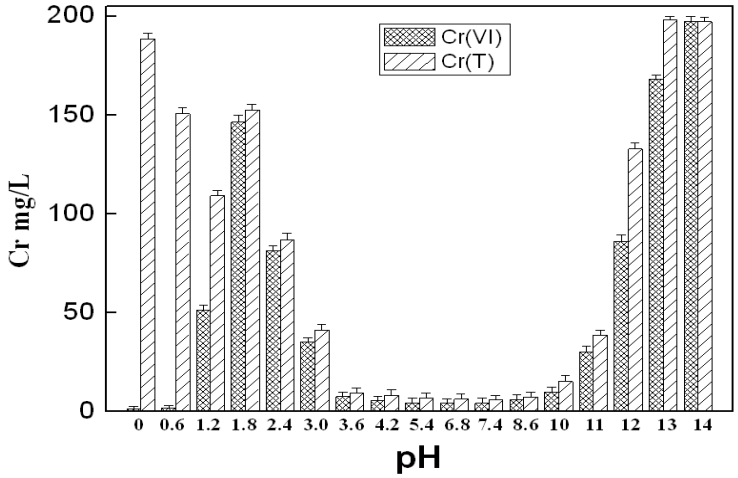

Figure S1. The influence of pH on the Cr(VI) reduction and Cr(III) immobilization capability of the cell debris of *O. anthropi*. The initial concentrations of Cr(VI) was 200 mg/L. The cell debris amount was 0.1 g/mL at wet weight and the interacting time was 24 h. The Cr(T) represents total Cr that remained in the supernatant.

2. The reduction and immobilization efficiency of Cr in different types of culture media.

The general strategy for Cr(VI) treatment by microbes was to reduce the Cr(VI) with the growth of bacteria simultaneously. As shown in Fig. S2a, we found that with the decrease of the concentration of Cr (VI), the total Cr level in the supernatant is constant, which remains almost the same as the initial concentration of Cr(VI). That means the reduced Cr could not be immobilized by the bacteria during the reduction process. If we increase the amount of cell debris into 0.1 g/mL at wet weight, the immobilizing amount of Cr(VI) can increase up to 39 % in LB medium (see Table 1). However, it is still difficult to achieve a full immobilization of the reduced Cr(III) in several types of bacterial culture media.

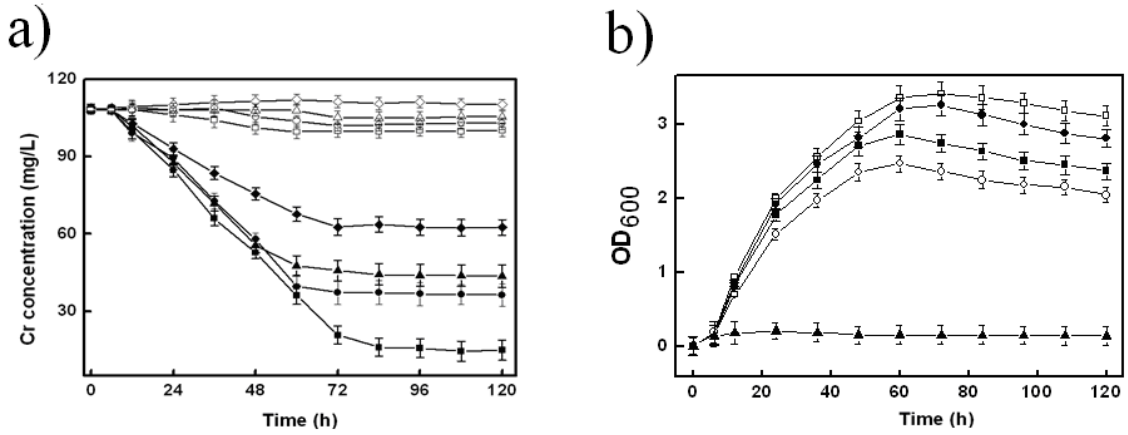

Figure S2. a) The reduction and immobilization efficiency of chromium by living *O. anthropi* cells in four types of bacteria culture media ((■,□) LB medium, (●,○) BT medium, (▲,△) PY medium, (◆,◇) BF medium). Solid symbols refer to Cr(VI) concentration in the solution, while open symbols refer to total Cr concentration. **b)** The determination of the optimal low cost recipe for growing *O. anthropi*. (▲) Original Tofu processing wastewater ; (○) 100% Tofu processing wastewater with pH set to 7.2 ; (■) 25% Tofu processing wastewater (pH7.2); (●) LB medium; (□) 25% Tofu processing wastewater + 1% saccharose (pH7.2).

3. T-test analysis of selective passivation experiments.

T-test was used to analyze the significance of difference of four kinds of passivation treatments. As showed in SI Table S3, there is no significant difference among E, E+A and A+E treatments. However, the p value is less than 0.001 between A and the other three types of treatments. It then addresses that the binding activity of the residual amido and carboxyl functional groups are significantly different with the test difference at the 0.001 level. In another word, the residual amido on the cell debris cannot immobilize Cr(III) after esterification treatment, while the residual carboxyl on the cell debris still have some capability to immobilize Cr(III) after acetylation treatment (about 25%). We speculate that in this situation, the residual carboxyl groups on the cell debris may adopt a chelation binding manner to Cr(III) with two carboxyl groups. Actually, the following competitive coordination experiment also reveal that small molecule with double carboxyl groups could indeed compete with cell debris for binding with Cr(III).

Table S3. T-test statistical results for the comparison of the binding ability of the cell debris to Cr(III) after different passivation treatments

The Comparison of different passivation treatment*	p value	Significance of difference
A and E	8.28×10^{-5}	Yes
A and A+E	2.29×10^{-5}	Yes
A and E+A	2.43×10^{-5}	Yes
E and A+E	0.97776	No
E and E+A	0.65225	No
A+E and E+A	0.72604	No

*A, the amido group was acetylated by the acetic anhydride; E, the carboxyl group was esterificated by acidic methanol; E+A, the cell debris was first esterificated and then acetylated; A+E, the cell debris was first acetylated and then esterificated.

4. The pilot-scale treatment of three types of Cr(VI)-containing wastewater.

4.1 The low cost culturing of *O. Anthropi*.

Organic waste was utilized as nutrition source for growing Cr(VI)-reducing bacteria such as *O. anthropi* and *P. citreus*. As shown in Fig. S2b, *O. anthropi* can grow very well in dilute Tofu (bean curd) processing wastewater containing 1% saccharose as culture medium, which facilitate the large-scale costless culturing of bacteria.

4.2 The pilot-scale treatments.

Based on the proposed strategy with two-step control of the bacteria culture medium, we performed 5 or 50 L pilot scale experiments for dealing with three types of typical Cr(VI)-containing wastewater, with two types of Cr(VI)-reducing bacteria as biomass. The process was shown in Fig. S3.

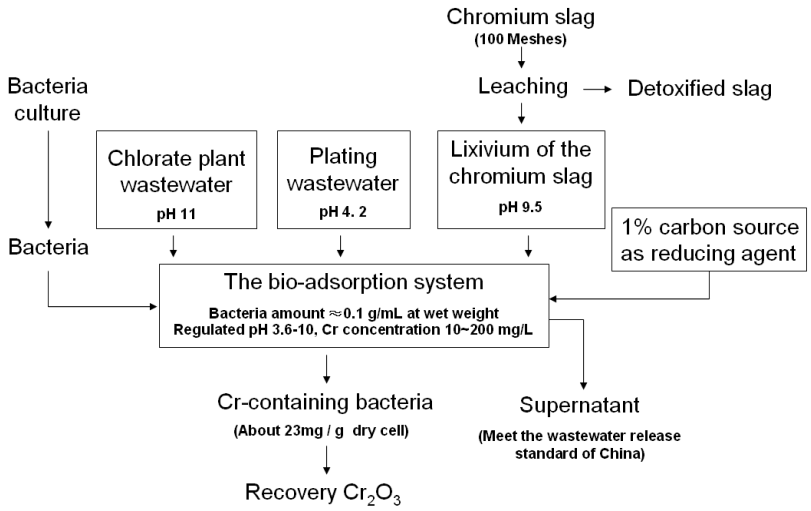

Figure S3. The process of pilot-scale treatment with three types Cr(VI)-containing wastewater.

The cells cultured from organic wastes were collected by filtration, and plunged into three types wastewater with different concentration of Cr(VI), and then 1% saccharose(carbon source) was added. If the pH value of wastewater is beyond the range of 3.6-10, it should be adjusted to 3.6~10 using HCl or NaOH before adding the bacteria. As shown in Table S4, with the co-existing of *O. anthropi* for 24 h, the three types of Cr-contaminated wastewater all met at the wastewater release standard of China (Cr(VI)≤0.5 mg/L, Total Cr≤1.5 mg/L). Another Cr(VI)-reducing bacteria, *P. citreus*, was also tested in 5 L pilot-scale experiment for chlorate plant wastewater, well immobilization of Cr was also achieved (Table S4).

Table S4. The pilot-scale remediation test on three types of classic Cr-contaminated wastewater

Bacteria	Wastewater	Scale (L)	Before treatment			After treatment		
			pH	Cr(VI)	Cr(T) ‡	pH	Cr(VI)	Cr(T)
O. anthropi	Lixivium of the chromium slag*	5	9.5	57.5±2.6	60±2.8	7.2	0.32±0.025	0.74±0.021
		50				7.0	0.35±0.026	0.82±0.023
	Chlorate plant wastewater†	5	11.0	102±3.2	112±2.2	7.5	0.28±0.012	0.68±0.015
		50				7.4	0.34±0.016	0.72±0.014

	Plating wastewater	5	4.2	107±2.4	118±2.4	6.8	0.24±0.012	0.69±0.018
P. citreus	Chlorate plant wastewater	5	11.0	102±3.2	112±2.2	7.6	0.36±0.018	0.72±0.022

***** Solid-liquid ratio is 20:1(W/V) in the leaching of the chromium slag process.

† The pH of chlorate plant wastewater was adjusted to <10 using HCl before adding the bacteria.

‡ The Cr(T) represents total Cr.

4.3 The recycle of Cr.

Since the amorphous Cr (III) could easily be re-oxidized into Cr (VI) in the natural environment, an ideal way to treat Cr(VI) pollution should not only transform it into insoluble form but also extract the Cr (III) from the environment. Here we show the possibility of recycling Cr via calcinating Cr-containing bacteria. We found the organic matter could be removed when we calcinated the Cr-containing bacteria at 900° C for 8 h. The main phase of the left solid powder was identified as Cr_2O_3 by XRD analysis (Fig. S4). We admit that, from the economy point of view, calcination might be an expensive way in real application, owing to the low immobilization efficiency of most bacteria. However, once a biomass with higher immobilization efficiency towards Cr (III) was found, this method will have practical value.

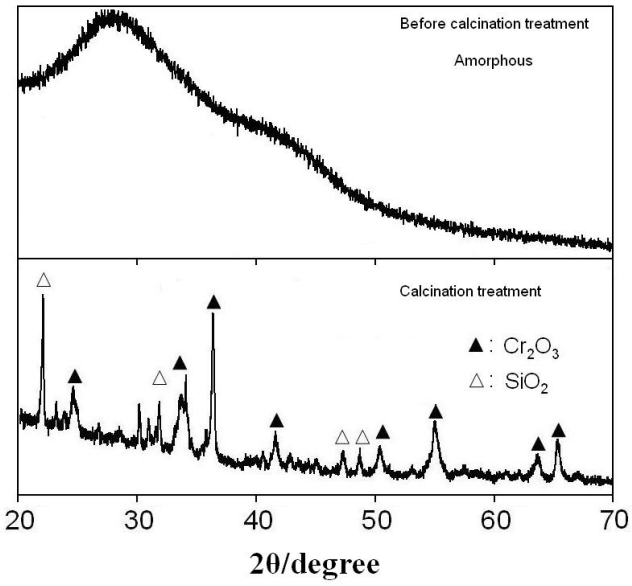

Figure S4. XRD data of the Cr-containing bacteria sample before and after calcination. It reveals that the main phase in the solid is Cr_2O_3 after calcination treatment.