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ScienceDirect
Trans. Nonferrous Met. Soc. China 27(2017) 932−938
Transactions of Nonferrous Metals Society of China
www.tnmsc.cn

Electrochemical response to biomass of bacterial cells of *Achromobacter* sp. CH-1 growth

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Received 30 May 2016; accepted 6 January 2017

Abstract: A simple and effective method has been developed to reflect the growth of bacteria CH-1 via the potential of the solution. The results indicate that during the bacterial cultivation, the biomass increases and the potential of the solution decreases over time. The relationship between biomass and potential of the solution could be expressed by the equation with constants of *a* and *b* which are all related to species, batch, number, and growth environment of bacterial. When the initial pH value is 10 and the initial biomass is 6.55×10^7 cell/mL, the correlated equation of the biomass and the potential of the solution could be divided into two segments. The growth of bacteria CH-1 is different under various experimental conditions, but the biomass is directly related to the potential of the solution regardless of the conditions of different initial pH values and bacteria number.

Key words: electrochemical response; Cr(VI); bacteria CH-1; biomass; potential of solution

1 Introduction

Chromium is one of the major trace heavy metal pollutants in the environment. Chromium in environmental waters typically comes from industrial pollution sources, including tanning factories, steel works, wood preservation and artificial fertilizers [1−4]. It is widely known that the toxicity and biological activity of the element depend on not only the total amount, but also its chemical form. Chromium species exist mainly in two different oxidation states in environmental water: Cr(VI) and Cr(III), which have contrasting physiological effects. Cr(III) is considered as an essential trace element for the maintenance of an effective glucose, lipid, and protein metabolism in mammals. On the other hand, Cr(VI) can be toxic for biological systems and cancerogenic in human beings.

The conventional methods for removal of Cr(VI) from wastewater include chemical precipitation, adsorption, and ion exchange, and so on [5,6]. In our previous works [7−22], the novel technologies for detoxification of chromium-containing slag and for removal of chromium-containing wastewater by microorganism *Achromobacter* sp. CH-1 were put forward, and then the correlated researches have been done.

During the process of bacterial cultivation, it is of great importance to control the growth of bacterial no matter what the relationship between its growth and product composition is. Therefore, the convenient, effective and quick means for the biomass measurement is fundamental to promptly determine the changing tendency of the bacterial growth and to adjust the cell metabolizing in the process of cell cultivation.

Nowadays, the conventional methods used in laboratory to measure the total amounts of bacteria are microscope direct notation, flat colony notation, photoelectric turbidimetry, smear straining method, minim calorimetry, photoluminescence method, electronic autocounter, and centrifugal volumetric method [23]. Moreover, there are other methods to characterize the bacteria amount by using some substances. For instance, the biomass for some special

Foundation item: Projects (51304251, 51504299) supported by the National Natural Science Foundation of China; Project (201509050) supported by Special Program on Environmental Protection for Public Welfare, China
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DOI: 10.1016/S1003-6326(17)60109-2

microorganisms could be estimated according to their specific lipid component contained in the cells. Within the scope of the environmental and ecological investigations, phosphatide is considered as the sign of the microorganism biomass in the sea and the bayou sediment. Electronic impedance methods based on the electrochemistry technology can be used to confirm the growth and breed situation of some bacteria in specific culture medium via testing the electronic impedance continuously. However, in spite of many approaches to measure the growth of cells, there are still many problems in the testing process because of the complexity of the physiological and biochemical characteristics of the cells and levity of the environment. Furthermore, biomass testing methods are still not practically used because their quality and precision are very poor, the in situ testing methods during the manufacture process are not mature yet and the relationship between the cell growth and the other parameters of the physiological and biochemical characteristics and so on is not clear.

Cr(VI)-reducing bacteria are widespread and Cr(VI) reduction occurs under both aerobic and anaerobic conditions [24,25]. During the numerous environmental parameters, the redox potential of the solution plays a very important role in the Cr(VI) reduction process by bacteria, and different bacteria can only reduce the Cr(VI) at different redox potentials in the aqueous solution [26]. During the industrial process, the parameters that can be obtained on-site are scanty and many physiological and biochemical characteristics parameters are still required to be tested. If the correlated relationship between the cell growth and some easily-testing parameters can be found, it is very useful to optimize the operation conditions, to magnify and to design the biochemical process via the changing tendency of the cell concentration reflected from some easily on-site testing parameters.

In this study, in order to characterize the growth situation of bacteria CH-1 via detecting the potential of the solution simply, the relationship between the subtotals of bacteria CH-1 (N) and potential (E) of the solution was established through testing N and E at different time.

2 Experimental

2.1 Bacterial strains

The strain studied in this work was *Achromobacter* sp. by gene sequencing of 16S rRNA and nominated as CH-1 separated from nature, which could reduce soluble and toxic chromate with high concentration to the insoluble and less toxic Cr(III) presented as $Cr(OH)_3$ precipitate in aerobic cultures, no Cr(VI) decreasing was observed in anaerobic cultures. In contrast to other chromium(VI) reducing microorganisms, CH-1 showed higher chromium(VI) reduction rate at high pH value. Reduction performed under alkaline conditions at pH value of 7 to 11, and optimum pH value was 10. The cultivation method was described in Refs. [8−11].

2.2 Medium and hexavalent chromium ions

Medium is composed of 4 g/L carbon source, 4 g/L nitrogen source, and 2 g/L NaCl. Cr(VI) solution with different concentrations of Cr(VI) using $K_2Cr_2O_7$ dissolved in water.

2.3 Analysis methods

Achromobacter sp. CH-1 was cultivated in a horizontal shaker with a speed of 120 r/min at 30 °C. Then, the cell number and solution potentials were determined after being cultivated for 2, 4, 6, 8, 10, and 12 h, respectively.

Cell number was monitored by microscope. The pH value was measured with LP115 pH meter. The set-up of potential measurement system is described in Fig. 1,

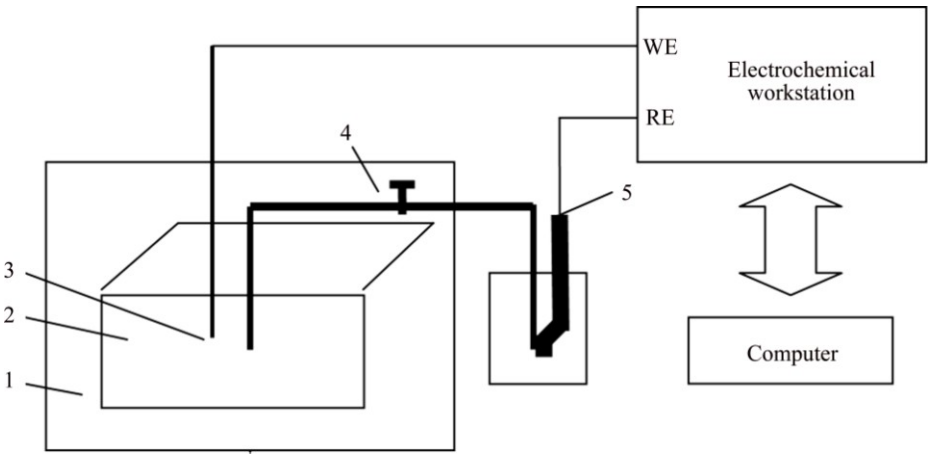

Fig. 1 Set-up of potential measurement system: 1—Water bath; 2—Electrobath; 3—Plantinum electrode (WE); 4—Salt bridge; 5—Reference electrode (RE)

which consisted of a polymethyl methacrylate electrobath with the sizes of 100 cm × 50 cm × 50 cm, a saturated calomel electrode (SCE) as a reference electrode, a platinum electrode as a working electrode, a water bath, an electrochemical workstation and a computer system. SCE and platinum electrode were connected with the electrochemical workstation (CHI660A) monitored by the computer. The solution potential was represented by the open circuit potential between SCE and the platinum electrode. All potentials in this work were expressed versus the potential of SCE. All experiments had three replicates.

3 Results and discussion

3.1 Relationship between ln *N* and *E*

The results of our previous research indicated that the optimal ability of reducing Cr(VI) in alkaline media by bacteria CH-1 could be obtained under the condition of initial pH 10, temperature 30 °C and the initial bacteria inoculation amount 20% (volume fraction). The bacteria CH-1 was cultivated under the optimal condition in this study. During the experimental process, the cell number (N) of the samples and the potential (E) of the solution were determined at regular intervals. The experimental results are listed in Table 1.

Table 1 Bacteria biomass and changes of solution potential

Time/h	E/mV	N/(10^7cell·mL^{-1})	ln[N/(cell·mL^{-1})]
0	−72.6	6.55	18.00
2	−75.1	7.80	18.17
4	−85.5	14.00	18.76
6	−98.2	25.50	19.36
8	−112.9	49.50	20.02
10	−130.7	84.50	20.55
12	−140.9	108.00	20.80
24	−200.0	380.00	22.06

As shown in Table 1, during the process of cell cultivation, the cell concentration increases and potential of the solution decreases over time. The relationship between the potential of the solution and logarithm of cells number (ln N) could be fitted in three ways, as shown in Fig. 2, Fig. 3 and Fig. 4, respectively. And the fitted equations are as follows:

$$-E=-467.56948+29.52356\ln N \quad (R=0.97487) \tag{1}$$

$$-E=1645.19916-183.75817\ln N+5.35867(\ln N)^2 \quad (R=0.99901) \tag{2}$$

$$-E=-3751.41836+628.07733\ln N-35.23523(\ln N)^2+0.6747(\ln N)^3 \quad (R=0.99975) \tag{3}$$

Obviously, according to the fitted line and the correlated coefficients, the liner correlation of the simple fitted equation is not satisfactory (Fig. 2), the quadratic fitted equation is good enough (Fig. 3), and the cubic fitted equation is optimal (Fig. 4). However, the cubic fitted equation is too complex to explain the physical significance of the constant and the coefficients.

Combining the characteristics of microorganism growth process with the physical significance of slope and intercept of the fitted line, the following fitted way (Fig. 5) is more preferable.

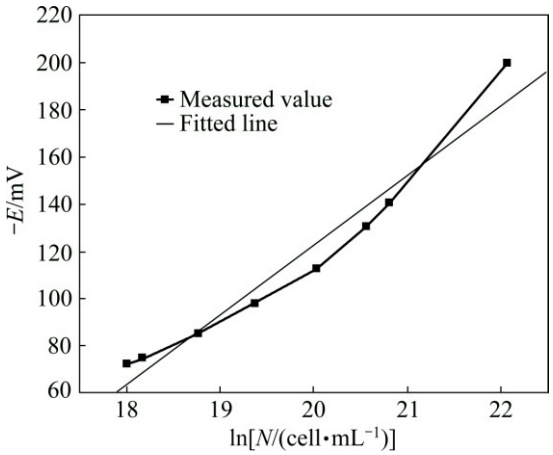

Fig. 2 Simple fitted curve of ln N−E

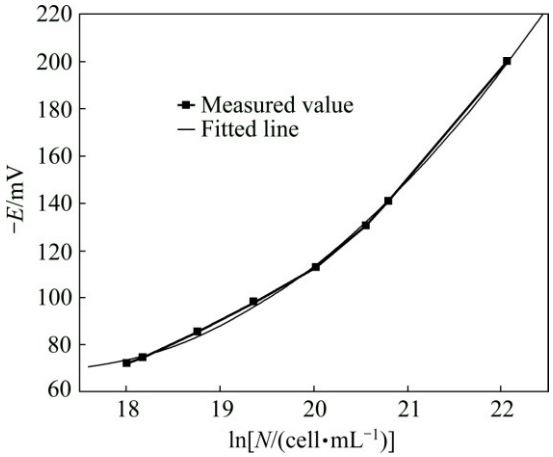

Fig. 3 Quadratic fitted curve of ln N−E

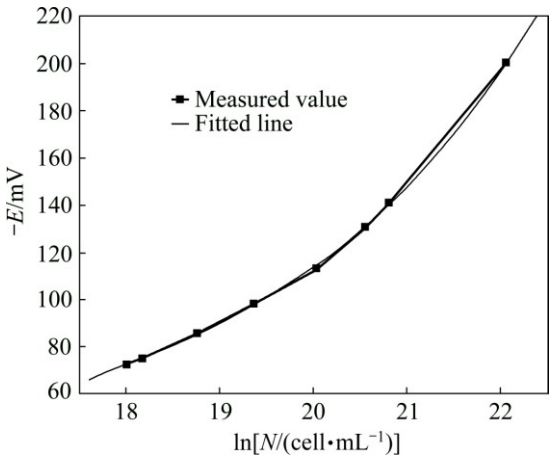

Fig. 4 Cubic fitted curve of ln N−E

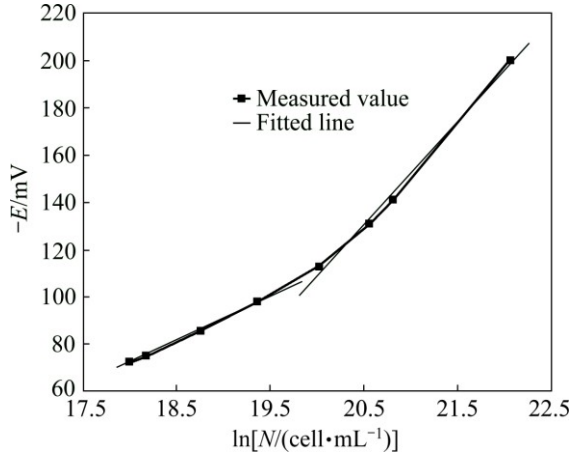

Fig. 5 Relationship between ln N and E

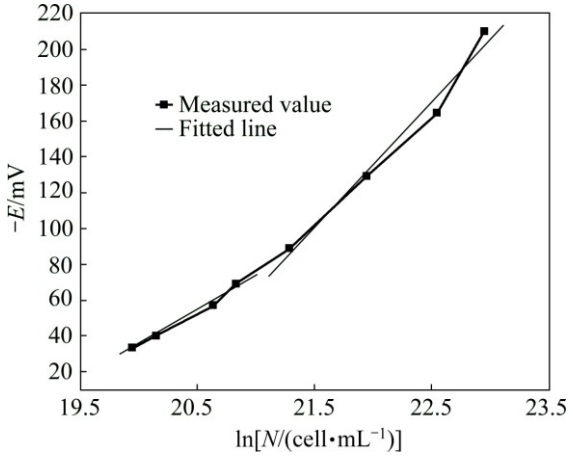

Fig. 6 Validation of relationship between ln N and E at optimized condition

It can be seen from Fig. 5 that with the growth of bacteria, the potential of the solution decreases slowly at the beginning and then speeds up later, which is similar to the growth curve of bacteria. The bacteria grow slowly in the lag phase while the potential of the solution decreases. However, in the logarithm phase, the bacteria grow rapidly and correspondingly the potential of the solution decreases rapidly. It can be analyzed from the two segments of Fig. 5 that the potential of the solution and the logarithm of cells are linear correlativity. The linear equation of former segment can be expressed as follows:

$$-E = -267.04+18.84\times\ln N \ (R=0.9983) \quad (4)$$

The linear equation of latter segment is

$$-E = -761.03+43.49\times\ln N \ (R=0.9972) \quad (5)$$

3.2 Validation of relationship between ln *N* and *E*

The bacteria CH-1 cultivation experiment under the optimal conditions of pH 10 and bacteria inoculation amount 20% was repeated to validate the relationship between the potential of the solution and the logarithm of cells. The results are shown in Table 2 and Fig. 6.

Table 2 Bacteria growth and changes of solution potential at optimized condition

Time/h	E/mV	N/(10^8cell·mL^{-1})	ln N
0	−33.4	4.55	19.94
2	−40.3	5.60	20.14
4	−57.2	9.15	20.63
6	−69.2	11.10	20.83
8	−88.8	17.50	21.28
10	−129.6	33.80	21.94
12	−164.3	61.50	22.54
24	−210.0	92.40	22.95

It can be concluded from the two segments of Fig. 6 that the potential of the solution and the logarithm of cells are also linear correlativity. The linear equation of former segment is

$$-E = -742.76+38.89\times\ln N \ (R=0.9940) \quad (6)$$

The linear equation of latter segment is

$$-E = -1404.26+70.00\times\ln N \ (R=0.9898) \quad (7)$$

Obviously, Eqs. (4)−(7) are of great difference under the same experimental conditions. The reason is that the activity of the bacteria is different in different batches. However, the linear correlativity between the potential of the solution and the biomass is similar, which can be described as

$$-E = a + b\ln N \quad (8)$$

where a is the electric charge quantity of unit cell concentration, b is the effect degree of cell concentration on electric charge quantity.

It can be seen from Eq. (8) that with the value of a becoming more negative, the electric charge quantity of unit cell concentration increases and the activity and reduction ability of cell becomes stronger; with the value of b increasing, the effect degree of cell concentration on electric charge quantity increases and the activity of cell becomes better. Both a and b are related to the species of bacteria, batch, amount and the growth environment of bacteria.

Although every experimental conditions and bacteria growth situation are different, the relationship between the potential of the solution and the biomass is similar. Therefore, we can gain the bacteria growth situation in site in the industrial process by measuring the potential of the solution and the biomass in every batch experiment and then calculating the constants a and b in the correlation equation.

3.3 Effect of initial bacteria inoculation amounts on relationship between ln *N* and *E*

Cultivating the bacteria CH-1 under the optimal conditions, the experiments were conducted under the conditions of same bacteria batch and initial pH value but different initial bacteria inoculation amounts.

Figures 7 and 8 show the relationship between the potential of the solution and the logarithm of cells with the initial bacteria inoculation amounts of 2.03×10^8 and 6.6×10^8 cell/mL. It can be deduced that the linear equations of the latter segments can be respectively expressed as follows:

$$-E=-1505.51+78.67\times\ln N \quad (R=0.9932) \tag{9}$$

$$-E=-1787.08+89.76\times\ln N \quad (R=0.9928) \tag{10}$$

Equations (9) and (10) illustrate that the initial bacteria inoculation amount has a little effect on the relationship between the potential of the solution and the logarithm of cells under the condition of same bacteria batch, pH value and other experimental conditions.

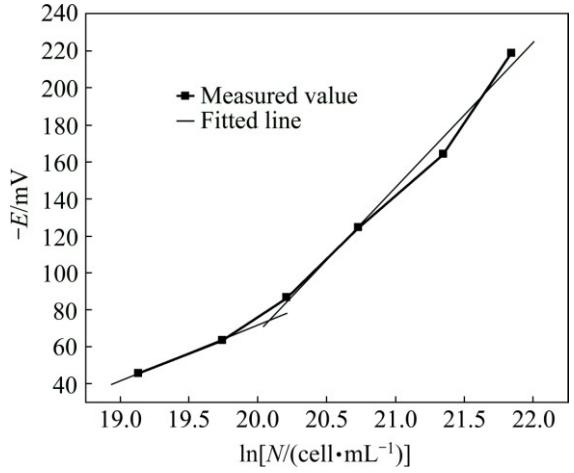

Fig. 7 Relationship between ln *N* and *E* with initial biomass of 2.03×10^8 cell/mL

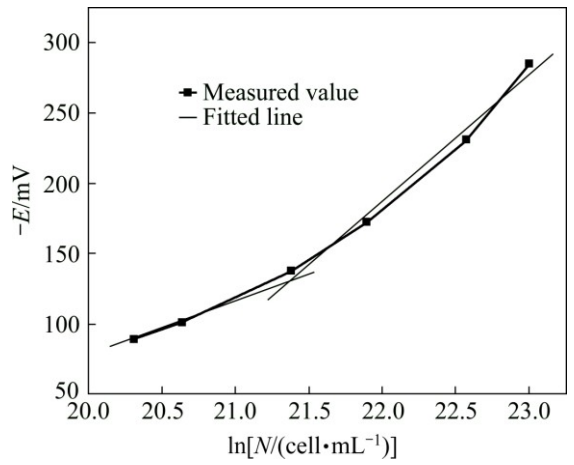

Fig. 8 Relationship between ln *N* and *E* with initial biomass of 6.6×10^8 cell/mL

3.4 Effect of initial pH values on relationship between ln *N* and *E*

The effects of the initial pH value on the relationship between the potential of the solution and the logarithm of cells are shown in Figs. 9 and 10.

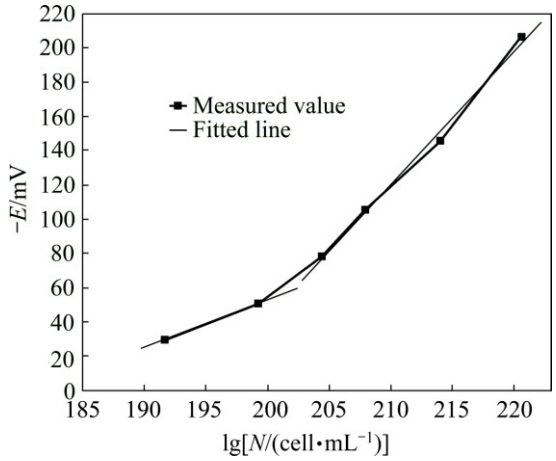

Fig. 9 Relationship between ln *N* and *E* at pH of 9

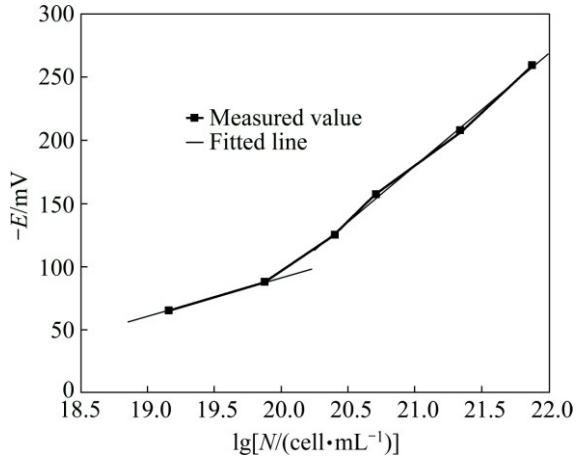

Fig. 10 Relationship between ln *N* and *E* at pH of 11

Similarly, the linear equations of the latter segments are

$$-E=-1511.81+77.72\times\ln N \quad (R=0.9974) \tag{11}$$

$$-E=-1690.28+89.04\times\ln N \quad (R=0.9988) \tag{12}$$

Equations (11) and (12) show that the initial pH value has a little effect on the relationship between the potential of the solution and the logarithm of cells under the conditions of same bacteria batch, initial bacteria inoculation amount and other experimental conditions. The values of *a* and *b* in Eqs. (11) and (12) reveal that the bacteria activity is better when the initial pH value is 11 than that of 9.

4 Conclusions

1) During the process of cell cultivation, the cell concentration increases and the potential of the solution

decreases with time course. There is a linear correlativity between the potential of the solution and the logarithm of cells when cultivating the bacteria CH-1 at the initial pH of 10 and bacteria inoculation amount of 6.55×10^7 cell/mL. The linear equation of the former segment is $-E=-267.04+18.84\times\ln N$ with R of 0.9983, the latter segment is $-E=-761.03+43.49\times\ln N$ with R of 0.9972.

2) The linear correlativity of the potential of the solution and the biomass is $-E=a+b\ln N$. Both a and b are related to the species of bacteria, batch, amount and the growth environment of bacteria, which are the key science and technology question because of their profound physical meaning.

3) Initial bacteria amount and initial pH value have a little effect on the relationship between the potential of the solution and the logarithm of cells. The bacteria activity is better when the initial pH value is 11 than that of 9.

4) Determination of the potential of the solution could quickly and simply response bacteria activity and growth of the system, and then the optimal controlling and adjusting means could be adopted to the detoxification process.

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Cr(VI)还原菌 *Achromobacter* sp. CH-1 生长量的电化学响应

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摘 要：溶液电势可简单有效地表征碱性介质中Cr(VI) 还原菌 *Achromobacter* sp. CH-1 的生长情况。*Achromobacter* sp. CH-1 培养过程中，溶液电势随着细菌数的增加而降低，溶液电势与细菌数对数呈线性相关，线性方程中常数 *a*、*b* 与细菌的种类、批量、数量和细菌的生长环境有关。初始 pH 为 10、初始细菌数为 6.55×10^7 cell/mL 时培养 CH-1 菌，溶液电势与细菌数的对数值的相关曲线可分两段表征。不同初始 pH 与不同细菌数条件下 CH-1 菌的生长情况不同，但细菌的生长量与溶液电势直接相关。

关键词：电化学响应；Cr(VI)；还原菌 *Achromobacter* sp. CH-1；生物量；溶液电势

(Edited by Xiang-qun LI)