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Potential-pH diagram for "*Leucobacter* sp. Ch-1–Cr–H_2O" system

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Abstract

Laboratory experiments were used to investigate the effects of initial pH and applied potential on the growth of *Leucobacter* sp. Ch-1 and Cr (VI) reduction and establish the pH–Eh diagram for *Leucobacter* sp. Ch-1–Cr–H_2O system. The results showed that the preferred initial pH for *Leucobacter* sp. Ch-1 growth was from 7.0 to 11.0. At this pH range, 56.2–99.5% of Cr (VI) was reduced by Ch-1 strain. The applied potentials from −700 to 0 mV, from −800 to +300 mV and from −800 to +400 mV at 7.0, 9.0 and 11.0 of initial pH values were favorable for the bacterial growth. The corresponding ranges of applied potentials for bio-reduction of Cr (VI) were from −200 to 0 mV, from −800 to +200 mV, and from −700 to +100 mV at above initial pH values. In the potential-pH diagram, the region of initial pH and Eh for Cr (VI) bio-reduction was included in the region for *Leucobacter* sp. Ch-1 growth and the stable region of $Cr(OH)_3$, which implied that Cr (VI) could be reduced to trivalent chromium existing in the forms of $Cr(OH)_3$ precipitate under the presence of *Leucobacter* sp. Ch-1 in alkaline condition. The results suggest that *Leucobacter* sp. Ch-1 has potential application for remediation of Cr (VI) contamination sites.

Keywords: Cr (VI); *Leucobacter* sp. Ch-1; pH; Applied potential; Potential-pH diagram

1. Introduction

The potential-pH diagram of "bacteria–metals–H_2O system" can provide a fundamental for bio-hydrometallurgy and bio-beneficiation. In fact, bioleaching of ores is also an electrochemical corrosion process. Whether the reaction of bacteria with ores is direct or indirect, it is closely related to the pH value and the potential of bioleaching system. The potential-pH diagrams express thermodynamic balance of reactions and thermodynamic stability of compounds. Furthermore, the potential-pH diagram of bacteria–metals–H_2O system can reflect the areas of bacteria activity, corrosion and stable region of ores and the products during the leaching of ores.

Becking et al. [1] reported the potential-pH diagram of *Thiobacillus ferrooxidans* growth. He pointed out that the optimum potential for this bacteria strain was between −400 and 800 mV, and pH value was between 1.5 and 3.0. Natarajan graphed the potential-pH diagram for "bacterial–copper pyrites–H_2O" system [2]. In addition, the potential-pH diagram for "bacteria–arsenopyrite–H_2O" system was reported to study the thermodynamics of bioleaching of arsenopyrite [3]. The changes of irons, arsenic and sulphur during the leaching of arsenopyrite, the bacteria activity areas, the stable existing areas of the products of ferric arsenate and sulphur during the bioleaching process were particularly pointed out.

Recently, bacteria were widely used in detoxification of heavy metal in metals-containing slag, particularly in chromium-containing slag [4–6]. In China, the accumulated amount of chromium-containing slag from metallurgical and chemical industries over a span of more than 30 years was more than 6 million tons, and 200–300 thousand tons are being discharged annually. Lack of appropriate disposal facilities had led to serious water and soil pollution. As reported in the previous literature, chemical stabilization and physical removal were documented for detoxification of chromium-containing slag [7]. However, these methods were not widely explored due to high cost and incomplete Cr (VI) removal. In recent years, more attentions were put on microbial method for Cr (VI) removal. Numerous species of bacteria having chromate reduction ability have been well documented, including *Acidithiobacillus*, *Bacillus*, *Enterobacter*, *Escherichia*, *Pseudomonas*, and *Shewanella* [8–13]. In our previous research, one novel strain *Leucobacter* sp. Ch-1 was isolated from chromate slag for detoxification of chromium-containing slag [14]. In order to obtain the highest

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reduction efficiency of Cr (VI), it is important to understand the effects of environmental conditions on Cr (VI) reduction by this bacteria strain. The hypotheses are that *Leucobacter* sp. Ch-1 growth and bio-reduction of Cr (VI) are dependent on pH and potential in environmental medium, and the regions of pH and Eh for Cr (VI) bio-reduction are different from that for chemical reduction. The outcome would help to provide the knowledge of thermodynamics of Cr (VI) reduction by bacteria. Therefore, the objectives of this study were to (i) investigate the effects of pH and applied potential on the growth of *Leucobacter* sp. Ch-1 and Cr (VI) reduction; (ii) determine the appropriate pH and Eh ranges; for Cr (VI) bio-reduction (iii) establish the pH–Eh diagram for *Leucobacter* sp. Ch-1–Cr–H_2O system.

2. Materials and methods

2.1. Bacterial strains

The strain was isolated from chromium-containing slag in Changsha, China, which was identified as *Leucobacter* sp. Ch-1 by gene sequencing of 16S rRNA and nominated as Ch-1. The 16S rDNA sequence was deposited at GenBank with accession no. EF 362778. The isolated *Leucobacter* sp. Ch-1 was inoculated in modified Luria Broth (LB) medium containing 10 g tryptone, 10 g NaCl, 5 g yeast extract, 0.1 g glucose and 4 g sodium lactate in 1 L distilled water at pH 9.0. The medium was autoclaved at 121 °C for 18 min. The cultured *Leucobacter* sp. Ch-1 was kept in a refrigerator at 4 °C prior to experiment.

2.2. Effect of pH on the growth of Leucobacter sp. Ch-1 and Cr (VI) reduction

For the experiment of *Leucobacter* sp. Ch-1 growth, the pH value of Luria Broth medium was adjusted to 5.0–12.0 using 1 mol L^{-1} NaOH and 1 mol L^{-1} HCl. *Leucobacter* sp. strain grown overnight was inoculated into the above culture medium and incubated at 30 °C by shaking with a speed of 150 rpm under aerobic conditions. Pure culture medium was used as control under the same conditions. After 2, 4, 7, 9, 12, 15, 18, 21, 23, 24, 28 and 36 h, suspension in each sample was taken under aseptic conditions and the cell number were enumerated. Each treatment had three replicates.

For the experiment of Cr (VI) reduction, Luria Broth medium was amended with 940 mg L^{-1} Cr (VI), the rest of the procedure was the same as described for *Leucobacter* sp. Ch-1 growth. At each sampling date, suspension in each sample was taken and Cr (VI) concentration was determined.

2.3. Effects of applied potential on the growth of Leucobacter sp. Ch-1 and Cr (VI) reduction

The culture medium inoculated with *Leucobacter* sp. strain grown overnight was put into a U-type glass notch in a water bath with 30 °C of constant temperature. The pH value of medium was adjusted to 7.0, 9.0 and 11.0 using 1 mol L^{-1} NaOH and 1 mol L^{-1} HCl. The applied potentials from −900 to +400 mV were imposed on the culture medium by a set of apparatus as described in Fig. 1. A control without applied potential was identically carried out. After incubation for 12 h, suspension in each sample was taken and the cell number was counted. Each treatment had three replicates.

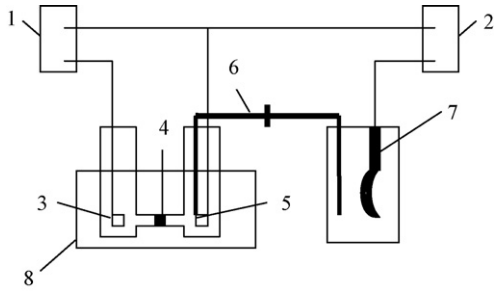

Fig. 1. Sketch of experimental apparatus by applied potentials. (1) voltage regulator, (2) potentiometer, (3) auxiliary electrode, (4) dissepiment, (5) working electrode, (6) salt bridge, (7) reference electrode and (8) constant temperature water tank.

In the experiment of Cr (VI) reduction, Luria Broth medium was amended with 120–140 mg L^{-1} chromium (VI), the rest of the procedure was the same as described for *Leucobacter* sp. Ch-1 growth as affected by applied potential. After 12 h incubation, Cr (VI) concentration was determined in suspension.

2.4. Analytical methods

2.4.1. Chromium (VI) concentration

The concentration of Cr (VI) was determined using diphenylcarbazide (DPC) method as described by Pattanapipitpaisal et al. [15]. A 0.1 mL of supernatant solution was diluted with water, made a 50 mL of volume, and then mixed with 2.0 mL of DPC and 0.5 mL of H_2SO_4 solution (1:1 v/v). After 10 min, absorbance at 540 nm was determined with a spectrophotometer (Hitachi U2010).

2.4.2. Cell numbers

For cell count supernatant containing bacterial cells was diluted into a series of dilution sequences (1 mL supernatant was added into 9 mL distilled water). A 0.01 mL of diluted sequence was inserted into the narrow slot between plate of haematimeter and a thin glass plate. The bacteria suspension was permeated into the counting chamber of haematimeter. After 2 min, the number of cells was counted with a microscope (Nikon, DXM1200). The counting procedure was carried out at a clean bench.

2.4.3. pH and potential

The pH value was measured with LP115 pH meter. The solution potential was measured with platinum electrode and a saturation calomel electrode was used as the reference electrode. All potentials in this paper were expressed versus the potential of saturation calomel electrode (SCE).

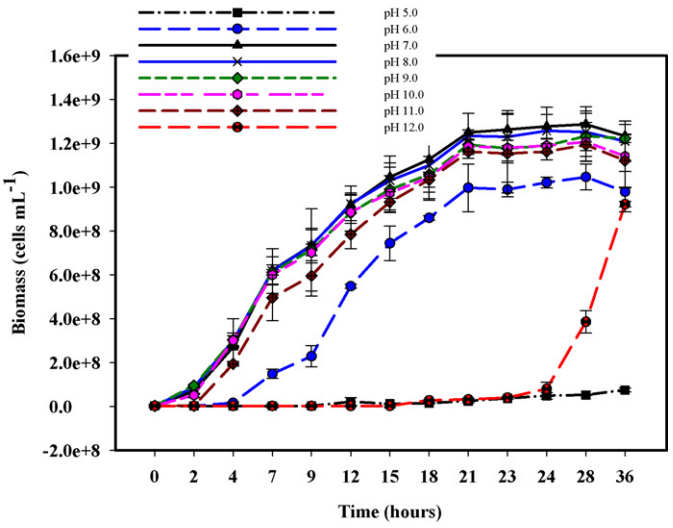

Fig. 2. Effect of initial pH values on the growth of *Leucobacter* sp. Ch-1.

3. Results and discussion

3.1. Effect of initial pH values on growth of Leucobacter sp. Ch-1 and reduction of Cr (VI)

Effect of initial pH values on growth of *Leucobacter* sp. Ch-1 is shown in Fig. 2. The initial pH values in culture medium were 5.0–12.0. When pH was 5.0, there were minor variations in biomass of *Leucobacter* sp. Ch-1 during the whole experimental procedure. Reversely, the number of Ch-1 cell rapidly increased before 15 h incubation at other pH values except for pH 5.0 and 12.0. Thereafter, cell number elevated gradually and the maximum cell number was found after 28 h incubation. Acid condition (pH lower than 7.0) showed lower cell number than that of neutral or alkaline condition except for pH 12.0 at all sampling dates. At pH 12.0, a low number *Leucobacter* sp. Ch-1 cell was obtained before 24 h. Thereafter, cell number linearly grew up until at the end of experiment. In this study, *Leucobacter* sp. Ch-1 was isolated from alkaline chromium-containing slag, but its pH value was lower than 11.0. Under extremely high pH condition (pH 12.0), there was long lag phase for *Leucobacter* sp. Ch-1.

Fig. 3 presents the changes of Cr (VI) concentration at different initial pH values. The present study, the Cr (VI) concentration in pure LB medium without *Leucobacter* sp. Ch-1 inoculation maintained consistent during the whole incubation procedure, indicating no Cr (VI) removal caused by the culture medium. It was noted that Cr (VI) removal in solution by *Leucobacter* sp. Ch-1 was dependent on the initial pH in culture medium. At pH 6.0 and 12.0, Cr (VI) concentration in solution did not differed within 32 h. However, Cr (VI) concentration remaining in solution gradually decreased from 940 mg L^{-1} at the beginning to 412, 163, 131, 5, 195 mg L^{-1} after 32 h incubation at pH 7.0, 8.0, 9.0, 10.0, 11.0, respectively. The corresponding removal efficiencies of Cr (VI) at above pH values were 56.2, 82.7, 86.0, 99.5, and 79.3%. At the end of experiment, initial pH 10.0 showed a significantly ($p < 0.05$) high Cr (VI) removal followed by pH 9.0, 8.0, 11.0 and 7.0, while significantly ($p < 0.05$) low Cr (VI) removal was found under initial pH 5.0, 6.0 and 12.0. The initial pH value for complete removal of Cr (VI) was 10.0 after 28 h incubation. With disappear of Cr (VI) in solution, the blue precipitation was found in alkaline solution in the present study. The image of scanning electron microscopy demonstrated that the precipitation exhibited a morphology structure with intimate, compact and non-crystal structure [16]. In our previous study, the electron paramagnetic resonance pattern of the precipitation was consistent with $CrCl_3$ [17]. Generally, trivalent Cr exists in alkaline solution as $Cr(OH)_3$. Thus, Cr (VI) removal by *Leucobacter* sp. Ch-1 could be regarded as Cr (VI) bio-reduction.

In the present experiment, *Leucobacter* sp. Ch-1 was isolated from chromium-containing slag with pH value between 9.0 and 11.0. Therefore, Ch-1 was an alkaliphile. Probably alkaline medium catalyzed reduction of Cr (VI) by *Leucobacter* sp. Ch-1. In previous studies, bacterial reduction of Cr (VI) under alkaline conditions like *Leucobacter* sp. Ch-1 was not well documented. Wang et al. have demonstrated that reduction of chromium in *Enterobacter* strain occurred at pH 6.5–8.5 and was strongly inhibited at pH 5.0 and 9.0 [18]. Urvashi and Datta reported that the maximum growth of *Ochrobactrum* sp. and chromate reduction was found at pH 7.0 [19].

3.2. Effect of applied potentials on growth of Leucobacter sp. Ch-1

At the initial pH of 7.0, +100 and +200 mV (SCE) of applied potential more or less restrained the bacteria's growth (Table 1). This inhibitory effect was more significant under +200 mV (SCE) of applied potential ($p < 0.05$). The number of *Leucobacter* sp. Ch-1 cell under +200 mV (SCE) declined by 55% as compared with control treatment (without applied potential). Reversely, when applied potential was controlled at −600 mV, the biomass increased from 1.02×10^8 cells mL^{-1} of initial cell number to 6.95×10^9 cells mL^{-1} after 12 h incubation. The applied potential of −600 mV showed 54% more cell number than that of without applied potential. But this stimulated influ-

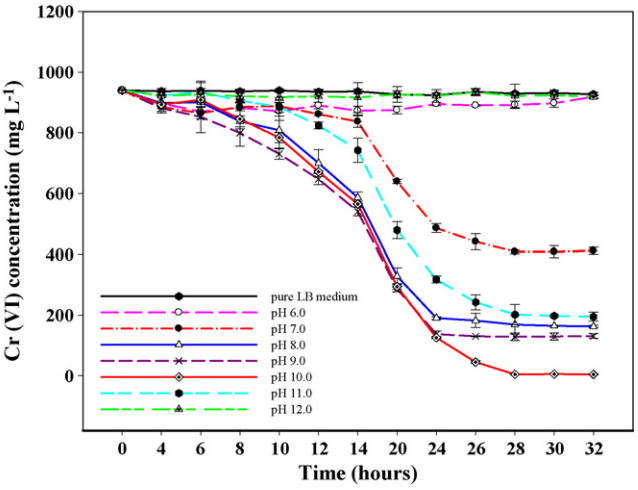

Fig. 3. Effect of initial pH value on reduction of Cr (VI) by *Leucobacter* sp. Ch-1.

Table 1
Effects of applied potentials on the growth of *Leucobacter* sp. Ch-1 at different initial pH values

Initial pH value	Eh (mV)	Initial biomass (cells mL^{-1})	12 h biomass without applied potential (cells mL^{-1})	12 h biomass with applied potential (cells mL^{-1})	Paired *t*-test* (*p* value)
pH = 7.0	−600	1.02×10^8	4.50×10^9	6.95×10^9	0.054
	−700	1.76×10^8	7.06×10^9	7.24×10^9	0.250
	−800	2.53×10^8	9.40×10^9	7.85×10^9	0.0001
	+100	2.97×10^8	8.25×10^9	8.09×10^9	0.593
	+200	4.32×10^8	2.17×10^9	9.86×10^8	0.023
pH = 9.0	−700	3.00×10^8	1.90×10^9	2.85×10^9	0.067
	−800	3.75×10^8	1.85×10^9	1.91×10^9	0.122
	−900	2.40×10^8	3.76×10^9	1.17×10^9	0.006
	+200	3.55×10^8	9.50×10^8	2.00×10^9	0.083
	+300	3.25×10^8	3.55×10^9	3.75×10^9	0.993
	+400	3.90×10^8	2.15×10^9	1.50×10^9	0.026
pH = 11.0	−700	2.53×10^8	6.75×10^9	7.55×10^9	0.0009
	−800	3.09×10^8	4.06×10^9	4.11×10^9	0.528
	−900	4.62×10^8	6.67×10^9	3.39×10^9	0.018
	+300	3.30×10^8	9.50×10^8	3.20×10^9	0.198
	+400	3.35×10^8	1.00×10^9	2.20×10^9	0.026
	+500	4.81×10^8	5.44×10^9	3.57×10^9	0.053

* Paired *t*-test between applied potential and without applied potential under each potential.

ence of negative applied potentials on cell growth was gradually impaired with decreasing applied potentials. Moreover, a significantly inhibitory influence of applied potential on *Leucobacter* sp. Ch-1 cell growth was obtained at −800 mV (SCE) ($p < 0.01$). The results revealed that applied potentials from −700 to 0 mV could be favorable for the bacteria's activity, and it was suitable for the bacteria's growth.

The similar influences of applied potential on the growth of *Leucobacter* sp. Ch-1 cell were obtained at initial pH 9.0 and 11.0 (Table 1). But the potential ranges for Ch-1 strain growth at initial pH 9.0 and 11.0 were wider than that at pH 7.0. For example, at initial pH 11.0, the favorable potential for *Leucobacter* sp. Ch-1 cell growth was from −800 to +400 mV, while the corresponding value at initial pH 7.0 was from −700 to 0 mV.

3.3. Effect of applied potentials on reduction of Cr (VI)

The Cr (VI) reduction in solution at initial pH 7.0 is shown in Fig. 4. At −200 mV of applied potential, the concentration of Cr (VI) in culture medium inoculated with *Leucobacter* sp. Ch-1 rapidly decreased from 140 mg L^{-1} at the beginning to 27 mg L^{-1} after 12 h. As compared with without applied potential, −200 mV of applied potential slightly declined Cr (VI) concentration. However, the difference of Cr (VI) remaining in solution between above treatments was not significant. Conversely, at −300, +100 and +200 mV, Cr (VI) concentrations in the applied potential treatments were significantly ($p < 0.05$) higher than that of without applied potential treatments, although Cr (VI) concentration in all treatments were lower than the initial concentration. Therefore the range of applied potentials for Cr (VI) reduction by *Leucobacter* sp. Ch-1 was from −200 to 0 mV at the initial pH 7.0.

In Fig. 5, the changes of Cr (VI) concentration in solution with initial pH 9.0 under different potential is shown. When *Leucobacter* sp. Ch-1 was inoculated, Cr (VI) reduction was observed under both applied potential and without applied potential because Cr (VI) concentration decreased from 128–132 mg L^{-1} at the initial values to 18–58 mg L^{-1} after 12 h. Moreover, Cr (VI) concentration under −700 mV applied potential was significantly ($\rho < 0.05$) lower than that of without applied potential. A slightly decreased Cr (VI) concentration was also found at −800 mV applied potential. However, applying +300 mV of positive potential and −900 mV of negative potential resulted in significantly ($\rho < 0.05$) inhibitory influences for Cr (VI) reduction. The results indicated that the favorable range of applied potentials for the reduction of Cr (VI) by *Leucobacter* sp. Ch-1 was from −800 to +200 mV when the initial pH value was controlled at 9.0.

As shown in Fig. 6, between −700 and +100 mV, there were no significant difference of Cr (VI) concentration between the

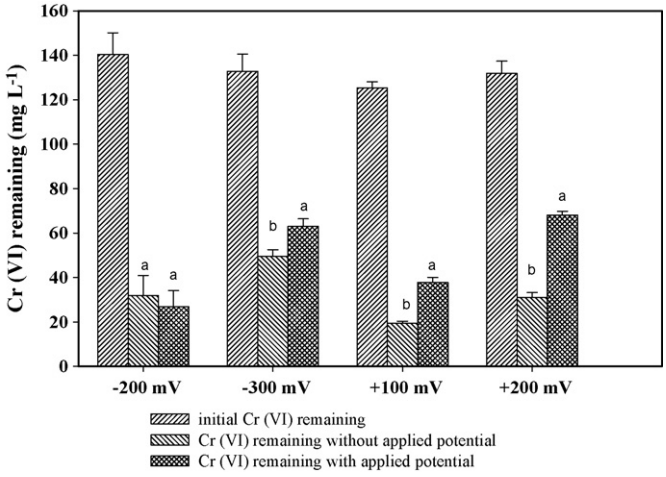

Fig. 4. Effect of applied potentials on Cr (VI) reduction by *Leucobacter* sp. Ch-1 at the initial pH 7.0 (values followed by different letters are significantly different under each applied potential at $p < 0.05$ using paired *t*-test).

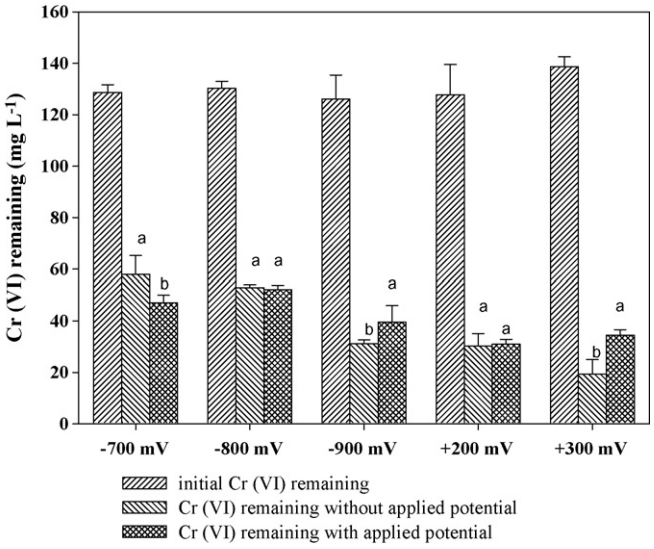

Fig. 5. Effect of applied potentials on Cr (VI) reduction by *Leucobacter* sp. Ch-1 at the initial pH 9.0 (values followed by different letters are significantly different under each applied potential at $p < 0.05$ using paired *t*-test).

treatments with applied potential and without applied potential in solution at pH 11.0, indicating minor influence of applied potential on Cr (VI) reduction by *Leucobacter* sp. Ch-1 during above potential range. Conversely, +200 and −800 mV of applied potential exhibited higher Cr (VI) concentrations in solution as compared with control treatment (without applied potential). The results suggested that favorable potential for Cr (VI) reduction was from −700 to +100 mV at initial pH 11.0.

3.4. The range of initial pH and Eh for Leucobacter sp. Ch-1 growth and Cr (VI) reduction

Based on the cell number of *Leucobacter* sp. Ch-1 and Cr (VI) concentration remaining in solution at different initial pH

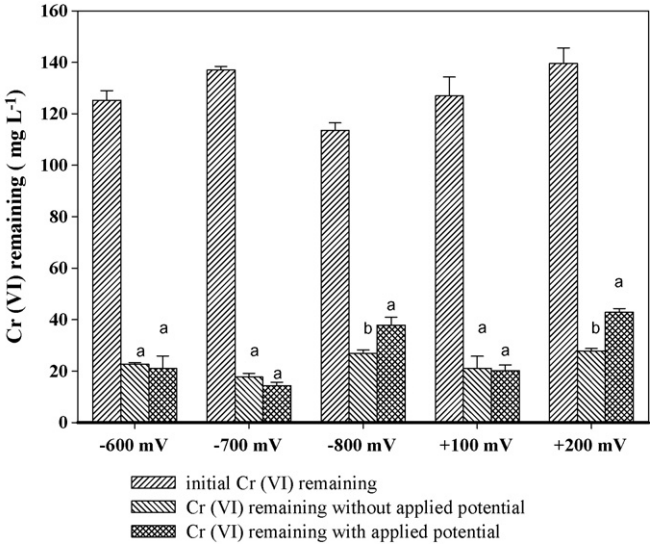

Fig. 6. Effect of applied potentials on Cr (VI) reduction by *Leucobacter* sp. Ch-1 at the initial pH 11.0 (values followed by different letters are significantly different under each applied potential at $p < 0.05$ using paired *t*-test).

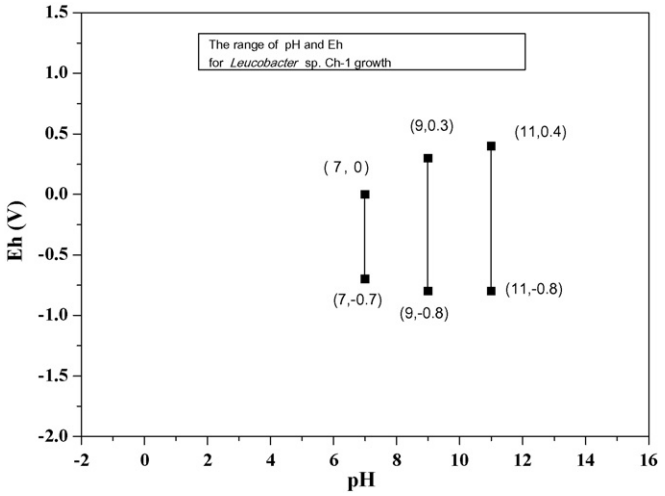

Fig. 7. The regions of initial pH and potential for *Leucobacter* sp. Ch-1 growth.

values and applied potentials, the range of bacteria growth and Cr (VI) reduction dependent on initial pH and Eh was graphed (Figs. 7 and 8). In Figs. 7 and 8, the range of both bacteria growth and Cr (VI) reduction widened with increasing initial pH value. Probably, Cr (VI) reduction by *Leucobacter* sp. Ch-1 was accompanied with participation of proton. It was noted that the region of bacteria growth at the absent of Cr (VI) was larger than that of Cr (VI) reduction. For instance, at pH 11.0, the potential for Ch-1 strain growth was from −800 to +400 mV, while the corresponding potential range for Cr (VI) reduction was from −700 to +100 mV. The similar trends were also found at initial pH 7.0 and 9.0. As compared with the region of initial pH and Eh for Ch-1 strain growth, the narrow range for Cr (VI) reduction may contribute to slight inhibitory influence of Cr (VI) on *Leucobacter* sp. Ch-1.

In the present study, most positive potentials impaired Cr (VI) reduction by *Leucobacter* sp. Ch-1 at all the tested initial pH values, while negative potential stimulated Cr (VI) reduction. Bacteria cell survival in ambient environment has been widely

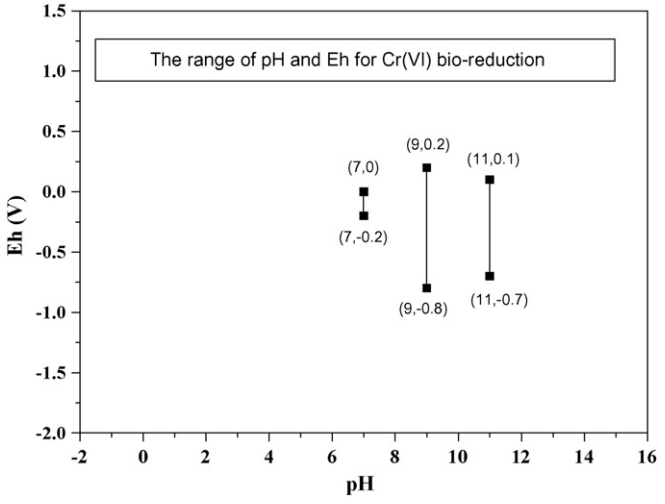

Fig. 8. The regions of initial pH and potential for Cr (VI) reduction by *Leucobacter* sp. Ch-1.

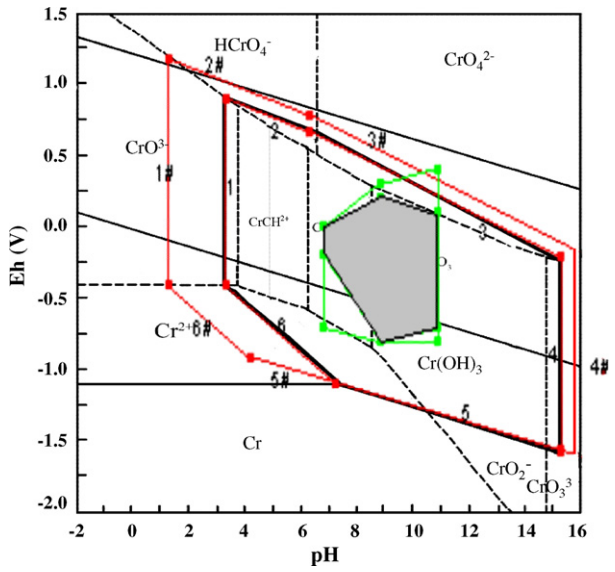

Fig. 9. Potential-pH diagram for *Leucobacter* sp. Ch-1–Cr–H_2O system. The region of pH and Eh encompassed with lines 1–6 represented the stable range of $Cr(OH)_3$ for 10^{-6} mol L^{-1} of Cr activity. The region encompassed with lines 1#–6# was the stable range of Cr $(OH)_3$ for 1 mol L^{-1} of Cr activity. The region included in green lines was the growth range for *Leucobacter* sp. Ch-1, and the grey area was the range of Cr (VI) bio-reduction. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

recognized. There is increasing evidence that *Escherichia coli* senses intracellular redox changes and migrates to a microenvironment with a preferred redox potential [18]. Redox potential is closely related to electron transport and metabolism of bacteria cell [19,20]. Becking et al. demonstrated that negative potential (from −500 to −1000 mV) stimulated enzymatic activities of *T. ferrooxidans*, while positive potential had inhibitory effects [1]. Therefore, extremely high and low potential inhibit the growth of bacteria cell.

3.5. Potential-pH diagram for "Leucobacter sp. Ch-1–Cr–H_2O" system

Potential-pH diagrams for Cr–H_2O system at 25–300 °C were reported by Lee [21]. In the present study, a potential-pH diagrams for"*Leucobacter* sp. Ch-1–Cr–H_2O" system was constructed based on the potential-pH diagrams for Cr–H_2O system, the diagram of *Leucobacter* sp. Ch-1 growth (Fig. 6) and Cr (VI) bio-reduction (Fig. 7).

In literature, 10^{-6} mol L^{-1} of Cr activity was used in drawing potential-pH diagrams for Cr–H_2O system [21]. Generally, the concentration of Cr ions varied from several moles to less than 10^{-6} moles per litre in waste water or groundwater. Therefore, the potential-pH diagram for the *Leucobacter* sp. Ch-1–Cr–H_2O system with chromium activities of 10^{-6} and 1 mol L^{-1} was graphed in this study. It was noted that the stable region of Cr $(OH)_3$ for 1 mol L^{-1} of Cr activity was relatively wider than that for 10^{-6} mol L^{-1}.

As shown in Fig. 9, the region of potential and initial pH for *Leucobacter* sp. Ch-1 growth was included in the region of Cr $(OH)_3$ stability. Furthermore, the region of Cr (VI) bio-reduction was encompassed within the regions of Cr $(OH)_3$ stability and *Leucobacter* sp. Ch-1 growth. The results indicated that Cr (VI) can be reduced to trivalent chromium existing in the forms of $Cr(OH)_3$ precipitate under the presence of *Leucobacter* sp. Ch-1 in alkaline condition.

4. Conclusions

The results in the present study showed that the growth of *Leucobacter* sp. Ch-1 was dependent on initial pH in culture medium. The *Leucobacter* sp. Ch-1 preferred to initial pH from 7.0 to 11.0. At the initial pH of 10.0, 99.5% of Cr (VI) can be reduced by *Leucobacter* sp. Ch-1 within 24 h.

There was a discrepancy of applied potential regions between *Leucobacter* sp. Ch-1 growth and Cr (VI) reduction. The region for *Leucobacter* sp. Ch-1 growth was from −700 to 0 mV, from −800 to +300 mV and from −800 to +400 mV at initial pH 7.0, 9.0 and 11.0, respectively. The corresponding ranges of applied potentials for reduction Cr (VI) were from −200 to 0 mV, from −800 to +200 mV, and from −700 to +100 mV.

The region of Cr (VI) bio-reduction was completely overlapped within the region of *Leucobacter* sp. Ch-1 growth and the stable region of $Cr(OH)_3$, which implied that Cr (VI) can be reduced to trivalent chromium existing in the forms of $Cr(OH)_3$ precipitate at the presence of *Leucobacter* sp. Ch-1 under alkaline condition. The results suggest *Leucobacter* sp. Ch-1 has potential application for remediation of Cr (VI) contamination sites.

Acknowledgements

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