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Heavy Metal Tolerance and Iron Reduction Rate of Acidiphilium Cryptum JF-5

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Abstract Acidiphilium cryptum JF-5 can overcome the low removal rates for ferric iron in common traditional sulfate-reducing bacteria (SRB) bioremediation methods treating acid mine drainage (AMD).High concentrations of heavy metal tend to inhibit the reduction ability of A. cryptum JF-5. Metal tolerance of A. cryptum JF-5 to aluminum, manganese, chrome, nickel, arsenic and zinc was studied. We found that A. cryptum JF-5 has the least tolerance to Cr, with the iron reducing rate reduced to 2% when the concentration of Cr was >5.0 mg/L. The iron reducing capacity of A. cryptum JF-5 was reduced to 48% and 41% respectively, when the concentrations of Ni and As were >5.0 mg/L. A. cryptum JF-5 performs best with Zn, Al and Mn. When the concentrations of Zn and Al reach to 50.0 mg/L, A.cryptum JF-5 still has 78% and 89% of the iron reducing rate. Furthermore, when the concentrations of Al, Zn and Mn increase up to 2000mg/L, A. cryptum JF-5 still has relative high iron reducing capacity, with reducing rate being 76%, 26% and 58% respectively. This provides a basis for how to apply A. cryptum JF-5 to SRB methods for treating AMD.
Keywords A. cryptum JF-5, metal tolerance, iron reducing rate, acid mine drainage

Introduction

Acidiphilum cryptum JF-5 strain was discovered by Professor K. Küsel and isolated from acidic lake sediments in 1999 (Küsel 1999). In acidic Fe-TSB medium, A. cryptum JF-5 bacteria can completely reduce 35 mm of Fe(Ⅲ) in about 7 days. The reduction rate of A. cryptum JF-5 is 2.93 nM Fe(Ⅱ) formed cell^{-1} d^{-1}. We can use A. cryptum JF-5 to overcome the low removal rates for ferric iron of traditional sulfate-reducing bacteria bioremediation method for acid mine drainage and improve removal rates of ferric iron and other heavy metals in AMD. AMD poses a serious risk to the environment as it often contains elevated concentrations of metals (iron, aluminium, zinc, manganese, nickel, chrome, and other heavy metals) and metalloids (of which arsenic is generally of greatest concern; Johnson 2005). AMD is characterized by low pH, often from 3 to 2 (Banks 1997). The low pH of AMD, increases the solubility of transition metals and so AMD often contains elevated concentrations (Hallberg 2010). When the metals exceed certain concentrations they can become toxic to microorganisms, such as A. cryptum JF-5, thereby deleteriously affecting reduction rates. In such cases, the various dissolved metallic constituents have both an individual and coupling effects on the activity of A. cryptum JF-5(Das 1997). However, the toxic effects on the activity of A. cryptum JF-5 have not been known clearly so far.

The objective of this study was to assess the tolerance of A. cryptum JF-5 to select dissolved metal/metalloid ions (Al^{3+}, Zn^{2+}, Mn^{2+}, Ni^{2+}, Cr^{6+} and As^{5+}) when present individually.

Materials and methods

The strain of Acidiphilum cryptum JF-5 used in this study is from Friedrich Schiller University of Jena (Germany). A. cryptum JF-5 can grow in an acid Fe-TSB liquid medium or an acid Fe-TSB solid medium, but in the former A. cryptum JF-5 grows faster. A. cryptum JF-5 can reduce ferric to ferrous in about 5 days in the absence of any other heavy metals turning the culture medium colorless. In the presence of other metals/metalloids where the capacity of A. cryptum JF-5 to reduce ferrous ion is decreased, this would increase time taken for the medium to become colourless. We cultured A. cryptum JF-5 in liquid medium with concentrations of each metal ranging from 0 (control) to 10 g/L for Al, Mn and Zn, 0 to 5 mg/L for Cr and 0 to 40 mg/Lfor Ni and As. When the control for each metal became colorless, the concentration of Fe(II) and the amount A. cryptum JF-5 bacteria in the medium was measured to show the tolerance to different metals. The ferrous ion and ferric ion were measured using the o-phenanthroline method (Dmitry 1993). The amount of bacteria was measured using a spectrophotometer set at a wavelength of 595 nm(F. Sánchez 2005). The various metal ions tested in this study were Fe^{3+} using $Fe_2(SO_4)_3$, Al^{3+} using $Al_2(SO_4)_3$, Zn^{2+} using $ZnSO_4$, Mn^{2+} using $MnSO_4$, Ni^{2+} using $NiCl_2$, Cr^{6+} using K_2CrO_4, and As^{5+} using Na_2HAsO_4 salts. All of the mediums were inoculated with A. cryptum and incubated at 30°C in anaerobic chambers (YQX-II, Shanghai botai of China).

All the experiments were carried out in duplicate. The same specimen determined consecutively 3 times to get the average. The average deviation between the replicates was found to be within ±5%.

Results and discussion

In order to quantify the tolerance of A. cryptum JF-5 to different metals the percent reduction was determined as ferrous ion concentration divided by the ferric ion concentration of the original medium.

As we can see from fig. 1, it is quite obvious that the percent reduction and the amount of A. cryptum JF-5 are positively correlated, with both decreasing with increasing of metal concentration.

As the concentration of Al^{3+} increases the percent reduction of Fe^{3+} decreased, however when the concentration reached 4 g/L the percent reduction of Fe^{3+} changed little but the amount of A. cryptum JF-5 increased substantially. This response was also seen for As and Ni but not for other metals where percent reduction of Fe^{3+} was closely followed by amount of A. cryptum JF-5. It perhaps because when these ion reach a certain concentration can form a complex with ferrous ion or ferric ion, which cannot be reduced but have less toxicity on A. cryptum JF-5.

Cryptum JF-5 performs better with high concentrations of Al^{3+}, $Zn^{2+}$and Mn^{2+} than Ni^{2+}, Cr^{6+} and As^{5+}. When the concentrations of Al and Zn reach to 50 mg/L, A. cryptum JF-5 still has a iron reducing rate of 89% and 78% . Furthermore, when the concentrations of Al, Zn and Mn are increased up to 2000 mg/L, A. cryptum JF-5 still has a relatively high iron reducing capacity, at 76%, 26% and 58% respectively. When a contrast is made in Al^{3+}, $Zn^{2+}$and Mn^{2+}, it's easy to draw a conclusion that Zn^{2+} has a greater influence to JF-5. When the concentrations of Al^{3+}, Zn^{2+} and Mn^{2+} reach 6000 mg/L, the percent reduction of Zn^{2+} is only 5%, while for Al and Mn it remains at 43% and 64% respectively.

Metal tolerance of A. cryptum JF-5 cell to Ni and As is better than for Cr. The iron reducing capacity of A. cryptum JF-5 was reduced to 48% and 41% respectively when the concentrations of Ni and As were 5 mg/L. It demonstrated that A. cryptum JF-5 cell has the lowest tolerance to Cr^{6+}. The iron reducing rate of A. cryptum JF-5 was reduced to 2% when the concentration of Cr was 5.0 mg/L. It may because A. cryptum JF-5 also can reduce Cr^{6+} to Cr^{3+}. When the concentration of Cr^{6+} reached 5 mg/L or much more, Fe^{2+} and A. cryptum JF-5 was nearly undetected. It indicated that A. cryptum JF-5 cannot survive in 5 mg/L Cr^{6+} or much more despite it can reduce Cr^{6+} to Cr^{3+}.

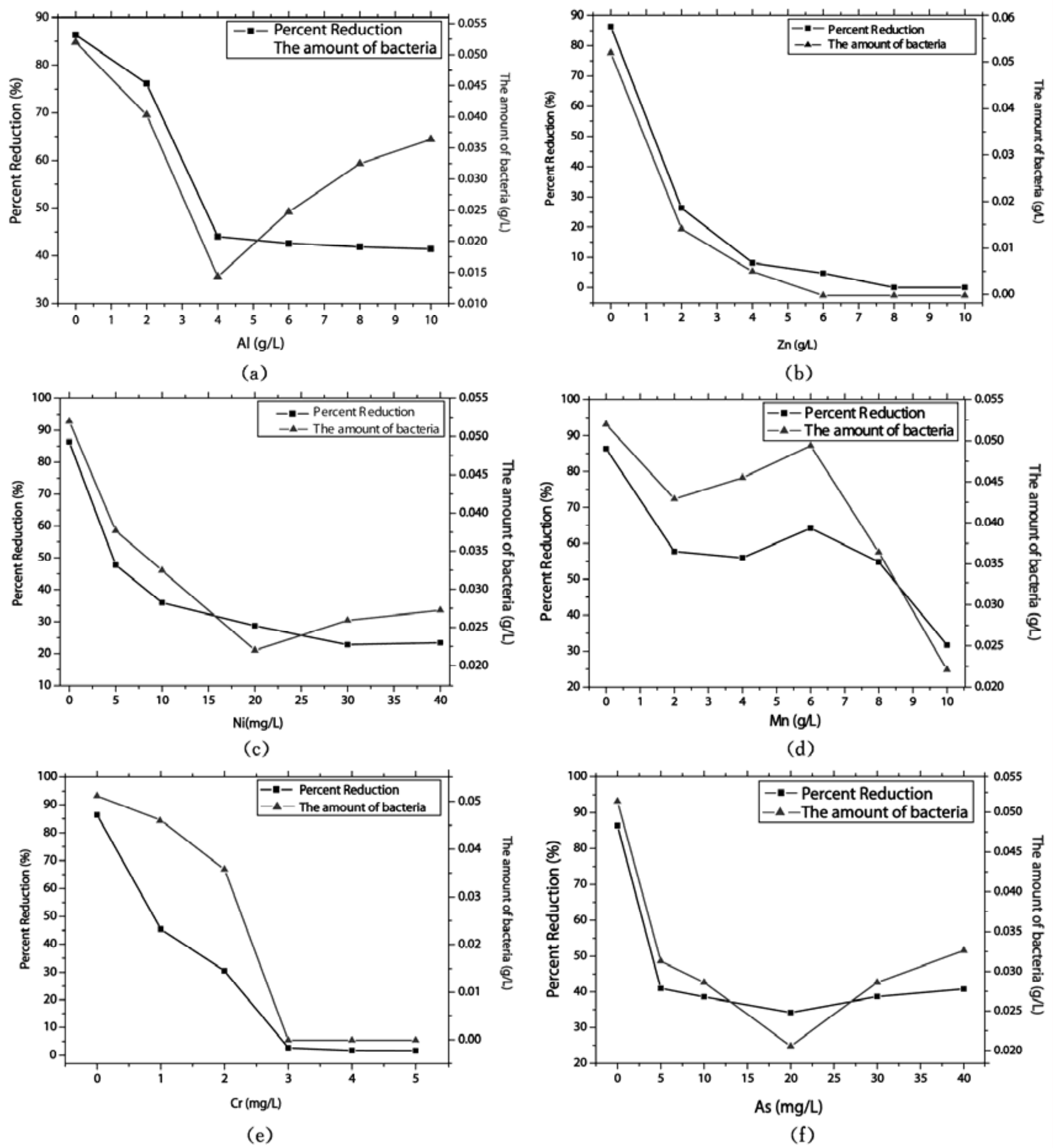

Fig. 1 *The percent reduction of Fe^{3+} and the amount of A. cryptum JF-5 with the increasing of concentrations of Al^{3+},Zn^{2+}, Mn^{2+}, Ni^{2+}, Cr^{6+} andAs^{5+}.*

Conclusion

The tolerance of A. cryptum JF-5 to select dissolved metal ions over a range of concentrations has been demonstrated in this work. The percent reduction of Fe^{3+} and the amount of A. cryptum JF-5 in the medium are closely correlated decreasing with increasing concentrations of metal ions.

Metal tolerance of A. cryptum JF-5 to Al^{3+}, $Zn^{2+}$and Mn^{2+} is better than Ni^{2+}, $As^{5+}$and Cr^{6+}. Among the group of Al^{3+}, Zn^{2+},Mn^{2+}, Zn^{2+} has a greater influence on A. cryptum JF-5 than Al^{3+} and Mn^{2+}. In the other group, metal tolerance of A. cryptum JF-5 to Ni^{2+} and As^{5+} is better than Cr^{6+} . A. cryptum JF-5 cell has the least tolerance to Cr^{6+}. The iron reducing rate of A. cryptum JF-5 is very small when the concentration of Cr^{6+} exceeded 3.0 mg/L.

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