Manganese waste water treatment by fungi derived from manganese slag

Yu-Zhu Ou-yang, Jian-Bing Cao, Xiao-Ming Li, Wei Zheng, Dong-Bo Wang and Yi Zhang

ABSTRACT

The aim of this study was to isolate a mould from the surface of manganese slag which had strong resistance and high adsorption of Mn^{2+}, and to determine the effects of initial Mn^{2+} concentration, incubation temperature, rotation speed and inoculation amount on adsorption of Mn^{2+} from manganese waste water solution. The result showed that a mould (A5) which was isolated from manganese slag had the adsorption rate of Mn^{2+} to 97.5% at the initial pH value 6, inoculation amount 2%, rotation speed 150 r/min, a concentration of Mn^{2+} 500 mg/L, and a temperature of 28°C cultivated for 50 h. As there is no research on adsorption of Mn^{2+} from manganese waste water by fungi before, this research showed a theoretical guidance on this field.

Key words | bioadsorption, domestication, mould

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INTRODUCTION

Manganese is important industrially because it serves as a desulphurising, deoxidising, and alloying element in the manufacture of steel and cast iron. The metal resources are being exhausted globally as the demand for industrially important metals increases.

Rich deposits of manganese ore bearing manganese values in excess of 35%, industrially preferred for processing, are found only in certain parts of the world, but in many instances such deposits are not available in the industrialised countries. China, as a developing country, needs to produce more and more steel, for which there is a huge demand for manganese. The demand for a host of manganese based chemicals like manganese sulphate, manganese carbonate etc. is also showing a rising trend. This would cause a tremendous upsurge in the processing of low-grade manganese ore and other resources such as mining tailing and manganese slag in the country. As the manganese resources are not so high, there is a need for their conservation and utilisation for utmost efficiency.

The mining and metallurgical activities that were particularly intensive during the last century resulted in the generation of huge amounts of mine tailings, including acid generating sulphidic tailings. Most of the tailings have been left in the mines of China without any management. Their improper management in the past resulted in the mobilisation of heavy metals to the surrounding environment, contributing to soil substrates contamination, soil texture destruction, a shortage of nutrients, ecological landscape destruction, groundwater pollution, biological diversity reduction, etc. The presence of toxic heavy metals in mine tailings caused lots of serious environmental problems. In order to resolve the above problems, it is important to develop a suitable and economical technology for removal of heavy metals from mine tailings.

Bioremediation of heavy metals has gained increased attention because it is innovative, environmentally friendly and economical (Tichy *et al.* 1998). Bioleaching processes are based on the ability of microorganisms to transform solid compounds and result in soluble and extractable elements which can be recovered. Metal solubilisation from solid wastes or other solids is achieved through a variety of *acidophilic* and *chemoautolithotrophic* bacteria

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such as *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans*. Other microorganisms such as *Leptospirillum ferrooxidans* were reported to bioleach zinc from the marmatite flotation concentrate effectively (Shi & Fang 2005) and the fungal treatment was also found to promote extensive leaching of chromium from preservative-treated wood (Reyes 2007). Because of the advantages of low cost, environmental friendliness and improved efficiency, bioleaching technology has been a great success for the mining industry (Shi *et al.* 2006). The use of microorganisms or their biomass for the recovery of metal from waste as well as the employment of plants for landfill applications has achieved growing attention (Umrania 2006). In recent years, bioleaching has proved to be a possible way to remove heavy metals from metal contaminated materials such as anaerobically digested sewage sludge, contaminated river sediment, spent nickel-cadmium batteries, tannery sludge, and incinerator fly ash (Seidel *et al.* 1998; Zhu *et al.* 2003; Chen & Lin 2004; Paul *et al.* 2004; Wong *et al.* 2004; Xu & Ting 2004; Fang & Zhou 2007). The study also proved that it was feasible to remove heavy metals from mine tailing with the use of the bioleaching remediation method (Liu *et al.* 2007).

The effectiveness of bioleaching is highly dependent on the physical, chemical and biological factors in the system (Chen & Lin 2001). Contributing factors such as the nature of contaminated material, substrate concentration, solids concentration, temperature, oxygen, pH, bacterial strain and cell concentration (Bosecker 1997), temperature, pH value and oxygen play an important role in the optimisation of the bioleaching process.

Therefore, the development of new technology is very important in order to recover metals from low-grade ores, slag and nodules that cannot be processed economically by conventional methods. The recovery of manganese from manganiferous ores by bioleaching with different kinds of microorganisms has been extensively investigated by many workers and, as a result, microbiological processes have been proposed to be less hazardous (Ehrlich 1987). The present study was initiated with the isolation of a fungus, *Penicillium citrinum*, capable of leaching manganese ore. However, there is no study that is based on the microorganism that exists in the manganese slag. This investigation isolated a fungus from manganese slag which had strong resistance and high adsorption of manganese, and determined the optimal condition for bioadsorption.

MATERIALS AND METHODS

Materials

The manganese slag under study comes from the manganese slag dam located 40 km south of Jishou city in central China's Hunan province. The manganese slag samples and mould-containing sludge were transported to the laboratory and stored at 4°C prior to their use. The characteristics of the manganese slag were determined as follows: the pH value of the manganese slag was 6.72; the content of Mn^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+} in the manganese slag was 3.55 g/kg, 1,400 mg/kg, 75.6 mg/kg, 1.5 mg/kg and 5.2 mg/kg respectively.

Czapek's culture medium was used for fungi isolation. This was prepared by dissolving 30 g sucrose, 2 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g KCl, 0.5 g $MgSO_4$, 0.01 g $FeSO_4$ in deionised water to 1 litre before autoclaving at 121°C for 20 min. For solid medium 15–20 g agar was added to the above medium before autoclaving.

Isolation of strains

Manganese slag was spread on a Petri plate containing Czapek's solid medium and was incubated at 27 ± 2°C. Spores from micro colonies on these plates were isolated by micromanipulation. The fungi spores were purified to aseptic conditions by streaking the spores repeatedly on the Czapek's medium agar plate with 20% NaCl (in order to ensure the aseptic environment). The purified fungi spores were transferred to liquid media. These liquid cultures were tested for bacterial contamination by plating on bacteriological media. Isolated and purified fungi cultures were identified according to morphological properties.

Domesticating strains

The mould was inoculated on Czapek's medium containing 500 mg/L Mn^{2+} at the inoculate amount of 2%, and then cultured at 28°C, 150 r/min for 120 h. The concentration of Mn^{2+} was then increased repeatedly to the inoculated

mould that had the adsorption ability; until it obtained the ideal domesticated strains.

Resistance experiment of strains

The mould was inoculated on Czapek's medium containing 500 mg/L Mn^{2+} at the inoculate amount of 2%, cultured at 28°C, 150 r/min for 120 h. Then the concentration of Mn^{2+} was increased from 2,000 mg/L, 3,000 mg/L, 4,000 mg/L to 5,000 mg/L, 200 μL cultured fluid was taken and spread in respective plates, then cultured at 28°C. The colonies were observed after 7 days. When there were no strains grown on the plate, the previous concentration of Mn^{2+} was taken as the limited tolerance concentration.

Bioleaching experiment

The bioleaching was conducted in 250 mL Erlenmeyer flasks with 100 mL of the same culture medium under different conditions. The flasks were agitated on a gyratory shaker. A blank run without inocula of fungi was also carried out in order to compare the results. During the bioadsorption process, the water loss due to evaporation was replenished daily with distilled water.

Chemical analysis

During the experiment, the pH value was measured at half-day intervals by a pH analyser (Model K100, Dr-Kornder). The waste water samples were taken from the reactor every 24 h. The samples were analysed for heavy metal Mn^{2+} concentrations by an Atomic Absorption Spectrophotometer (AAS) (ModelZEEnit700, Analytik Jena AG). All treatments and controls were replicated in duplicate.

Adsorption rate calculation

The adsorption rate was calculated according to the following formula:

$$C = \frac{(C_0 - C_1)}{C_0} \times 100\%$$

C: adsorption rate; C_0: initial concentration; C_1: concentration after adsorption.

RESULTS AND DISCUSSION

Isolation and identification of the strains

The microorganisms were isolated as described above. The isolated strains were initially identified according to the morphological characteristics. The result is shown in Figure 1.

The morphological characteristics of the fungi are large and dense, slightness, villous, opaque, dried, rough and with white villous aerial hypha. It had a blank front, dark green back, and was pigment water-soluble. We decided it was a mould.

Mn^{2+} resistance experiment

We isolated five fungi from the manganese slag, but different strains had different resistances to the manganese ion. The five strains' resistances to manganese are shown in Table 1.

It can be seen from Table 1 that only A5 was able to survive in a medium of 5,000 mg/L manganese concentration. Most of the strains were able to survive at 2,000 mg/L manganese concentration only, but A4 was not able to survive at any of the four concentrations

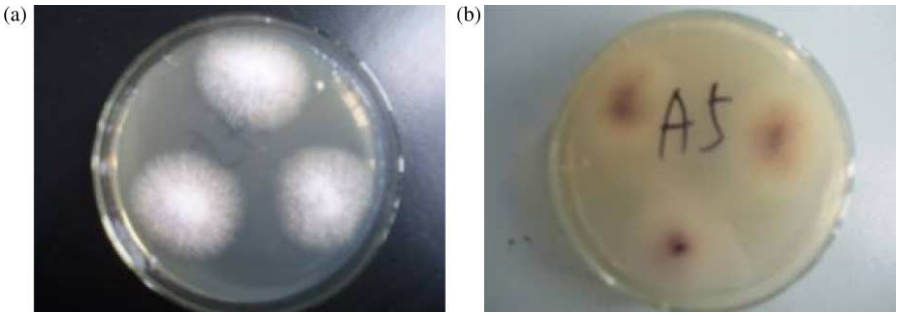

Figure 1 | The morphology of A5 strain: (a) front, (b) back.

Table 1 | The manganese resistance result of the strains

	Mn^{2+} concentration (mg/L)			
Strain number	**2,000**	**3,000**	**4,000**	**5,000**
A1	+	+	+	−
A2	+	−	−	−
A3	+	−	−	−
A4	−	−	−	−
A5	+	+	+	+

Note: " + " able to survive, " − " unable to survive.

of the experiment. Therefore, we chose A5 as the experimental strain.

Domesticating strains

The A5 strain was domesticated six times in order to obtain the high manganese resistance strains. The strains grew well during the domesticating process. After inoculating, we calculated the adsorption rate of every offspring. The adsorption rate of every passage is shown in Table 2.

From Table 2, it can be seen that the first offspring showed the highest adsorption rate with 94.36%. With the increase of manganese concentration, we did not obtain higher adsorption offspring, because the strains were isolated from the residue piled up for more than 10 years, and were grown under a high manganese concentration, and had been domesticated for stability and high efficiency. In this research we took the first passage to do the adsorption characteristic influence tests.

Adsorption characteristic experiment

Effect of temperature on Mn^{2+} adsorption rate

The effect of temperature on Mn^{2+} adsorption rate at the end of the incubation period is shown in Figure 2. The experiments were performed at various temperatures (22°C, 25°C, 28°C, 31°C and 33°C) and at 2% inoculate, pH value 6, the initial Mn^{2+} concentration 200 mg/L, and a rotation speed 150 r/min for 144 h. The adsorption rate was determined by the concentration of manganese in the broth. As shown in Figure 1, the effect of temperature was indistinct. Five temperatures showed the same trend and there was little difference in the adsorption rate. However, the adsorption rate increased constantly with time. A5 had a prodigious adsorption rate for the first 40 h. Incubated at 28°C for 47 h, the adsorption rate reached 97.1%, and then the concentration showed a minute difference because the organism adsorbed the Mn^{2+} and at the same time the decaying of the organism released the adsorbed Mn^{2+} (Figure 3).

Effect of the initial concentration of Mn^{2+} on adsorption rate

At the optimum temperature, the effects of Mn^{2+} adsorption rates on A5 were investigated at 50 mg/L, 100 mg/L, 200 mg/L, 500 mg/L, 1,000 mg/L and 3,000 mg/L Mn^{2+} initial concentration and at 2% inoculate, pH value 6, 28°C, rotation speed 150 r/min for 144 h. In the experiment, the adsorption rate of A5 increased until stabilisation and remained at the same concentration of Mn^{2+} with time. A different Mn^{2+} concentration had a tremendous effect on the adsorption rate. The A5 strain showed obvious adsorption when a Mn^{2+} concentration of 1,000 mg/L was allowed, but as the concentration exceeded this figure the adsorption rate died down quickly. The adsorption rate was 93.3, 65.2 and 9.1%, respectively, at the concentration of 500 mg/L, 1,000 mg/L and 3,000 mg/L for 143 h. The Mn^{2+} concentration increased from 500 mg/L to 1,000 mg/L and 3,000 mg/L, while the adsorption rate decreased to 30.12 and 90.25% respectively. During the experiment, the A5 strain grew well at the Mn^{2+} concentration of 3,000 mg/L, but the adsorption rate was the lowest, because A5 let out manganese voluntarily using a chemosmosis inverted pump which depended on metabolism.

Table 2 | The adsorption rate of the offspring

Offspring number	**1**	**2**	**3**	**4**	**5**	**6**
Mn^{2+} concentration (mg/L)	500	800	1,000	2,000	3,000	4,000
A5 adsorption (%)	94.36	93.28	82.54	34.37	11.34	5.74

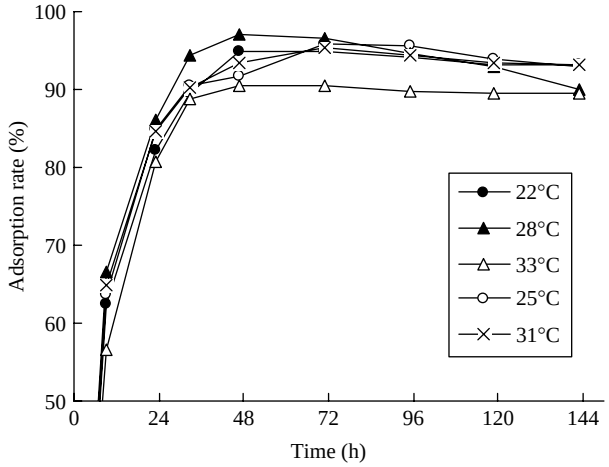

Figure 2 | Effect of temperature of Mn^{2+} on adsorption rate.

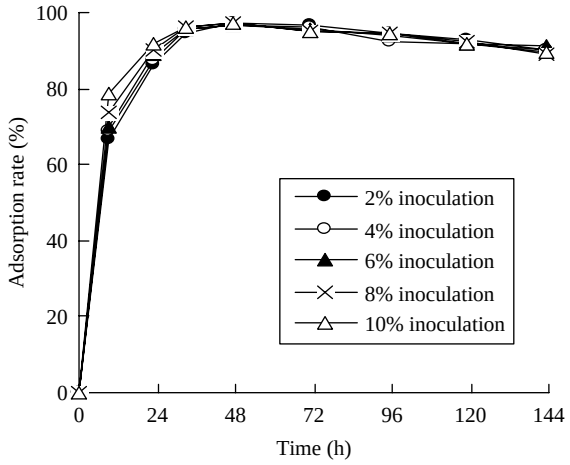

Figure 4 | Effect of inoculation amount on adsorption rate.

This function ensured that A5 was able to survive in a high Mn^{2+} concentration environment. Thus it can be seen that A5 had high adsorption at the initial Mn^{2+} concentration 500 mg/L.

Effect of inoculation amount on adsorption rate

The effect of the inoculation amount on the adsorption rate by the A5 strain is shown in Figure 4. The experiments were performed with 2, 4, 6, 8 and 10% inoculation amounts and 500 mg/L initial manganese concentration, pH value 6, 28°C, rotation speed 150 r/min for 144 h. The adsorption rate increased gradually with time. The 2% inoculation amount had the highest adsorption rate, which was 97.1% at 47 h. The rate then decreased slightly, because in the adsorption system, when the strains are not saturate by metal, the adsorption amount increases rapidly, but when the amount of adsorbent reaches a certain quantity, there is no obvious effect on the removal rate to continue an increase of the adsorbent amount. However, the microorganism had exhibited a short growth cycle and a fast growth rate, and the biomass had a burst of growth in a short time. Although it had a different initial inoculation amount, the system would reach an ultimate biomass in a short time. Therefore, the inoculation amount had little effect on the adsorption rate.

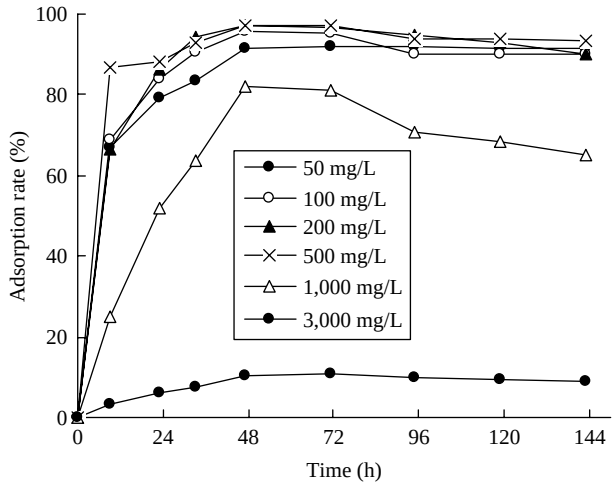

Figure 3 | Effect of the initial concentration of Mn^{2+} on adsorption rate.

Effect of rotation speed on adsorption rate

The effect of rotation speed on adsorption rate by strain A5 is shown in Figure 5. The experiment was performed at 0 r/min, 50 r/min, 100 r/min and 150 r/min rotation speed and 500 mg/L initial manganese concentration, pH value 6, 28°C, 2% inoculate for 144 h. The adsorption rate increased rapidly, and then decreased slowly. Static cultured and 150 r/min cultured, the adsorption rate was 83.6% and 97.1% respectively for 47 h. This showed that A5 had a low requirement regarding rotation speed, but an environment with sufficient oxygen supply was beneficial to Mn^{2+} adsorption. High rotation could lead to plasmatorrhexis. So we chose 150 r/min as the optimum rotation speed.

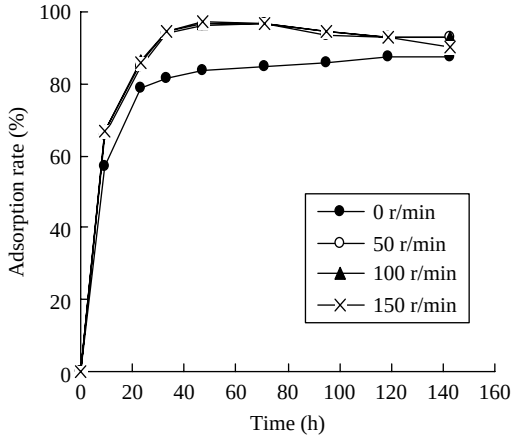

Figure 5 | Effect of rotation speed on adsorption rate.

Culture medium acidity regulated by A5

Different species of microorganism have their own optimum growth pH values. The environmental pH value will have a direct and indirect effect on microorganism cells. For example: it will affect the uptake of nutrients, enzyme adaptability in metabolism reactions, toxicity of environmentally harmful material and so on. But at the same time, the life course of the microorganism will change the external environmental pH value. The culture medium acidity regulation by A5 at different pH values is shown in Figure 6. In this figure the pH value decreased obviously before 48 h culture when the initial pH was 7, 8 and 9.6. After 48 h it tended to be stable. However, the pH increased obviously in 48 h when the initial pH was 4, and it tended to be stable after 48 h. The pH did not change a lot when the initial pH was 6. Although it had different initial pH values,

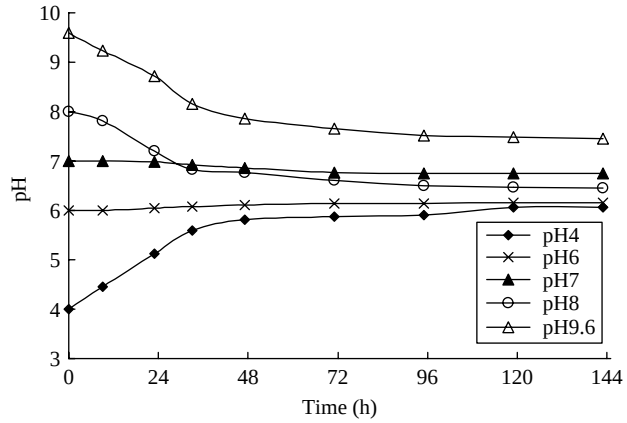

Figure 6 | Culture medium acidity regulated by A5.

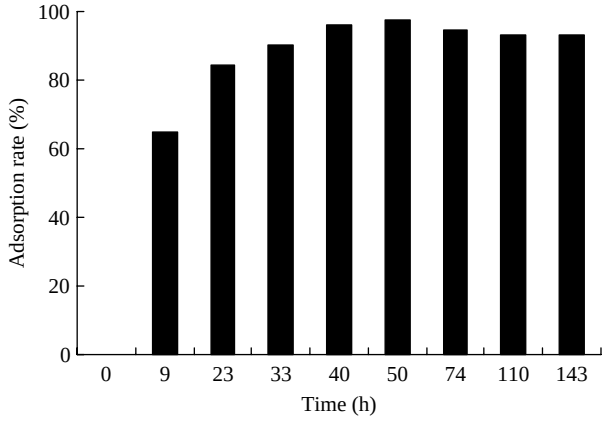

Figure 7 | The adsorption rate change curve of A5 at optimal conditions.

the pH value after culturing was 6.15–6.75. This can explain why A5 can change the harsh pH environment and showed that it is significant for environmental remediation.

Optimal adsorption condition series experiment

In order to examine the adsorption effect of the strain under optimal conditions, the A5 strain was cultured at pH 6, 2% inoculation amount, 28°C, 150 r/min rotation speed, an initial Mn^{2+} concentration 500 mg/L, and then the Mn^{2+} concentration was measured at specific intervals, and the adsorption rate calculated. The result was shown in Figure 7. The adsorption rate increased rapidly with time. The adsorption rate reached a maximum of 97.5% after 50 h, and then decreased a little. Because A5 was carrying out in vivo adsorption, during the early growth period the strain reproduced quickly and the biomass increased rapidly, which led to a rapid increase of the adsorption rate, until it reached the maximum. Along with the constant reproducing of A5, the biomass increased constantly, and Mn^{2+} was adsorbed by the microorganism. When the growth of A5 reached the stable stage, the biomass no longer increased, and the adsorption rate was nearly constant.

CONCLUSIONS

In this study, a mould (A5) which has high resistance to manganese was isolated from electrolytic manganese waste residue. And we discussed the optimum conditions of

adsorption of manganese ion in manganese waste water. The result showed that the manganese adsorption rate reached 97.5% at 28°C, with an initial pH 6, 2% inoculation amount, 150 r/min, initial Mn^{2+} concentration 500 mg/L for 50 h. The strain has high adsorption properties at 22–33°C and Mn^{2+} concentration 50–1,000 mg/L. It also grows fast and has a low oxygen supply rate. This result can be directly used in manganese waste water treatment and polluted soil remediation.

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