Bio-Remediation of Acid Mine Drainage in the Sarcheshmeh Porphyry Copper Mine by Fungi: Batch and Fixed Bed Process

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Abstract

Acid mine drainage (AMD) containing high concentrations of iron and sulphate, low pH and variable concentrations of heavy metals leads to many environmental problems. The concentrations of Cu and Mn are high in the AMD of the Sarcheshmeh porphyry copper mine, Kerman province, south of Iran. In this study, the bio-remediation of Cu and Mn ions from acid mine drainage was investigated using two native fungi called Aspergillus niger and Phanerochaete chrysosporium which were extracted from the soil and sediment samples of the Shour River at the Sarcheshmeh mine. The live fungi was first harvested and then killed by boiling in 0.5 N NaOH solution. The biomass was finally dried at 60 $^{\circ}C$ for 24 h and powdered. The optimum biosorption parameters including pH, temperature, the amount of biosorbent and contact time were determined in a batch system. The optimum pH varied between 5 and 6. It was found that the biosorption process increased with an increase in temperature and the amount of biosorbent. Biosorption data were attempted by Langmuir and Freundlich isotherm models and showed a good match. Kinetic studies were also carried out in the present study. The results show that the second-order kinetics model fits well the experimental data. The biosorption experiments were further investigated with a continuous system to compare the biosorption capacities of two systems. The results show that biosorption process using a continuous system increases efficiency up to 99%. A desorption process was eventually performed in order to recover Copper and Manganese ions. This process was successful and fungi could be used again.

Keywords: Acid mine drainage, Aspergillus niger, Biosorption, Copper, Phanerochaete chrysosporium, Sarcheshmeh

Introduction

With the ever increasing industrial activities during recent times, many aquatic bodies are receiving loads of heavy metals and organic chemical wastes that far exceed the water quality criteria for wastewater discharge designed to protect the environment, animals and humans [1].

Acid mine drainage (AMD) and heavy metal pollution are considered to be the most serious water pollution problems in mining areas. Such drainages containing iron sulphates and other components can affect the quality of the receiving water bodies. Many types of mining sites, such as coal mining, metalliferous mining, backfilled opencast mining, quarries, coal washing wastes as well as tailings dams and overburden waste materials contain iron sulphide minerals, in particular pyrite (FeS_2).AMD occurs when such sulphide minerals in rock are exposed to air and water, changing the sulphide to sulphuric acid [2]. This acidic drainage can dissolve heavy metals found in the waste rocks and tailings such as lead, zinc, copper, manganese, arsenic, selenium, mercury, and cadmium ions and discharge into ground and surface water. AMD can destroy aquatic life and habitat. Moreover, AMD generation and subsequent metal leaching are a particular concern because they can continue indefinitely, causing environmental damage long after the mine

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operation has ended, and it costs a lot to mitigate the damage caused by AMD.

The overall reaction, catalyzed by microbes, can be given as:

$$FeS_2 + \frac{7}{2}O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \quad (1)$$

The dissolved Fe^{2+}, SO_4^{2-} and H^+ represents an increase in the total dissolved solids and a decrease in pH. In many environmental settings, the consequence of AMD can be moderate to severe [3].

Effective, low-maintenance and inexpensive treatment systems are necessary to treat AMD. There are many alternative methods to treat or remediate AMD. The most common method is the chemical precipitation that is based on the actively adding alkalinity and neutralizing materials and settling the resulting precipitates. This method is known as active treatment system. The advantages of lime treatment systems are neutralising acidity, removal of iron and aluminium salts and the reduction in sulphate concentration [4]. However, active treatment systems often produce large amounts of gypsum sludge contaminated by heavy metals and need additional treatment facilities, therefore such systems are generally expensive.

Another technology that is more passive in nature is the use of passive anoxic limestone drains, PALD, [5]. This method has been developed for pre-treatment of acid mine drainage, in which acid drainage is generally passed through a drain including crushed limestone under anoxic conditions for avoiding the precipitation of metal hydroxides within the drain and on the limestone gravels. A successful treatment of AMD using anoxic limestone drains can be achieved if this method is combined with constructed wetlands [6].

Constructed wetlands are often cost-effective, low-maintenance passive treatment systems for AMD [7]. Such systems are successful in removing metals from AMD. However, wetlands are not always reliable during all seasons of the year for water quality improvement. A wetland is a chemically complex system wherein many physical, chemical and biological processes occur. Although many of these processes are not fully understood, they have been shown to be effective in metal removal, in particular iron, and in the raising of *pH* as well as sulphate reduction [8].

Two kinds of constructed wetland systems which have been reported by Saharan et al. [9] are aerobic and anaerobic wetland systems. In aerobic wetlands, AMD treatment is dominated by the processes occurring in the shallow surface layer while in anaerobic wetlands, by the chemical interactions taking place within an organic substrate such as compost bioreactors. An aerobic wetland is commonly constructed to treat mine drainages that are net alkaline [10]. Passive bioremediation systems that utilise a combination of aerobic and anaerobic wetlands have been used for full-scale treatment of AMD [11]. However, these methods are not economic in Iran and also leave huge amounts of gypsum sludge.

Another method for treatment of AMD is based on the biological process. Many microbial species such as bacteria, fungi and yeast are known to be capable of adsorbing heavy metals on their surface and/or accumulating the metals within their structure. There is a possibility to use micro-organisms in the removal of toxic metals from AMD and even in the recovery of such metals using their biosorption properties [12].

Many research works have been carried out in the past to investigate pyrite oxidation, AMD generation and heavy metals prediction in the Sarcheshmeh porphyry copper mine, southeast Iran. Doulati Ardejani et al. [13] studied the impact of AMD on the quality of the surface and groundwater at Sarcheshmeh copper mine. Chemical analysis of the water samples indicated pyrite oxidation and AMD generation in the mine and waste dumps and their serious impact on the quality of water bodies. The pH of the water samples in the Shour River originated from the Sarcheshmeh mine varied between 2 and 3.9 and the concentrations of the most toxic metals were above their allowable limit. The concentration of SO_4 in both surface and groundwater samples were very high. However, the pH of groundwater samples varied from 6.3 to 7.2.

Rooki et al. [14] used back propagation neural network (BPNN), general regression neural network (GRNN) and multiple linear regression (MLR) methods to predict heavy metals including Cu, Fe, Mn, Zn in the Shur River impacted by AMD at Sarcheshmeh porphyry copper mine. Seifpanahi Shabani et al. [15] developed a numerical finite volume model to simulate biosorption of copper and manganese ions from acid mine drainage (AMD) at Sarcheshmeh porphyry copper mine using Aspergillus Niger fungal. A sensitivity analysis of the major parameters affecting biosorption process was performed. They found that the biosorption process is most sensitive to the maximum adsorption capacity. Its lower values resulted in delays in biosorption mechanisms. The model was also sensitive to the reactor length. The increase in the reactor length considerably increased the biosorption process.

The literature review thus far has shown that the impact of the long-term acid and metal leaching problems associated with mining activities, in particular, at the Sarcheshmeh copper mine has not been completely solved. In order to solve such problems, the aspect of AMD treatment using a cost-effective method has to be considered to minimise the socio-economic and environmental impact of mine drainages. Among the treatment methods, biosorption process has been shown to be more efficient and economic.

In the present work the biosorption of Cu and Mn as two most abundant metals from AMD of the Sarcheshmeh copper mine was investigated using two native fungus species i.e. Aspergillus niger (As.) and Phenarocate crysosporium (Ph.) extracted from the sediments of the Shour River at Sarcheshmeh mine.

Site Description

As a case study, the biosorption of Cu and Mn from AMD of the Sarcheshmeh copper mine was considered. This copper mine is located about 160 km to southwest of Kerman and 50 km to southwest of Rafsanjan in the Kerman province, Iran. The average elevation of the mine is 1600 m. The mean annual precipitation of the site varies from 300 to 550 mm. The temperature varies from +35 °C in summer to -20 °C in winter with plenty of snow. A rough topography is predominant at the mining area. Figure 1 shows the geographical situation of the Sarcheshmeh copper mine.

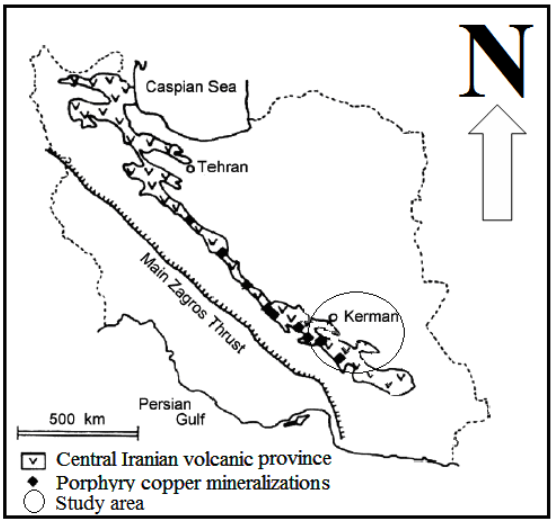

Figure 1: Sarcheshmeh porphyry copper mine (modified after Shahabpour and Doorandish [18]; Rooki et al. [14])

The shape of ore deposit in the Sarcheshmeh mine is oval with 2300 m length and 1200 m width approximately. This deposit is associated with the late Tertiary Sarcheshmeh granodiorite porphyry stock [16]. The porphyry is a member of a complex series of magmatically related intrusives emplaced in the Tertiary volcanics at a short distance from the edge of an older near-batholith-sized grano-diorite mass.

Open pit mining method is used to extract copper deposit in the Sarcheshmeh. A total of 40,000 tons of ore (average grades 0.9 % Cu and 0.03 % molybdenum) is approximately extracted per day in the Sarcheshmeh mine [17].

Material and Methods

AMD Preparation

AMD from the Sarcheshmeh copper mine was sampled in five discrete times, placed in 20-liter standard containers and transferred to the laboratory. Table 1 gives some chemical parameters of AMD that was measured before biosorption process.

Table 1: AMD chemical analysis results

Parameter	Concentration
Fe^{2+} (ppm)	0.097
Ni(ppm)	0.003
Pb(ppm)	0.65
Ag(ppm)	0.057
Al (ppm)	0.24
Mo (ppm)	0.29
Cu (ppm)	8.265
Mn (ppm)	10.401
pH	5.4
BOD_5(mg/l)	225 mg/l
COD (mg/l)	350 mg/l
D.O (mg/l)	1.54
Turbidity(NTU)	167.9

Biomass Production

Two native fungus species extracted from the sediments of the Shour River propagated separately in potato dextrose agar. The culture was maintained on slants and was incubated for 5–7 days at 33±2°C. Once inoculated, fungi were cultivated in 100 ml medium contained in 250-ml Erlenmeyer flasks, which were agitated on a shaking incubator at 150 rpm and 30°C. After 4 days, the fungal cells were harvested by filtration using filter paper Whatman No. 42 [19]. Living biomass was boiled in 0.5N NaOH solution for 15 min and then washed with generous amounts of deionized water until the pH after washing received to the near-neutral range (7.0±7.2) [20]. The biomass was then dried at 60°C for 24h and powdered. The dried biomass with particle sizes less than 75 mm preserved for bio-remediation process.

Batch Biosorption Experiments

Batch forms of kinetic and isotherm sorption experiments were conducted separately to evaluate the effects of pH, time, and temperature and biomass concentration on the removal of Cu and Mn ions.

A known quantity of dried biomass was added to AMD samples in 250 ml Erlenmeyer flasks before pH adjustment. The pH of each solution was adjusted to the required value (3, 4, 5 and 6) using 0.1N NaOH and H_2SO_4 acid. All the experiments were kept in a shaker at 150 rpm and various ranges of temperature from 20°C to 40°C for two hours (equilibrium time). Different amounts of biomass (in grams) were added to each solution, and then the effect of time variation was studied in the range of 10-120 min.

In order to investigate the effect of biomass concentration on the biosorption process, 100 ml solutions containing heavy metals which were determined before experiment was prepared with an optimal pH. The necessary amount of the biomass was then added to each solution. Subsequently, the Erlenmeyers were kept in a shaker at 150 rpm and an optimum temperature for two hours (equilibration time). After this time, the concentrations of the metals were measured in the solutions. Samples which were taken at certain time intervals filtered using a filter paper for removing the suspended biomass and analysed for residual metal concentration. The metal concentration in the supernatant solution was determined by an atomic absorption (Model Varian 432). The amount of adsorbed metal ions per unit biosorbent (mg of Cu and Mn to g of dry biosorbent) was obtained using the following expression:

$$q = \frac{(C_0 - C_e)V}{M} \qquad (2)$$

where,

q = amount of metal adsorbed on the biomass (mg/g);

C_o = initial metal ion concentration in solution (mg/l);

C_e = equilibrium metal ion concentration in solution (mg/l);

V = volume of the medium (l);

M = amount of the biomass used in the reaction mixture (g).

The percent biosorption of Cu and Mn was calculated as follows:

$$Biosorption\ (\%) = \frac{(C_i - C_f)}{C_i} \times 100 \qquad (3)$$

where, C_i and C_f are the initial and final Cu and Mn concentrations, respectively.

Absorption Isotherm

An adsorption isotherm demonstrates the relationship between equilibrium concentrations of adsorbate in the solid phase q, and in the liquid phase C at a constant temperature. The adsorption isotherms are often obtained in the laboratory using batch tests in which the equilibrium data are attempted by various isotherm models. There are many different isotherm models that have been suggested for the adsorption of solutes in a liquid solution onto a solid surface. Langmuir adsorption expression is one of the main models which are applied to describe the equilibration relationship between the metal ions and active points on the biomass. In Langmuir model, it is assumed that a certain number of active sites have been distributed on the cell surface, which have the same affinity to form bonds and the adsorption occurs in a monolayer, and no reaction takes place among the adsorbed molecules. In this isotherm, the energy of adsorption is constant and there is no migration of the adsorbate molecules in the surface of biosorbent. The Langmuir model can be described as follows [21]:

$$q = \frac{bq_{\max} C_e}{1 + bC_e} \qquad (4)$$

A linear expression for the Langmuir isotherm can be expressed as follows:

$$\frac{1}{q} = \left(\frac{1}{bq_{\max}}\right)\left(\frac{1}{C_e}\right) + \left(\frac{1}{q_{\max}}\right) \qquad (5)$$

where,

$q_{\max}$ = maximum metal uptake corresponding to the saturation capacity (amount of metal ions per unit weight of biosorbent to form a complete monolayer on the surface) (mg/g);

b= energy of adsorption (the ratio of adsorption / desorption rates) (l/mg);

q = amount of metal adsorbed on the biomass (mg/g);

C_e = equilibrium (residual) metal concentration in solution (mg/l).

The constants $q_{\max}$ and b are the characteristics of the Langmuir isotherm and can be determined from Equation 4. A plot of 1/q versus 1/Ce gives a straight line with a slope of $(1/bq_{\max})$ and an intercept of $(1/q_{\max})$.

In this study, Freundlich expression was also applied for the biosorption of Cu and Mn ions. The empirical Freundlich adsorption isotherm is obtained on the assumption that the sorption takes place on a heterogeneous adsorbent surface, where the sorption energy distribution decreases exponentially. This model is also applicable to multi-layer adsorption and is expressed by the following equation [22]:

$$q = K_f\, C_e^{\frac{1}{n}} \qquad (6)$$

where, K and n are the Freundlich constants which represent the adsorption capacity and adsorption intensity of the sorbent, respectively.

Equation 6 can be linearised as follows:

$$Ln\, q = Ln\, K_f + \left(\frac{1}{n}\right) Ln\, C_e \qquad (7)$$

Freundlich constants can be determined from Equation 7.

Adsorption Kinetics

It is very important to predict the rate at which pollutant is removed from aqueous solutions in order to design an appropriate treatment system based on adsorption process. Kinetic models are used to analysis the experimental data. The rate of biosoprtion process can be identified with the kinetic adsorption data. Many kinetic sorption models such as fractional power [23], zero and first order [24], pseudo-first order [25], Elovich [26], second order [27], pseudo-second order [28] and Lagergren [29] have been previously studied for heavy metal ions adsorption. Correlation coefficients (R^2) are used to confirm the experimental data with the model. If R^2 value is closed to unity, it means that model used for the kinetics of Cu and Mn biosorption is justified.

In this research, two kinetic models including pseudo-first order (Lagergren model) and pseudo-second order models were examined. Pseudo-first order kinetic model explains the biosorption rate based on the adsorption capacity. It is generally expressed as follows:

$$\ell og\,(q_e - q_t) = \ell og\, q_e - \frac{K_1}{2.303}(t) \quad (8)$$

where,

q_e = amount of metal adsorbed at equilibrium (mg/g);

q_t = amount of metal adsorbed at time t (mg/g);

K_1 = pseudo-first order rate constant (min^{-1});

t = time (min).

A plot of $log\ (q_e - q_t)$ versus t presents a linear relationship from which K_1 and q_e are the slope and the intercept, respectively.

The pseudo-second order model applied to biosorption as an adsorption kinetic can be written as follows:

$$\frac{dq_t}{dt} = K_2(q_e - q_t)^2 \quad (9)$$

where,

q_e = amount of metal adsorbed at equilibrium (mg/g);

q_t = amount of metal adsorbed at time t (mg/g);

K_2 = pseudo-second order rate constant (g mg^{-1} min^{-1});

t = time (min).

Integrating Equation 9 with the boundary conditions (q=0 to $q=q_t$ at $t=0$ to $t=t$) yields a linear equation as follows:

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{t}{q_e} \quad (10)$$

A plot of t/q_t versus t gives a straight line with a slope of $(1/q_e)$ and an intercept of $(1/K_2 q_e^2)$.

Fixed Bed Absorption Column Studies

A series of experiments were conducted with various natural and simulated wastewater and fungal biomass columns. The experiments involved four parts as follows:

- biosorption of single-metal from AMD;
- investigating the effect of presence of other metal ions on biosorption of one defined metal ion;
- studying the effect of flow rate on breakthrough curves and
- the desorption of metal ions.

Immobilization of Fungal into Alginate Beads

A 2 % (w/v) of sodium alginate was prepared in hot distilled water (60 ± 1 °C). After cooling, 4.5 g of biomass (dry weight) was mixed with 225 ml sodium alginate solution (2 % of biomass concentration, w/v). The mixture was dropped into 0.2 M $CaCl_2$ solution with a sampler and stirred to avoid aggregation of the beads. The resultant beads 2 mm each in diameter were cured in 0.2 M $CaCl_2$

solution at 4 °C for 24 h to complete gelatin. For the preparation of blank Ca-alginate beads, similar procedures were used at the absence of fungal biomass [30].

Experimental Set-Up

Continuous flow sorption experiments were performed in a glass column (2.5 cm internal diameter and 50 cm height). All the experiments were conducted at the room temperature. 4.5 g (dry weight) of immobilised fungal beads was packed into the column with a bed depth of 30 cm (Figure 2). The pH of metal solution was adjusted to optimum pH of each metal biosorption which was obtained in batch experiments. AMD flow rate was adjusted to 6 ml/min. Samples were taken from the effluent at pre-determined time intervals and analysed for the remaining concentration of metal ion in the effluent. The experiments were continued until a constant effluent concentration of metal ion was obtained.

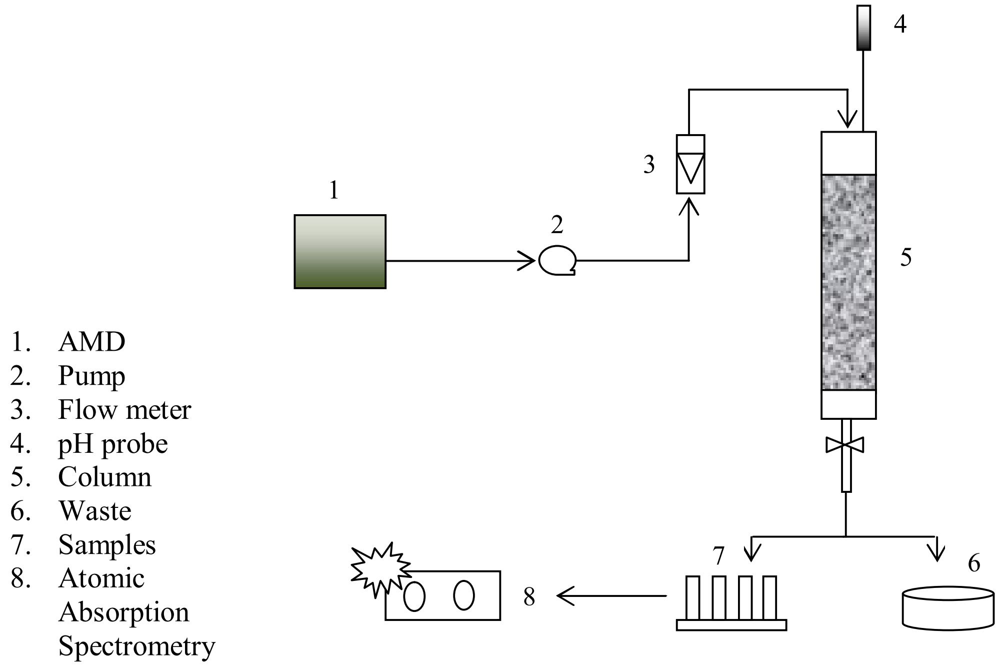

Figure 2: Experimental Set-up

Calculation of the Column Biosorption Capacity

The amount of metal ion removed by the pre-treated biomass was calculated from the experimental breakthrough curve using the following formula [31]:

$$q = \frac{C_0 Q}{1000 m_s} \int_0^t \left(\frac{1 - C|_{z-L}}{C_0} \right) dt - \frac{V_L \varepsilon C_0}{m_s} \qquad (11)$$

where,

q = average concentration of metal ion removed by the biomass (mg/g);

C_0 = initial concentration of metal ion in the solution (mg/L);

Q = volumetric flow rate (ml/min);

m_s = biomass dry weight (g);

$C|_{z-L}$= concentration of metal ion in outlet of the column (meq/L);

t = time (min);

V_L = fixed bed volume (cm^3) and

ε = column void fraction.

(11)The integral part of Equation 11 was solved numerically by the trapezoidal rule, using the experimental data from the breakthrough curve.

Mathematical Models

Several models describe the dynamic behaviour of biosorption in a fixed bed system based on the concentration profiles. In this study, the Yoon-Nelson equation was applied to biosorption columns.

Yoon–Nelson Model

The Yoon–Nelson equation is expressed as follows [32]:

$$\frac{C_e}{C_0}=\frac{\exp(k_{YN}t-\tau k_{YN})}{1+\exp(k_{YN}t-\tau k_{YN})} \quad (12)$$

where,

k_{YN} = rate constant (L/min) and

τ =*the* time required for 50% of adsorbate breakthrough (min).

This model involves a plot of $\ln(C_e/(C_0\text{-}C_e))$ versus sampling time (t). The parameters k_{YN} and τ can be obtained using the non-linear regressive method.

Results and discussions

Biosorption of heavy metals onto microbial biomass is considerably influenced by a number of factors; in term of the specific surface properties of the microbial cell wall and the physical and chemical properties of the adsorbent such as metal ions concentration, temperature, pH and the amount of biomass [33].

Effect of pH on Biosorption

pH of the solution containing a metal plays an important role on the biosorption capacity. pH values of the metal solutions (100 ml) were changed from 3.0 to 8.0 . The metal solutions were then contacted with the pretreated biomass (0.1 g) for 1 h on a rotary shaker at 150 rpm and 30 °C. After that, sample was filtered and used for analysing the dissolved metal concentrations in order to determine optimum pH value, for biosorption of each metal.

It is clear in Figures 3 and 4 that the maximum biosorption of Cu and Mn occurred at the pH range between 5.0 and 6.0 on both A. niger and Ph. chrysosporium. Although not illustrated well, the adsorption of metal ions per unit weight of biosorbent increased gradually when pH increased from 3 to 6, in general. It is due to the presence of negative functional groups like Phosphates and Carboxyl on the fungal cell's wall [34]. The biosorption of Cu and Mn ions decreased above pH 6. This may be due to the hydrolysis of metals and complex formation reactions [35].

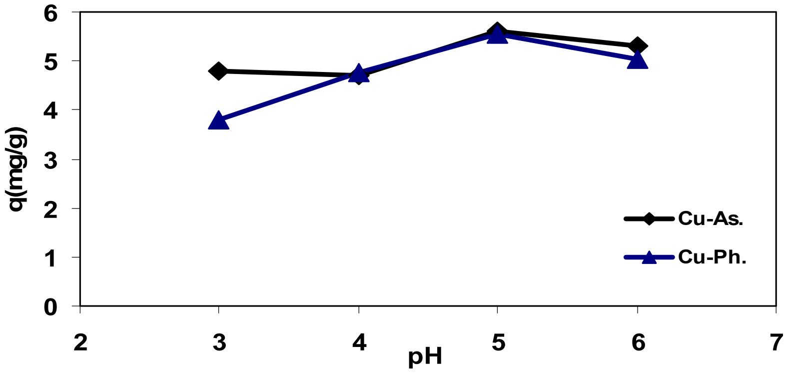

Figure 3: Effect of pH on Cu biosorption on *A. Niger* and *Ph.* Chrysosporium

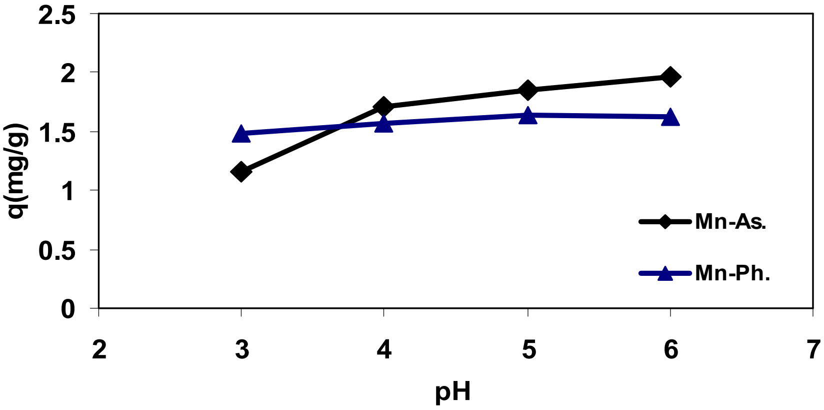

Figure 4: Effect of pH on Mn biosorption on *A. Niger* and *Ph.* Chrysosporium

Batch Kinetic Experiments

The purpose of this experiment was to determine the contact time required to attain the equilibrium between dissolved and solid-bound sorbate (*i.e.* ions). Equilibrium time is a function of many factors, including type of biomass (number and kind of biosorption sites), size and form of biomass, physiological state of biomass (active or inactive, free or immobilised), as well as the metal involved in the biosorption system.

Batch kinetic studies were conducted to determine the equilibrium time for biosorption of Cu and Mn at optimum pH value. Pretreated biomass (0.1 g) was added to metal solutions (100 ml) on a rotary shaker at 150 rpm and 30 °C. Samples were collected at different time periods varied from 10 min to 2 hours and then filtered. Filtered samples were analysed for dissolved metal concentrations in order to determine the optimum contact time.

Figures 5 and 6 show results for biosorption of Cu and Mn obtained with the milled biomass. Optimum contact time for Cu sorption was 30 min while it was 60 min for Mn sorption. During these time periods, 84.5% and 67% of Cu was absorbed by A. niger and Ph. Chrysosporium respectively. Results also show that over 120-minute period, 96% and 97% of total sorption of Mn occurred by A. niger and Ph. Chrysosporium respectively. So, this time was used for kinetic studies. Figure 5 shows that sorption can be divided into two stages: one in which the sorption rate is very high (79% of biomass saturation capacity for sorption of Cu was reached in a contact time of 15 minutes), followed by a second stage with a much lower sorption rate. This behaviour has been reported by other researchers. Crist et al. [36] and Crist et al. [37], observed that proton uptake by algal cells consists of two processes, a fast surface reaction and a slow diffusion of protons into the cells. As Figures 5 and 6 shows, A. niger was more successful in the biosorption process compared with Ph. Chrysosporium. In order to investigate kinetic effects, Lagregren pseudo-first and pseudo-second order equations were used to calculate kinetic constants. The results are given in Table 2. The high value of R^2 for pseudo-second order model for both biosorbent types suggests that the biosorption of Cu and Mn on *A. niger* and *Ph.* Chrysosporium can be well described by pseudo-second order kinetics model.

Effect of Temperature on Cu and Mn Biosorption

Effect of temperature on Cu and Mn uptake capacity of *A. niger* and *Ph. chrysosporium* was studied. The metal solutions (100 ml) at optimum pH value were contacted with the pretreated biomass (0.1 g) for 1 h on a rotary shaker at 150 rpm. As Figures 7 and 8 show the removal of both metal ions increased with rising temperature up to 40 °C. Since the biosorption of Cu and Mn was endothermic thus the amount of adsorption increased with increasing temperature [38].

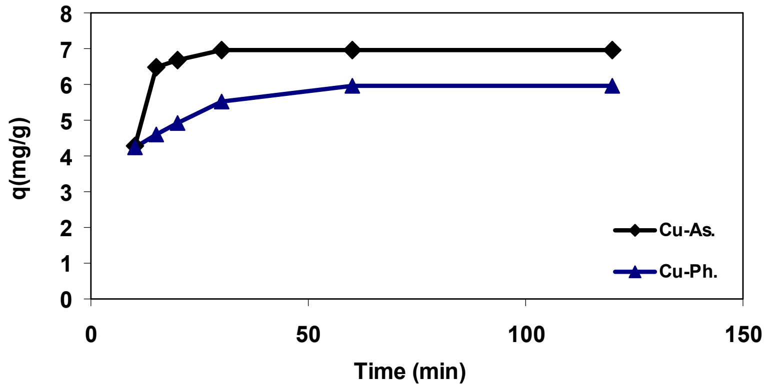

Figure 5: Effect of contact time on Cu biosorption on *A. Niger* and *Ph.* Chrysosporium

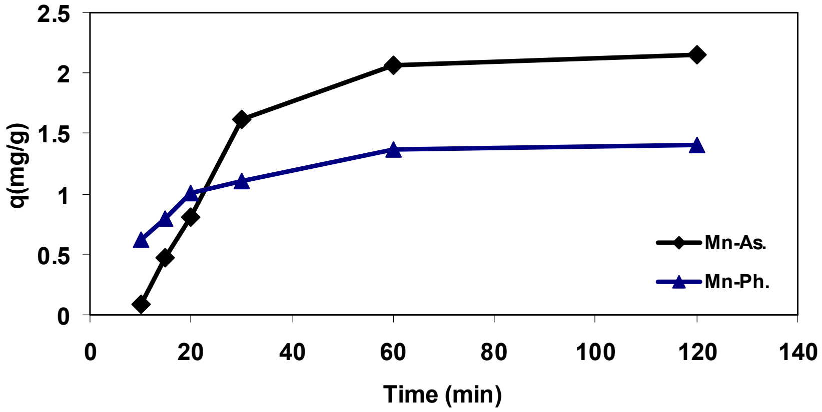

Figure 6: Effect of contact time on Mn Biosorption on *A. Niger* and *Ph.* Chrysosporium

Table 2: Kinetics constants

Metal ion	Biomass	Pseudo-first order model Constants		Pseudo-second order model constants	
		K_1	R^2	K_2	R^2
Cu	As.	0.204967	0.958	0.051523	0.997
Cu	Ph.	0.105938	0.983	0.035262	0.999
Mn	As.	0.057575	0.957	0.012810	0.993
Mn	Ph.	0.057575	0.993	0.004384	0.997

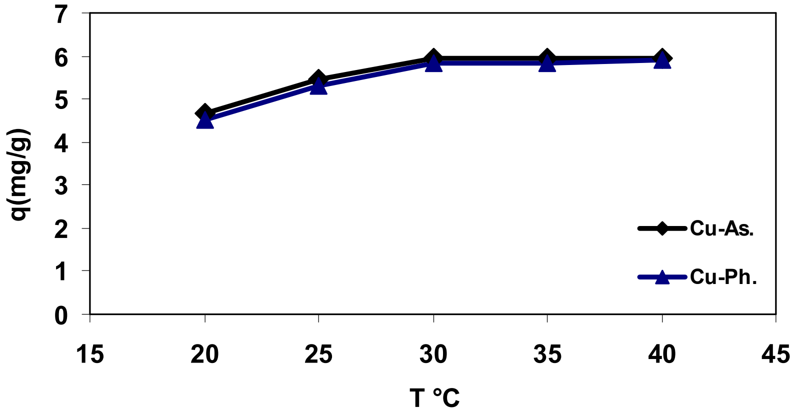

Figure 7: Effect of temperature on Cu biosorption on *A. Niger* and *Ph.* Chrysosporium

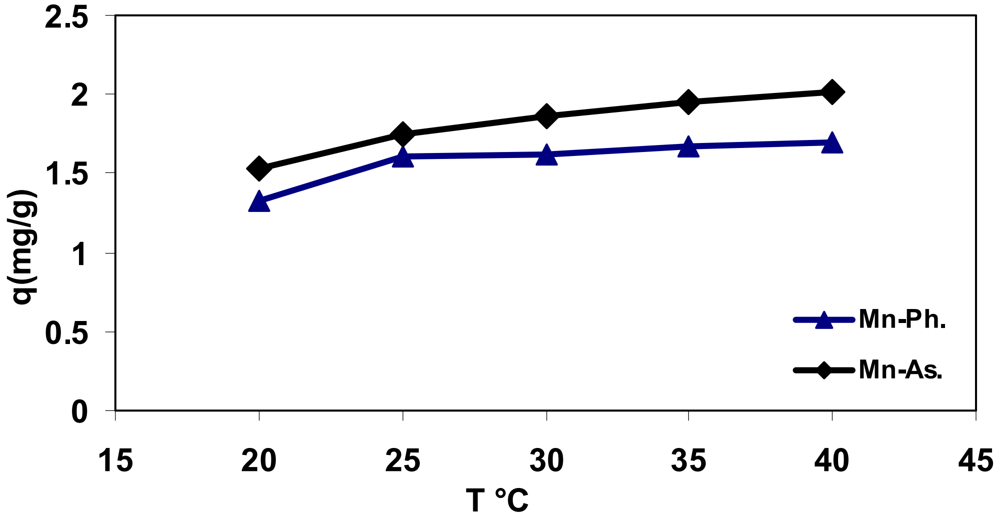

Figure 8: Effect of temperature on Mn biosorption on *A.Nniger* and *Ph.* Chrysosporium

Effect of Biosorbent Concentration

A concentration range of 1-4 g/lit was used for biomass in the solution to find out the metal biosorption capacity of the treated biomass. Four samples containing varying amounts (0.1-0.4 gr) of pretreated biomass were contacted with known volumes (100 ml) of metal solutions. Isotherm studies were conducted for Cu and Mn in the separate solutions at optimum pH value.

Results on the influence of biomass dose showed that the amount of metal bound per g of biomass in mg g^{-1} increased as the biomass concentration was increased. It is due to an increase in the adsorption surface area.

Increasing trend in biosorption of chromium, nickel, calcium, and iron with rising concentrations of A. niger has been reported by previous researchers as well [39]. The present observations (Figures 9 and 10) are in conformity with those reported by Basci et al. [40] for Cu biosorption by wheat shells.

Figure 9 shows that the maximum biosorption efficiency was obtained at 3 g/L of biosorbent for Cu ion biosorption and further increases in biosorbent concentration decreased the maximum removal of this metal. This can be explained by forming aggregates during the biosorption process which take place at higher biomass concentrations; causing a decrease in the effective adsorption area. It has been suggested that several factors including pH, ionic strength, temperature, metal ion in solution and biomass concentration explain the decreased adsorption capacity at increasing biomass.

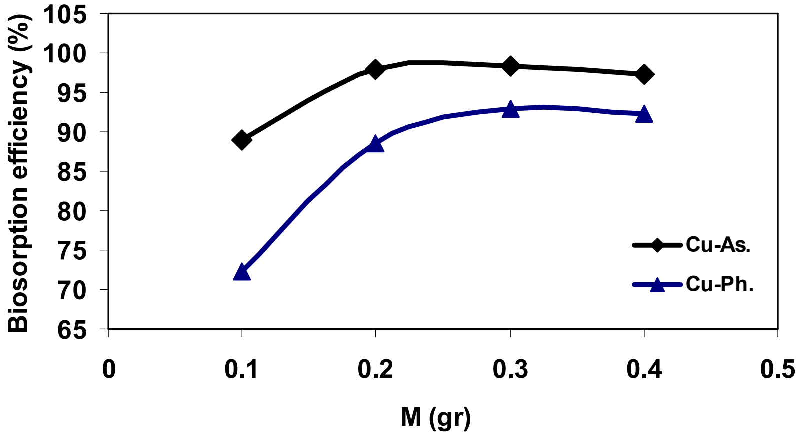

Figure 9: Effect of biosorbent concentration on Cu biosorption on *A. Niger* and *Ph.* Chrysosporium

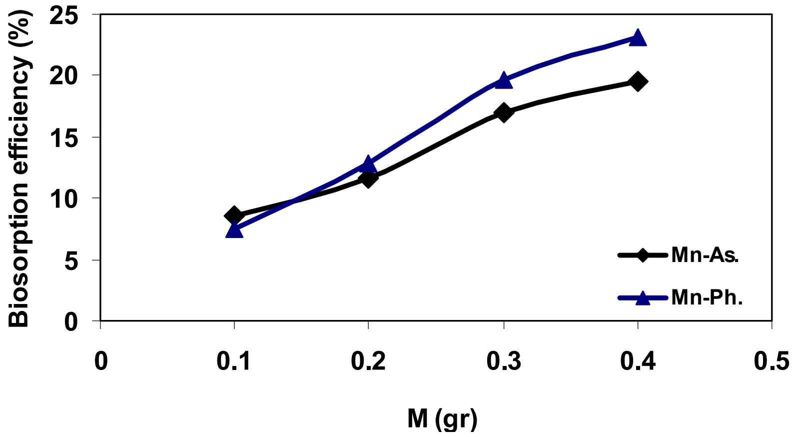

Figure 10: Effect of biosorbent concentration on Mn biosorption on *A. Niger* and *Ph.* Chrysosporium

Biosorption Isotherm

Langmuir and Freundlich sorption models were selected for the estimation of maximum metal biosorption by the biosorbent. The empirical parameters of these two isotherms along with the correlation coefficients (R^2) are given in Table 3. Results show that the biosorption process follows well the Langmuir isotherm model.

Desorption and Regeneration

The regeneration of the biosorbent is expected to be a key factor in accessing the potential of the biosorbent for commercial application. The capacity of the biomass to adsorb metal ions was determined by repeating the adsorption experiments in five successive cycles. HNO_3 (10 mM) solution was used as a desorption agent [41]. The observations show that higher than 97% desorption was obtained at the end of five adsorption-desorption cycles. The biomass conserved well metal adsorption capacity even after five cycles. Desorption ratio can be expressed as:

$$R_{Des} = \frac{q_d}{q_a} \times 100 \tag{13}$$

where,

R_{Des} = desorption ratio (%);

q_d = amount of metal ions desorbed to the elution medium (mg/g) and

q_a = amount of metal ions adsorbed onto the beads (mg/g).

Fixed Bed Study

The characteristic parameters of the fixed bed biosorption were examined to obtain data to optimise the processes in fixed bed column for biosorption of Cu and Mn.

Effect of Volumetric Flow Rate

The breakthrough curves at two different flow rates are shown in Figure 11. It is well illustrated that a breakthrough curve generally occurred faster with higher flow rate. Breakthrough time to reach saturation increased significantly with a decrease in the flow rate. For a lower rate of influent, metal ions had more time to contact with biomass and resulted in higher removal of metal ions in the column [42]. At higher speeds, more data containing reading errors were observed. As well shown in Figure 11, for lower flow rate (4 ml/min), experimental data fit well to the calculated curve.

Effect of Co-Adsorption

Industrial wastewater usually contains more than one type of metal ions. Therefore, the effect of co-adsorption of multiple metal ions on biosorption of individual ions was also evaluated [43].

For this purpose, a synthetic single-ion solution of Cu was prepared using $CuSO_4$. Table 4 compares the removal of Cu from AMD with a synthetic single-ion solution. This table shows that when there are different metal ions in AMD, column will be saturated sooner so τ in AMD column is less than the synthetic single-ion solution column. The reason is that sorption sites will be occupied by other metal ions.

Table 3: Linearised parameters of Langmuir and Freundlich isotherms for Cu and Mn biosorption on *A. Niger* and *Ph.* Chrysosporium

Metal ion	Biomass	Langmuir			Freundlich		
				Parameter			
		q_{max}	b	R^2	K_f	n	R^2
Cu	As.	8.598	2.103	0.9969	7.195	19.685	0.8373
Cu	Ph.	8.183	1.808	0.9952	6.597	15.504	0.7992
Mn	As.	2.381	0.347	0.9998	0.854	3.905	0.8493
Mn	Ph.	1.823	0.305	0.9912	0.499	2.319	0.9764

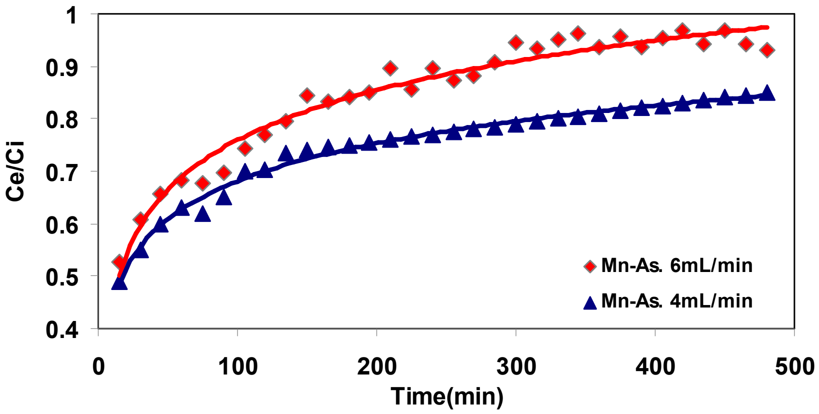

Figure 11: Breakthrough curves for biosorption of Mn onto immobilised biomass of *A.Niger a*t two different flow rates

Table 4: Comparison of τ in AMD and synthetic single-ion solution

Metal ion	Biomass	τFor AMD	τ for a synthetic single-ion solution
Cu (II)	As.	1005	1930.5
Cu (II)	Ph.	1464	2501

Table 5: Yoon-Nelson model parameters

Metal ion	Biomass	τfor AMD	τ for a synthetic single-ion solution
Cu (II)	As.	1005	1930.5
Cu (II)	Ph.	1464	2501

Kinetics Model of Biosorption

A simple theoretical model developed by Yoon–Nelson was applied to investigate the breakthrough behaviour of metal ions on fungal biomass. So, the values of k_{YN} (a rate constant) and τ (the time required for 50% of adsorbate breakthrough) could be obtained using a non-linear regressive analysis [44] from Equation 12. The values of k_{YN} and τ are given in Table 5.

Table 5 shows that biosorption rate was much faster (about 4 times) for Mn and the column was also saturated sooner. It also shows that the rate of biosorption is 2 times faster for A. niger than that for Ph. Crysosporium.

Desorption and Reuse

Once the biomass was saturated with metal ions in the column, it was important to regenerate the biomass for the recovery of metal ions as well as the use of the biomass for further biosorption [45].

The column desorption studies were carried out by passing a 0.5 M HNO_3 solution with a flow rate of 1 mL/min. The average residence time in the column was approximately 180 min.

Conclusions

The present study evaluated the removal of Cu and Mn from AMD of the Sarcheshmeh porphyry copper mine using A. niger and Ph. Crysosporium cells. The pretreatment of biomass was performed by boiling in 0.5N NaOH solution for 15 min. Live or dead fungal biomass can be used for the removal of toxic metal ions. The use of dead cells seems to be more advantageous than using live cells in metal ion removal. It was seen that initial pH, temperature and initial metal ion concentration highly affected the biosorption capacity of the sorbent. A. niger and Ph. chrysosporium were sensitive to pH. A wastewater with a pH between 5.0 and 6.0 can be effectively considered for metal ion removal. Wastewaters with a pH lower than 5.0 would require pH adjustment. Biosorption capacity increased as metal ion concentration and temperature increased. Optimum contact time for biosorption of Cu was 30 min while it obtained 60 min for Mn biosorption. The equilibrium biosorption of Cu and Mn ions onto the biosorbent were tested using Langmuir and Freundlich models. Langmuir model accurately fitted the experimental data. Desorption of heavy metals were easily performed, and the biomass could be regenerated and used again. Kinetic studies showed that second-order kinetic equation can truly represent biosorption kinetic process. It was seen that biosorption of metals is a two phase process. It is rapid first and then it will be gentle. Subsequent studies showed that, presence of other metal ions will reduce sorption capacity of one particular metal ion. Furthermore, higher flow rate will lead to sooner breakthrough that can save more time. Using a continuous system for biosorption, increases efficiency, especially for Mn. It became possible to adsorb 99% of Cu and 50% of Mn. However, in a batch system, only 20% of Mn was adsorbed. In general, Cu was absorbed better than Mn, and A. niger has a higher capacity of sorption comparing the biosorption by Ph. Chrysosporium.

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