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Removal of Zn(II) by modified *Rhizopus arrhizus*

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ABSTRACT

This study shows the potential of modified *Rhizopus arrhizus* (MRA) for the removal of Zn (II) in water and to investigate the effect of various parameters such as temperature, initial pH, initial concentration, and co-ions. Additionally, kinetic studies and adsorption isotherms have been studied in order to investigate the mechanism of the biosorption process. The results revealed that biosorption on MRA could substantially remove the Zn(II) in water. The biosorption have been found to be pH dependent. The uptake of Zn(II) on MRA increased with the increase in pH. The effect of initial concentration was studied by varying it from concentration 2 to 100 mg/l. The results indicated that the uptake of Zn(II) increases with the increase in initial concentration. The temperature variation (5–40˚C) showed Zn(II) uptake on MRA follows an increasing trend. The process was found to be independent of the presence of co-ions. The kinetics studies showed that the uptake of Zn(II) on MRA follows pseudo-second-order kinetics. Results further revealed that the adsorption of Zn(II) on MRA fitted best on the Freundlich isotherm model (R^2 = 0.998). Therefore, it is concluded that MRA is an affective biosorbent for the removal of Zn(II) in water.

Keywords: Biosorption; Kinetic and equilibrium studies; *Rhizopus arrhizus*; Freundlich isotherm mode

1. Introduction

Presence of heavy metals in aquatic systems has been identified as a lethal consequence of various industrial activities. Heavy metals like lead, chromium, mercury, nickel, zinc, manganese, and copper, when digested in excessive quantities, lead to acute diseases [1–3]. Zinc is an essential element required in trace amounts for the growth of living. The highest acceptable limit of zinc in drinking water is 5 mg/l and becomes toxic when its intake goes up to 100–500 mg/d [4]. Major sources of zinc in industrial wastewater are mainly processes involving acid mine drainage, processing of natural ores, municipal

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wastewater, galvanizing plants, etc. It is an unremitting waste which can accumulate throughout the food chain [5,6].

Heavy metal removal has been practiced decades before the usage of various conformist techniques such as reverse osmosis, ion exchange, membrane separation, precipitation, and evaporation. These techniques are economical when employed to remove heavy metal loads at high concentrations. However, they are quite expensive when practiced at lower metal concentration [7–10]. Therefore, in order to meet the stringent wastewater quality standard, it is indispensable to have some unconventional techniques which should be cost-effective and environment-friendly.

Biosorption is a technique which has been found to be effective at low concentration [8]. The characteristic feature of biosorption is the removal of a substance by dead biomass. Various biomass materials like sludge, dead micro-organisms, and agri-wastes are being tested for the removal of various heavy metals from synthetic as well as real wastewater [9,11]. *Rhizopus arrhizus* (RA) belongs to the Fungi class of micro-organisms and has been extensively investigated. It was investigated that cell wall of RA is of great importance as it plays a dominant role in the removal of metals. Different compounds that take part in metal sequestration are chitosan-chitin entities, amino acids, lipids, various uranic acids having carbonyl groups, and functional groups having ionizable parts like phosphate [3,10,12].

Current investigation reconnoiters the potential of modified *Rhizopus arrhizus* (MRA). It was selected because it can be produced inexpensively and has a nonpathogenic effect on both humans and animals [10]. The objective of the existing work is to study the effects of pH, contact time, initial concentration, temperature, co-ions, desorption with HCl, and the reuse of MRA on the uptake of Zn(II). Kinetic and equilibrium modeling was performed using classical models. The correlation coefficient (R^2) was used to show the goodness of the fit.

2. Materials and methods

2.1. Development of modified Rhizopus arrhizus (MRA)

Cells of RA were immobilized on a slab made of polyurethane with dimensions of 80 mm × 25 mm × 10 mm with 20 mesh no. An L-shaped supporting stainless steel wire was used to place a prerogative slab of polyurethane foam, horizontally, at the bottom of a 500-ml conical flask. These flasks, secured with cotton wool and concealed with aluminum foil, were autoclaved for 20 min. The growth medium, restraining glucose (30 g/L), yeast extract (10 g/L), malt extract (10 g/L), and double distilled water, were prepared in a 2-l flask, covered with cotton wool and an aluminum foil on the top. The flask was then sterilized (autoclaved at 121˚C, 15 psig for 20 min) and was left to cool. About 100 ml of growth medium was poured into sterilized conical flasks containing convolute polyurethane foam under clean conditions in a flow cabinet.

A 0.5% V/V inoculums of spore suspensions of *Rhizopus arrhizus* was added by means of a micropipette to 100 ml liquid medium in each flask. The flasks were then incubated at 300˚C and 200 rpm in an orbital shaker for around 48 h. The mycelia, while growing in the bulk liquid, were entrained in the open pore network of the foam matrix, which at the end of growth were immobilized within the foam matrix. The medium was then drafted, washed twice with double distilled water, and inactivated with 1% V/V formaldehyde to prepare the modified *Rhizopus arrhizus* (MRA) and stored for further experimentation.

2.2. Chemicals and biosorption studies

Zinc solution was prepared by dissolving a precalculated quantity of analytical grade zinc salt. Effect of pH was anticipated between 2 and 7 using a 100 ml analyte with a 100 ppm copper concentration at 30 ± 2˚C and 200 rpm and 80 min. To study the effect of contact time, 100 ml analyte with 30 ppm zinc concentration was used. Samples were taken out at different intervals of time, ranging from 5 to 80 min. Effect of the initial zinc concentration was studied between 2 and 100 ppm. Impact of temperature was observed between 5 and 40˚C. Unconsumed metal concentration was discerned using an atomic absorption spectrophotometer.

1 M solution of KOH and HCl was used to maintain the pH. In spite of this, the pH of 6.5 was used in the testing of all samples. For each experiment, immobilized biomass was thoroughly scrubbed with double distilled water to discard any formaldehyde solution before the biosorption experiments.

3. Results and discussion

3.1. Effect of initial pH

Fig. 1 elucidates that the removal of Zn(II) is affected by the initial pH of the solution and the variation in the pH during the study. It shows that uptake of Zn(II) by MRA increased with increase in pH. Trend of following the uptake of Zn(II) has two distinct regions. In the first region, as the pH

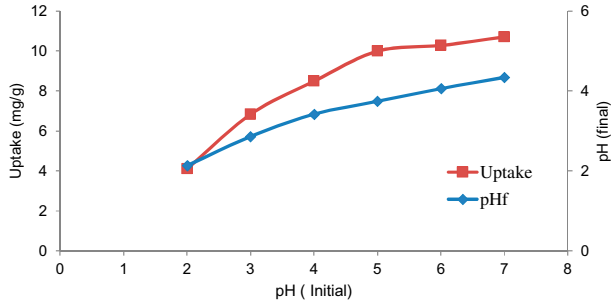

Fig. 1. Effect of initial solution pH on the Zn(II) uptake and final pH.

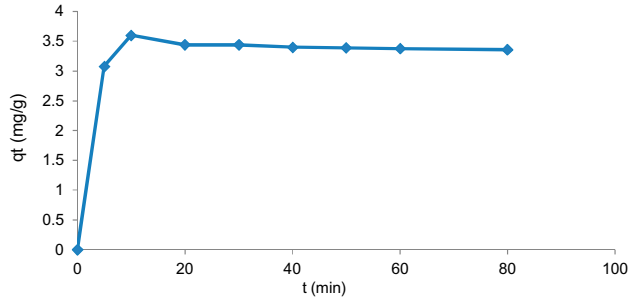

Fig. 2. Effect of contact time on Zn(II) MRA.

increased from 2 to 5, an appreciable increase in Zn(II) uptake was observed; from 4.10 to 9.99 mg/g, as shown by the linear portion between pH 2 and 5. However, in the second region, uptake increased only by 0.71 mg/g, when pH increases from 5 to 7. A low pH value is an indication of higher concentration of H^+ ions in the system. These ions are highly mobile and can occupy any vacant negatively charged space. Thus, due to the protonation of active sites of MRA less amounts of Zn(II) were picked up by it. However, uptake of 4.10 mg/g at pH 2 is an indication that ion exchange of H^+ is not the only mechanism for the sequestration of Zn(II) by MRA. At a higher pH, the binding groups of MRA contains a negative charge due to deporotonation, resulting in a higher Zn(II) uptake, which has been observed [13]. Fungi of each group have its distinct composition and structure of its cell wall. However, it has been found that two basic types of binding compounds may be identified in each type i.e. glucosamines (a Chitin-chitosan unit, proteins, and amino acids) and ionizable binding groups (phosphate and carbonyl groups of uronic acids) [10,14]. In addition to uptake of Zn(II) for a given value of initial pH, the corresponding final pH is also helpful in understanding the removal mechanism. Generally, the value of initial pH was greater than the final value of the pH, which may be due to exchange of H^+ ions with Zn(II). It supported the hypothesis of the existence of multiple Zn(II) removal mechanisms by MRA [10,15]. Since the uptake between pH 6 and 7 was not appreciable, a pH value of 6.5 was taken as optimum for the study of other conventional parameters.

3.2. Biosorption kinetics

For the designing and selection of optimum operating conditions for batch metal removal processes, one of the most important factors is the kinetics of metal biosorption. Fig. 2 shows the biosorption kinetics of Zn(II). Time progression of the metal biosorption depends on the nature of biosorbent. It is already known that for mesophilic organisms, equilibrium appears in less than 15 min and the removal is independent of its metabolism [16]. However, in the current study, more than 90% (3.08 mg/g) of the equilibrium uptake (3.36 mg/g) was achieved within 10 min as shown in Fig. 2. Similar results have been reported elsewhere. Biosorption of Zn(II) onto MRA involves multiple steps as can be seen in Fig. 2. The initial rapid step attributes to the removal of the Zn(II) by the vacant binding sites present on the surface of MRA followed by a slower step indicating intraparticle diffusion. Equilibrium was achieved in 60 min, as no improvement in the uptake was observed after this length of contact time. Similar pattern has been observed elsewhere [10].

Biosorption kinetics was simulated through Elovich, pseudo-first-order and pseudo-second-order kinetics. The correlation coefficient calculated for pseudo-first order was very low i.e. 0.209; shows inapplicability of this model for the Zn(II) biosorption onto MRA.

Equation for pseudo-second-order kinetics developed for the sorption process is given below [17]:

$$q_t = q_e\left(\frac{q_t k_2 t}{1 + q_t k_2 t}\right) \tag{1}$$

Linearized form of above equation is:

$$\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{k_2 q_e^2} \tag{2}$$

There are two main concepts behind the application of this equation. First, the amount of binding site occupied by the metal ions has a direct relation with the biosorption uptake, while the second is the chemisorption, the rate-controlling step. A plot between t/q_t and

t is shown in Fig. 3. q_e (3.359 mg/g) calculated from the slope of the graph approximately equals to the observed $q_{e,exp}$ (2.96 mg/g), indicating an excellent fitting ($R^2 = 0.9883$) of this model to the kinetic data. Rate-controlling step can be found out with help of the following simple model given by Weber and Morris [18] as:

$$q_t = k_{id} t^{0.5} + C \quad (3)$$

Weber and Morris show that if the graph between q_t and $t^{0.5}$ appears to be a straight line, then intraparticle diffusion would be the rate-controlling step. Where in the above case Fig. 4 shows multiple linearity of the data, which is an indication that intraparticle diffusion is not the rate-controlling step. So it may be concluded that Zn(II) onto MRA is due to chemisorption, which also limits the rate of biosorption.

3.3. Effect of initial concentration

The effectiveness of a biosorbent can be judged by its interaction with the metal solution of various known initial concentrations. Therefore, a number of experiments were conducted with initial concentration of Zn(II) varying from 2 to 100 mg/l. Fig. 5 represents a typical trend of q_e vs. C_e. It shows a weak interaction of MRA and Zn(II). However, the uptake of Zn(II) increases from 0.613 to 56.352 mg/g when its initial concentration is varied from 2 to 100 mg/l. This revealed the vocation of Zn(II) on empty sorption sites. Langmuir and Freundlich isotherms were employed to reveal Zn(II) sequestration mechanism by MRA. Langmuir isotherm is based on the simple assumption that all the binding sites are similar and metal rests upon the biomass surface in a monolayer. A linearized form of Langmuir isotherm is as under [18]:

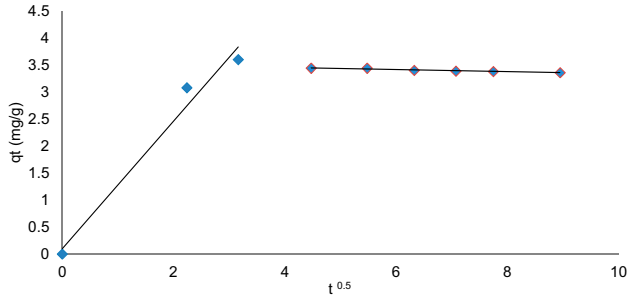

Fig. 4. Pore diffusion model for the Zn(II) biosorption on MRA.

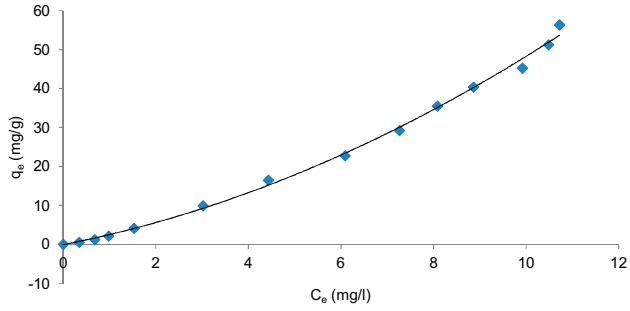

Fig. 5. Effect of initial concentration of the Zn(II) on uptake by MRA.

$$\frac{C_e}{q_e} = \frac{1}{bq_{max}} + \frac{C_e}{q_{max}} \quad (4)$$

q_{max} and b are Langmuir constants, and represents the maximum biosorption capacity and the affinity of a biosorbent for the adsorbate. These parameters are calculated from the slope and intercept of the graph of C_e/q_e vs. C_e as shown in Fig. 6. Values of q_{max} and b were 48.9027 mg/g and 0.05083, respectively. Langmuir model showed a fairly good fitting to the data

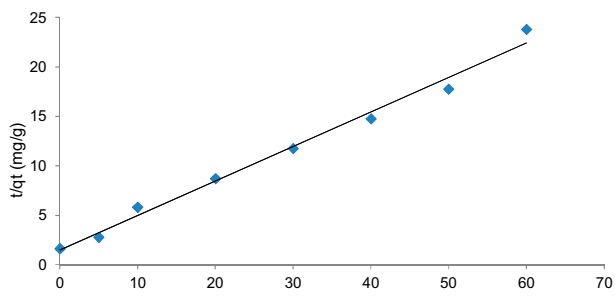

Fig. 3. Pseudo-second-order kinetics for the Zn(II) uptake on MRA.

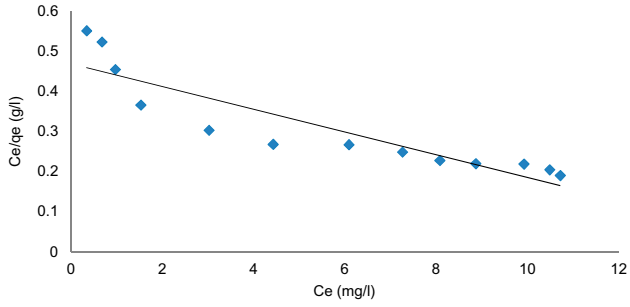

Fig. 6. Application of the Langmuir model for Zn(II)-loaded MRA.

due to the aid of fit R^2, the coefficient of correlation, was 0.816. This showed the possibility of the removal of Zn(II) by MRA in the form of a single layer. Since the R^2 is not unity, there is a need to search for a better description of the process. On contrary to the Langmuir model, Freundlich model is based on binding site heterogeneity and can be written in the following linearized form [18]:

$$\log q_e = \log K_F + \frac{\log C_e}{n} \quad (5)$$

K_F and n are Freundlich constants, and can be calculated from the slope and intercept of the graph between $\log q_e$ and $\log C_e$ as show in Fig. 7. K_F and n were 2.43 and 0.7859, respectively. The value of the correlation coefficient was 0.9988 which showed that the Freundlich model described the Zn(II) biosorption onto MRA better than Langmuir. This is in agreement with the fact that multiple binding sites (carboxylic, amines, carbonyl, and phosphate groups) exist, as discussed earlier in Section 3.1.

3.4. Effect of temperature

Fig. 8 shows the effect of temperature on the biosorption of Zn(II) onto MRA. It can be seen that effect of temperature is relatively less on the uptake as compared to the pH. However, an increase in uptake can be observed with increase in temperature. This indicates that the process of Zn(II) removal by MRA is endothermic. A number of reasons may be associated to the increase in the uptake: (1) increase in the number of active sites due to bond rupture at higher temperatures, (2) increase in the kinetic energy of the metal ion resulting in ease of approach of Zn(II) to binding sites of MRA, (3) ease in attaining the activation energy resulting in the formation of stable complexes, and (4) widening of pores facilitating pore diffusion [16,19,20]. Since the process for the biosorption of Zn(II) onto MRA is complex as pointed in Section 3.1, a combination of aforementioned mechanisms may be there to promote the uptake of Zn(II) onto MRA. Since the increase in uptake from 5 to 40°C is just 1.36 mg/g, it may be assumed that the biosorption of Zn(II) onto MRA was mainly taken place by some manner of energy-independent mechanisms.

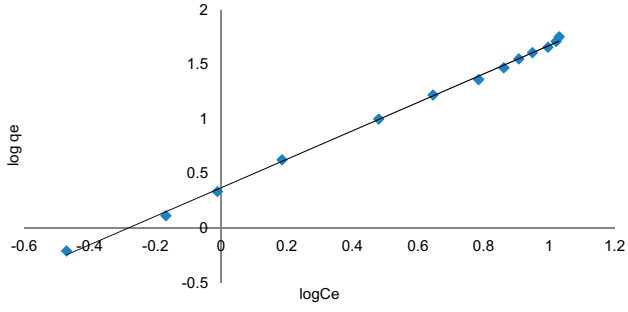

Fig. 7. Application of the Freundlich model for Zn(II)-loaded MRA.

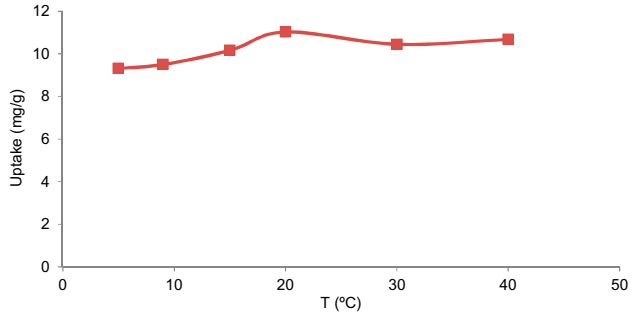

Fig. 8. Effect of solution temperature on the uptake of Zn(II) on MRA.

4. Conclusions

From the above discussion it can be inferred that the MRA appears to be a good biosorbent for the removal of Zn(II) ions in water. The following facts can be concluded: (1) the biosorption of Zn(II) ions uptake increases from 4.10 to 10.70 mg/g by increasing the initial pH from 2 to 7, respectively, (2) the Zn (II) ions uptake is expected to follow pseudo-second-order kinetics model, (3) it was analyzed that by increasing the temperature from 5 to 40°C the metal uptake gradually increased from 9.32 to 10.68 mg/g, (4) metal ion uptake increased from 0.613 to 56.352 mg/g varying initial concentration from 2 to 100 mg/l, and (5) the experimental data best fitted in Freundlich isotherms showing R^2 value close to unity.

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