The use of *Aspergillus oryzae* for Iron removal from wastewater

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ABSTRACT : Heavy metals are natural elements in the earth's crust. Some of these heavy metals are known as pollutants and are toxic, therefore, their amount in water should always be under control. One of this expulsion toxic elements is iron. As known microorganisms perform an important function in the bioremediation of contaminated water. Among of these microorganisms that are capable of bioremediation of heavy metals are *A. oryzae*. The aim of this study is to investigate the conditions for bioremediation of one of these heavy metals iron in water. Results showed that powder *A. oryzae* can be used for removal of iron from polluted water. As the dried A. oryzae in concentration 1g/100 ml was found to be more effective in the removal of iron from water at pH 3 with contact time 90 minutes. the *A. oryzae* successfully removed iron and has ability to be regenerated and reuse in the removal process.

Keywords: mm*Aspergillus oryzae*; Biosorption; Bioremediation; Iron; Water treatment; Adsorption kinetics.

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I. INTRODUCTION

Heavy metal ions are highly increased and spread in our environmental as a result of urbanization and industrialization such as electroplating, steel manufacturing, wood preservation, tanning and glass manufacturing (Brusseau et al., 2019; Cao et al., 2018; El-Beltagi et al., 2020; Olawale S.A., 2021; Qasem et al., 2021; Su et al., 2019; Wang et al., 2020; Wang, Y. and Huang, K., 2020; Zglobicki W., 2022; Zhang Y and Duan X., 2020). The presence of such as metal ions in the environment generally induces physiological changes in water and soil. These toxic metals accumulated in crops via food chain; entrance our bodies, and cause several illnesses (Azimi A and Azari A., 2017; Bolisetty et al., 2019; Fierro et al., 2021; Huang et al., 2019; Jai et al., 2018; Moldovan et al., 2021; Taseidifar et al., 2017).

Iron is one of the most spreading toxic heavy metals. It is the reason of about up to 5% of the earth's crust. Iron is indeed essential as it plays an important role in electron transfer process and enzymatic activities (Prashanth et al., 2015; Bourzama et al., 2021; WHO, 2017) but in high dosages, it can cause several troubles for human health (Marsidi, 2018). Hence, iron must be uptake or transformed to less toxic form in water before using in irrigation or before being discharged to the environmental. There is no health-based guideline in all the world for the concentration of iron in drinking water. However, based on taste and nuisance considerations the World Health Orgnisation (WHO) and the U.S. Environmental Protection Agency (EPA) recommend that the concentration of iron in drinking water should be less than 0.3 (WHO, 2017; EPA, 2000).

Several mechanisms are used to remove or decrease the amount of iron from water such as oxidation with chlorine and potassium permanganate treatment with limestone, ion exchange, activated carbon, liquid-liquid extraction, chemical precipitation, some filtering materials and bioremediation (Hassouna et al., 2018; Renu et al., 2017; Shamim S, 2018; Tran et al., 2017; Zglobicki W., 2021). These physical and chemical processes are expensive in case of lower concentration of heavy metal (Li et al., 2013). The biological processes are economical and high efficiency for remediating single or binary metal solutions (Bulgariu and Bulgariu, 2012; Mishra and Malik, 2012). Different fungi biomass and other microorganisms were used as biosorbents for biosorption of heavy metal due to its ability of the regeneration and recovery of the metals besides it is highly efficient, economic and a friend of the environment (Daiz-Alarcon et al., 2019; Farrag et al., 2017; Hassouna et al., 2018; Kalak et al., 2020; Krishna et al., 2019; Kulakovskaya et al., 2018; Musah et al., 2022; Wang Y and Huang k, 2020; Zada et al., 2021). Different fungi are not utilized only in the fermentation industries (antibiotics, organic acids, enzyme production and food industries), but are also known for its applicability for biosorption of heavy metals (Brown et al., 2001). It should be mentioned that biosorption process depend on different factors such as biomass dosage, contact times, initial concentrations of heavy metal and physic-chemical conditions. Several studies before provide the ability of *A. oryzae* in removal of different heavy metal

such as copper, cadmuim, lead, zinc, nickel, manganese and aluminum etc (Ankita C. and Jayanthi A., 2016; Gunjal A. et al., 2017; Gunjal A., 2021; Mahmoud et al., 2017; Mohammad et al., 2012; Sinha et al., 2019).

The aim of the present study was to examine the physicochemical properties of different types of mycelia of fungi *A. oryzae*. Furthermore, the purpose was to study the efficiency of bioremoval of iron (Fe^{+3} ions) from aqueous solution by different biomass under different conditions of adsorbant dosage, initial concentration, pH and contact time. Additionally, the biosorption kinetics, equilibrium and Langmuir, Freundlich and Tamkin isotherms were analyzed. Also, the nature of iron biosorption was studied.

.II. Materials and Methods

2.1. Materials:

2.1.1. Reagents and materials

Potato dextrose medium (PDA), Ferric nitrate ($Fe(NO_3)_3.9H_2O$, assay 99%) [Alpha Chemika, India], EDTA (assay 99%) [ADWIC, Cairo], Magnesium Sulphate ($MgSO_4$, assay 98%) [Adwic, Cairo], Ammonium Chloride (NH_4Cl, assay 99%) [Adwic, Cairo], Ammonia Solution (assay 33%) [Adwic, Cairo], Sodium Acetate Anhydrous ($NaCH_3COO$, assay 99%) [Adwic, Cairo], Acetic Acid (glacial assay 98%) [Alpha Chemicals, Cairo], Salicylic Acid (assay 99%) [Adwic, Cairo], Acetone (assay 99%) [Adwic, Cairo], Hydrochloric Acid (HCl, assay 30-34%) [Researchlab, India], Sodium Hydroxide (NaOH, assay 98%) [Alpha Chemicals, Cairo] and Bi-distilled water was used for preparation and dilution of all the prepared solutions.

2.1.2. Instruments:

Atomic Adsorption Spectrophotometer (AAS) [model 969 AA Spectrometer, Unicam, 1999) and pH meter.

2.2. Apparatus and procedure:

2.2.1. Preparation of fungal biomass

Aspergillus oryzae was isolated from plants "*Endophyetic fungi*" from *Ficus elastica* (El-Sayed et al., 2021). These fungal isolates were morphological & molecular identified (El-Sayed et al., 2019) and stored as fungal stocks cultures as slope culture at 4°C.

Potato dextrose medium (PDA) was used for the growth of *A. oryzae*. The slants were incubated for 7 days at c. The cultures were maintained at 4°C and sub-cultured every 14 days. A plug (4-mm diameter, equal 2 $\times 10^7$ spore) was inoculated into a sterilized potato dextrose medium. The flasks were incubated for 14 days at 30°C. At the end of incubation period, the fungal biomass was filtrated by filter paper, washed several times with distilled water, and dried in oven at 55°C for 24h and ground with a mortar to make powder biomass.

2.2.2. Preparation of heavy metal solution

The stock solution of Fe^{+3} ions (0.1 M) was prepared by dissolving 10.1 g of $Fe(NO_3)_3.9H_2O$ in 250ml of distilled water. Other concentrations prepared from the stock solution by dilution varied between (10^{-2}, 10^{-3}, and 10^{-4}).

2.2.3. Analytical methods

Calculation of removal percent (%)

The amount of Fe^{+3} before and after the adsorption were determined by either titration against EDTA or atomic absorption spectrophotometer analysis.
The removal percent were calculated by using the following equation:
$$\text{Removal percent (\%)} = ((C_i - C_e)/C_i)*100 \quad (1).$$

Effect of powder biomass weights

Several powder biomass weights (0.5, 1, 1.5, and 2g) for *Aspergillus oryzae* were tried. The Fe^{+3} removal was studied by addition of the weighed amount of the powder biomass to 100 ml of (10^{-2} M) of Fe^{+3} solution and shaked for 60 minutes. Then, the percent of Fe^{+3} removal was calculated as indicated before.

Effect of contact time

The effect of contact time on efficiency of Fe^{+3} removal was studied. Different time intervals (30, 60, 90, 120, and 150 minutes) for *A. oryzae* were tried. The Fe^{+3} removal was studied by addition of 1g of

Aspergillus oryzae to 100ml of (10^{-2} M) of Fe^{+3} solution and shaked. Then, the percent of Fe^{+3} removal was calculated as indicated before.

Effect of initial Fe^{+3} concentrations

The effect of varied initial Fe^{+3} concentrations on efficiency of Fe^{+3} removal was studied. Different initial Fe^{+3} concentrations (0.527, 5.53, 46.69 and 437.51mg/100ml) for *A. oryzae* were tried. The Fe^{+3} removal was studied by addition of 1g of *A. oryzae* to 100ml of Fe^{+3} solution and shaked for 90 minutes. Then, the percent of Fe^{+3} removal was calculated as indicated before.

Effect of initial pH value

The effect of initial pH value was studied. Different initial pH values (2.5, 3, 3.5, and 4) were tried. The Fe^{+3} removal was studied by adjusting the pH of 100ml of (10^{-2} M) of Fe^{+3} solution by using 0.1 N NaOH. Then, added 1g of *A. oryzae* to 100ml of Fe^{+3} solution and shaked for 90 minutes. Then, the percent of Fe^{+3} removal was calculated as indicated before.

Effect of presence of NaCl as inert salt

The effect of addition of 0.4g of NaCl (assay 99.5%) to 100ml of (10^{-3} M) of Fe^{+3} solution was studied. The Fe^{+3} removal was estimated for optimum fungal dry weight 1g of *Aspergillus oryzae*. The mixture was shaked for 90 minutes at the solution pH. Then, the percent of Fe^{+3} removal was calculated as indicated before.

Regeneration of biomass

The biomass obtained after the desorption process was washed with (0.1 N) hydrochloric acid (assay 30-34%). Then, it was thoroughly washed several times by distilled water to get the neutral pH of washed solution. Then, they were dried and re-suspended in 100ml of (10^{-3} M) of Fe^{+3} solution and shaked for 90 minutes for A. oryzae at the solution pH. The reloading capacity was calculated as following equation:

The reloading capacity = amount of Fe^{+3} removal in the second cycle / amount of Fe^{+3} removal in the first cycle (2).

2.2.4. Isotherm models of biosorption

Iron biosorption was analyzed using Langmuir and Freundlich isotherms to study the adsorption behavior to investigate the performance of the biosorption process under different operating conditions. The adsorption isotherm models were applied at different induced Fe^{+3} concentrations. The amount of Fe^{+3} removal on the fungal biomass q (mg/g) was calculated according to the following equation:

$$q = (C_i - C_e)V/W \quad (3)$$

Where, C_i is the initial concentration of Fe^{+3} ions before adding fugal biomass (mg/L), C_e is the equilibrium concentration of Fe^{+3} ions after adding fungal biomass (mg/L), V is the volume taken from Fe^{+3} ions solution (L) and W is the amount of fungal biomass taken (g).

The Langmuir adsorption isotherm is utilized to describe chemisorptions process when the adsorbent and the adsorbate formed ionic or covalent chemical bonds (Langmuir, 1918). This model can be written in linear form:

$$1/q_e = 1/q_{max} + (1/q_{max} \, k_a)(1/C_e) \quad (4)$$

Where, q_e is the equilibrium amount of adsorbate on fungal biomass (mg/g), q_{max} is the maximuim amount of adsorbate on fungal biomass (mg/g) and k_a is the Langmuir constant (the maximum adsoption capacity in mg/L). The magnitude of a dimensionless constant R_L was used to determine the quality of Langmuir adsorption isotherm (the separation factor) can be calculated by the following equation:

$$R_L = 1/(1 + C_0 \, k_a) \quad (5)$$

The parameter R_L indicates the shape of the isotherm accordingly:

Value of R_L	Type of isotherm
$0 < R_L > 1$	Favorable
$R_L > 1$	Unfavorable
$R_L = 1$	Linear
$R_L = 0$	Irreversible

The empirical Freundlich adsorption isotherm is utilized to describe adsorption on a heterogeneus surface (Freundlich, 1906). This model can be written in linear form:

$$\log q_e = \log k_f + 1/n \, (\log C_e) \qquad (6)$$

Where, k_f is the Freundlich constant (the adsorbent capacity in mg/g) and n is the Freundlich coefficient (the adsorbent intensity in mg/L).

The Temkin Isotherm contains the factor that taking into the account of adsorbent adsorbate interactions. That is utilized to describe the assumption that a fall in the heat of sorption is linear rather than logarithmic, as shown in Freundlich isotherm (Temkin and Pyzhev., 1940). This model can be written in linear form:

$$q_e = B \ln A_T + B \ln C_e \qquad (7)$$

$$B = [RT / b_T] \qquad (8)$$

Where, A_T is Temkin isotherm equilibrium constant (L/g), B is cocstant related to heat of sorption (J/mol), R is universal gas constant (8.214 J/mol/K), T is temperature at 298K and b_T is Temkin isotherm constant.

III.RESLUTS And DISCUSSIONS

3.1. Effect of fungal powder biomass weights on Fe^{+3} removal

The effect of fungal powder biomass weights on Fe^{+3} removal was examined for (0.5, 1, 1.5, and 2g) for *A. oryzae*. Figure (1) shows that the percent of Fe^{+3} removal by *A. oryzae* was increased by increasing the biomass weights till reaching maximum (32.68%) at 1g. Then, the percent of Fe^{+3} removal decreased.
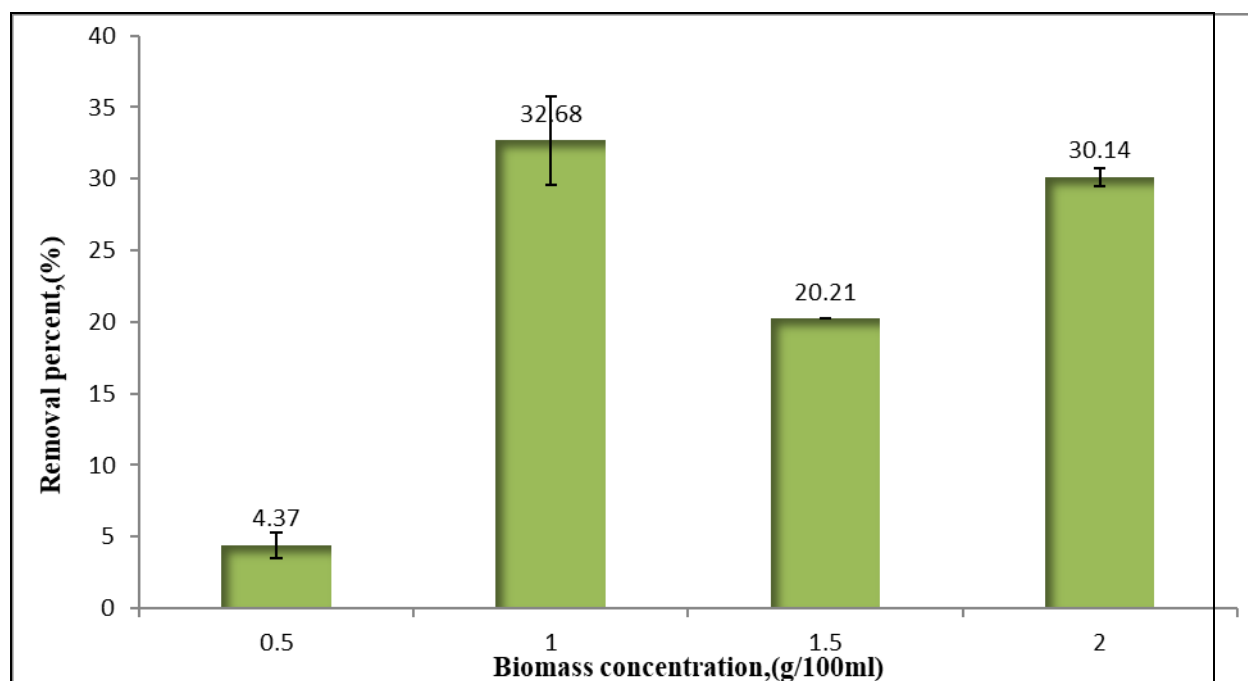

Fig. (1): Effect of Biomass of *Aspergillus oryzae* on Fe^{+3} removal at solution pH and contact time 90 minutes.

This increase as occurred in our results was explained due to the increase in the availability of free active sites binding on the absorptive surface area of the adsorbent. These results were investigated in other studies as (Siwi et al., 2018) reported that the initial concentration of biosorbent increased the removal efficiency was increased, (Mondal et al., 2017) reported that the biosorption of chromium gradually increased by increasing the biomass of Aspergillus nigar and (Saad et al., 2019) reported that the amount of copper (II) uptake by Aspergillus tamarii increased as the initial biomass concentration increased. Then, occur decreasing which mostly explained due to the decrease in the active sites binding at high biomass dose as a result of the partial cell aggregation. These results were investigated in other studies as (Selatina et al., 2004) reported that the decrease in biosorption of metal was occurred due to the clogging of the biomass which lead to decrease the number of available active sites binding for metal ions at higher concentrations or in sufficiency of metal ions in

solution with respect to available binding sites. (Hajahmadi et al., 2015) reported that the adsorption of zinc, cobalt and cadmium increased by increasing the Aspergillus nigar dosage till reaching the maximum then it was decreasing. Therefore, this observation indicates that the optimum level of fungal powder biomass weight that should be used in the following experiments was 1g/100ml.

3.2. Effect of contact time on Fe^{+3} removal

The effect of contact time on Fe^{+3} removal by fungal biomass was examined at (30, 60, 90, 120 and 150 minutes) for *A. oryzae*. Figure (2) shows that the percent of Fe^{+3} removal and *A. oryzae* increased by increasing the contact time till reaching the maximum at 90 minutes (55.94%). Then, they decreased by increasing the contact time more than 90 minutes.
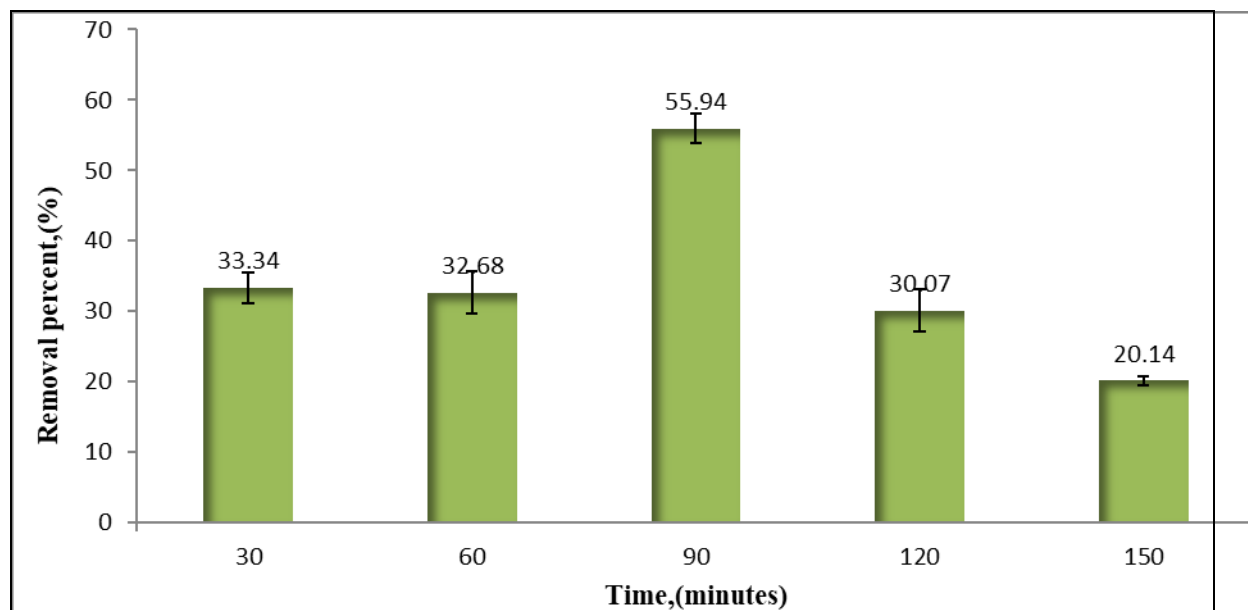

Fig. (2): Effect of contact time on removal of (10^{-2} M) of Fe^{+3} ions at solution pH, room temperature and using 1g/100ml of *A. oryzae*.

These obtained results were explained due to the change in the solution pH by time which reverse the process from desorption to adsorption. (Yu et al., 2000) explained this due the present of vacant active sites binding in the out surface of adsorbent, but by passing the time the active sites binding was saturated. Therefore, the adsorption process occurred in the inner surface instead of the outer surface. Due to the smaller inner surface area, the increased contact time causes efficiency to decrease. (Zareh et al, 2022 and Darama et al., 2021) reported that the process of zinc ions removal increased by increasing time contact till reached equilibrium time then decreased.

3.3. Effect of initial Fe^{+3} concentration on Fe^{+3} removal

The effect of initial Fe^{+3} concentration on Fe^{+3} removal by the fungal biomass was examined for (0.527, 5.53, 46.69 and 437.51mg/100ml) for *A. oryzae*. Figure (3) shows that the amount of Fe^{+3} removal by *A. oryzae* was increased by increasing the initial Fe^{+3} concentration till reaching the maximum at 5.53mg/100ml (84.65%). Then, it decreased by increasing the initial Fe^{+3} concentration.
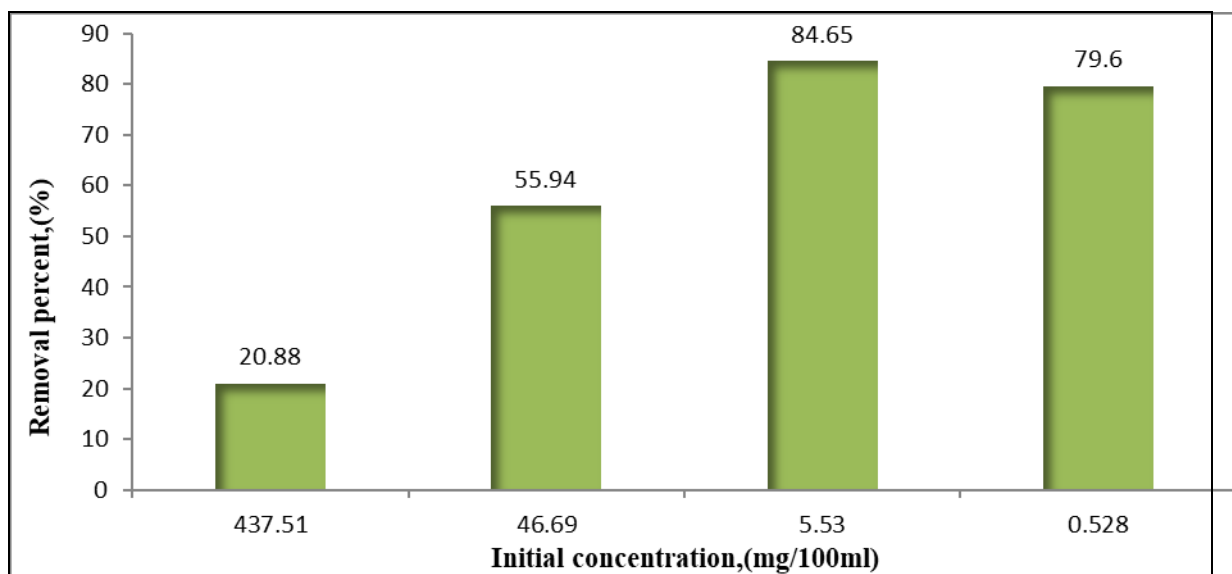
Fig. (3): Effect of initial Fe^{+3} concentration on Fe^{+3} removal using 1g/100ml of A. oryzae at the solution pH, room temperature and contact time 90 minutes.

These results were explained due to the proportion of the free active sites binding compared to the initial number of Fe^{+3} ions in the lower concentration are more, thereby tending to an increase in biosorption process. For higher concentration these sites become less and occupied, thereby tending to a decrease in biosorption process. The results of the present study agreed with several previous studies (Fawzy et al., 2022, Fawzy, 2020, Hajahmadi et al., 2015, El-Saied et al., 2017 and Hassouna et al., 2018).

3.4. Effect of initial pH values of solution on Fe^{+3} removal

The effect of initial pH on Fe^{+3} removal was examined by varying the pH value (2.5, 3, 3.5 and 4). From this study, the pH value suitable for the best removal percentage was found. Figure (4) shows that the optimum pH value for A. oryzae was at pH<3. Above this pH, ferric ions were converted easily to ferric hydroxide and the percent of Fe^{+3} removal could not be measured accurately (El-Naggar et al. 2018).

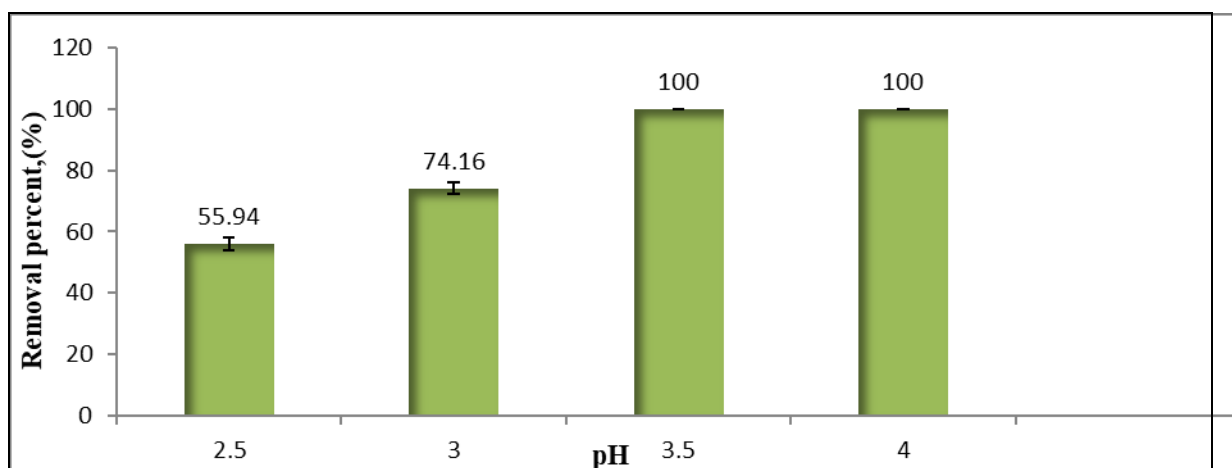**Fig. (4): Effect of initial pH values of (10^{-2} M) of Fe^{+3} solution on Fe^{+3} removal using 1g/100ml of A. oryzae at room temperature and time contact 90 minutes.**

3.5. Effect of presence of NaCl as inert salt on Fe^{+3} removal

The effect of addition of NaCl on the Fe^{+3} removal percent was decreased by (5.84%) in case of A. oryzae. In the presence of the inert salt, this decrease was explained due to the competitive effect of Na$^+$ ions on binding sites. (Aranda-García et al., 2020) explained that the vacant binding sites of the adsorbent tend to be saturated by the free Na$^+$ ions, thus, inhibited the biosorption of metal and the electrostatic forces between metal and the binding sites decreased because of an increase or expand in the electrical diffused double layer in the presence of NaCl. (El-Saied et al., 2017) explained that the formation of metal chloro-complexes due to the

presence of the free Cl- ions. The obtained results of the present study agreed with several previous studies (Djemmoe et al., 2016; Verma et al., 2016; Flores-Chaparro et al., 2017).

3.6. Regeneration of biomass

Regeneration of the used biomass is of importance for recycling purposes. 0.1N HCl was applied for the regeneration for fungal biomass. It worked like a desorbing agent that removes the binded ferric ions. The percent of Fe^{+3} removal decreased from 84.65% to 33.62%. (Xiao et al., 2010) explained this because of the competitive effect of the remained free H^+ ions. In spite of the Fe^{+3} desorbed biomasses were washed several times with DDW till the washed solution pH reaching neutral, the remained free H^+ ions on the fungal biomass surface compete with the vacant sites binding. Therefore, the Fe^{+3} removal decreased because of the decrease in the available free binding sites on the adsorbent fungal biomass. (Jaafarzadeh et al., 2014) reported that the biosorption capacity of cadmium decreased after desorbed by 1M of hydrochloric acid.

The reloading capacity = amount of Fe^{+3} removal in the second cycle / amount of Fe^{+3} removal in the first cycle   (9).

3.7. Isotherm models of biosorption

The adsorption isotherm models were evaluated for iron at the solution pH and contact time of 90 minutes with 1g of A. oryzae. The obtained results were used to fit the Langmuir, Freundlich and Temkin adsorption models by ignoring the extremely low value of Fe^{+3} concentration. The isothermal constant was calculated to find out the adsorption capacity of the different fungi for Fe^{+3}. The values of isothermal constants (K_a, K_f and A_T) and correlation coefficients R^2 were showed in the Table 1 for Langmuir, Freundlich and Temkin isotherms models. That results indicated that the biosorption data was best fitted in Freundlich as compared to Temkin and Langmuir models. The Langmuir constant (K_a), Freundlich constants (K_f and n) and Temkin constant (A_T and B) values were determined from slope and intercept of the plot.

The Langmuir model assumes that the maximum amount of Fe^{+3} adsorbate on the homogenous surface of fungal biomass (biosorbent) take place in saturated monolayer form. The monolayer saturation capacities, q_{max} fungi is 50 mg/g for *A. oryzae*. In case of the values of R_L were 0.9396, 0.5971, 0.1493 and 0.0184 for the initial Fe^{+3} concentrations 0.527, 5.53, 46.69 and 437.51 mg/100 ml. The R_L values indicate that sorption was more favorable for the lower initial metal ion concentrations than for the higher ones.

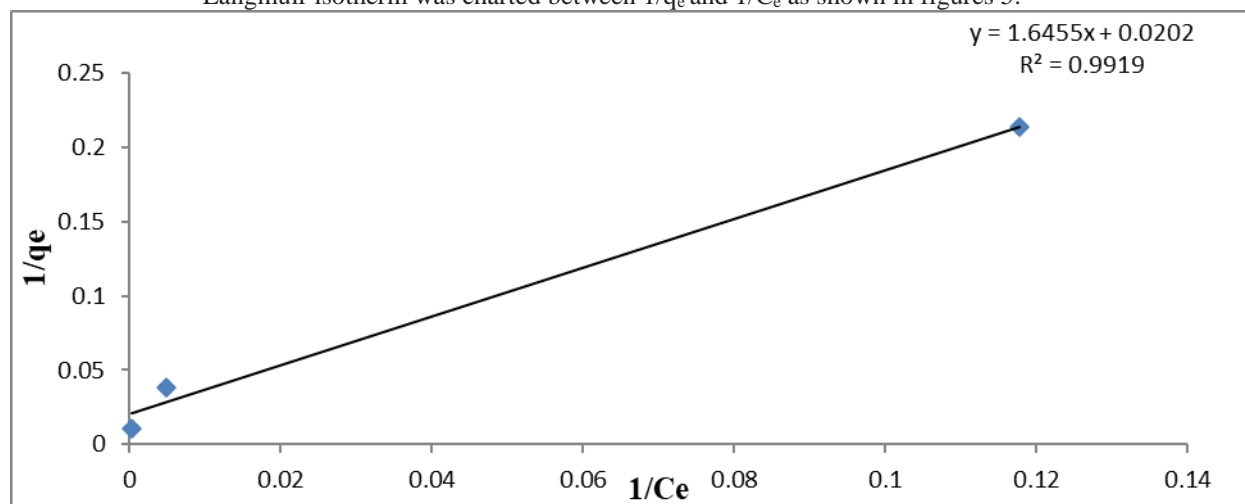Langmuir isotherm was charted between $1/q_e$ and $1/C_e$ as shown in figures 5.

Fig. 5.: Langmuir adsorption isotherm of Fe^{+3} in case of A. oryzae.

Freundlich isotherm was charted between log q_e and log C_e as shown in figures 6.
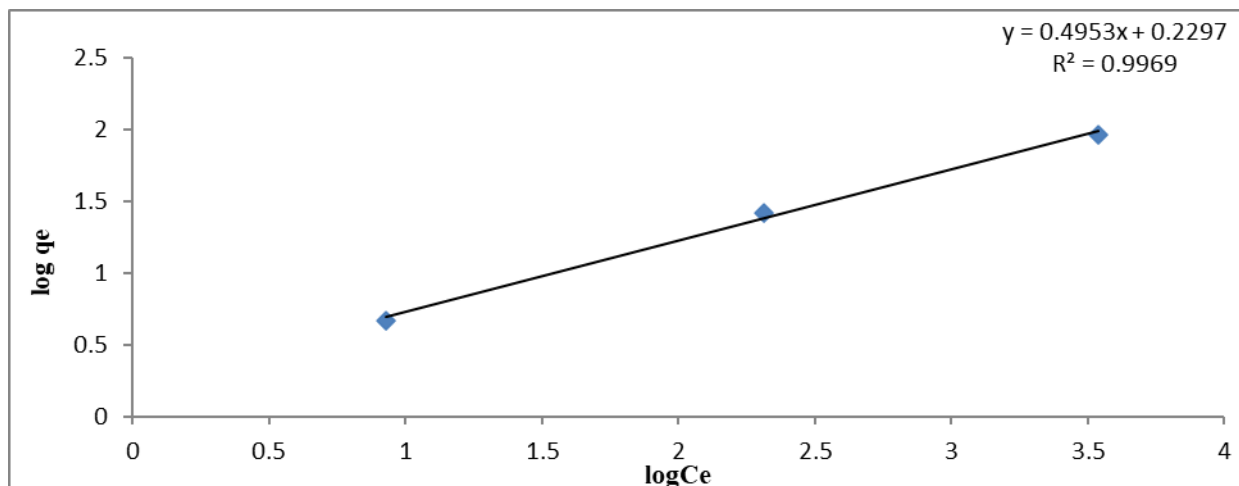
Fig. 6.: Freundlich adsorption isotherm of Fe^{+3} in case of Aspergillus oryzae.

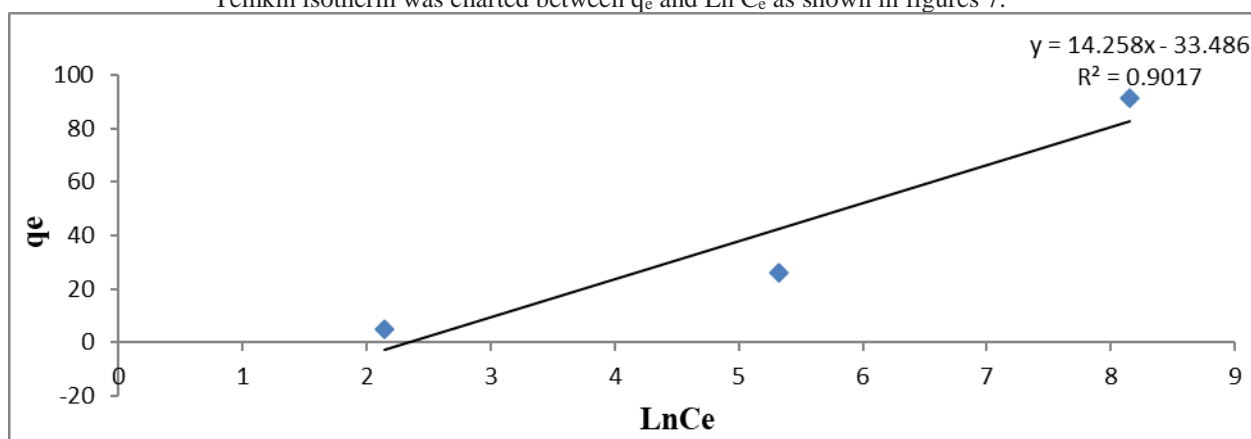Temkin isotherm was charted between q_e and Ln C_e as shown in figures 7.

Fig. 7.: Temkin adsorption isotherm of Fe^{+3} in case of A. oryzae.

Table 1: Isotherm parameter for the biosorption of Fe^{+3} by A. oryzae.

		Aspergillus oryzae
Langmuir isotherm model	q_{max}	50
	k_a	0.0122
	R^2	0.9919
Freundlich isotherm model	n	2.02
	k_f	1.694
	R^2	0.9969
Temkin isotherm model	A_T	0.0955
	b_T	173.767
	B	14.258
	R^2	0.9017

IV. Conclusions

In the present study, fungi powder biosorbent of A. oryzae was applied successfully for the sorption of iron metal from wastewater. The biosorption process was dependent on the amount of biomass, the contact time, the initial concentration of iron in water and the initial pH of solution. Biosorption increased by increasing the amount of biomass, contact time and concentration of iron till reach maximum then decreased. While at lower pH enhanced the efficiency of biosorption process. It was seen that although the addition of NaCl salt (increasing ionic strength) affected the adsorption yield and equilibrium uptake negatively, the fungi still have a considerable potential for the uptake of iron from saline waters. The sorption data fitted into Langmuir,

Freundlich and Temkin isotherms models. Which Freundlich Adsorption model was found have the highest regression value and hence the fit.

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